ABSTRACTS

ABSTRACTS FIRST NORTH - SOUTH CONFERENCE AND WORKSHOPS ON PHARMACOGENETICS (BEATING THE GENE : FROM THE BENCH TO THE BEDSIDE)

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Plenary Lecture 1

An Introduction to New Developments in Population PK/PD Modelling

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Population modelling seeks to describe the variability in drug behaviour both between and within subjects. On the one hand, we want to understand the behaviour of the drug, and the mechanisms that underlie it. On the other hand, we also want to use this information in a way that is maximally useful for planning initial and subsequent therapy in the next patient who comes to us who seems to be a member of that population. Parametric population modelling programmes assume that the shape of the parameter distribution in the population is either normal, lognormal, or multimodal. Most currently available software for this, such as NONMEM and the USC*PACK iterative 2 stage Bayesian programme, use either the first order (FO) or the first order, conditional expectation (FOCE) approximation to compute the likelihood or the conditional probabilities. These approximations destroy statistical consistency. Because of this, there is no guarantee that these methods will get results closer to the truth if more subjects are studied. The results may actually get worse with more subjects. Further, they are not very precise. However, there are now newer parametric population modelling programmes that do have both consistency and precision, such as the software of Lavelle in France, and the PEM programme of Leary at USC. Nonparametric (NP) population modelling programmes compute the entire most likely parameter distribution, without any constraints as to the assumption of normal, lognormal, multimodal, or any other shape. They simply obtain a discrete joint probability density that is most likely given the raw data and the error model used. Since the density is discrete, there is nothing to integrate, and there is no need for approximation. Integration is simply replaced by summation. Because of this, nonparametric modelling programmes such as NPEM and NPAG are consistent and precise. Studying more patients is guaranteed to give better results. Further, NP population models are uniquely well suited to develop maximally precise dosage regimens using the method of “multiple model” dosage design. The NP software is now capable of making any linear or nonlinear model of a drug having a single response, such as phenytoin, for example, or of the induction period of carbamazepine.

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Plenary Lecture 2

Pharmacogenetics of Psychotropic Drugs with Focus on CYP2D6

Leif Bertilsson

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At a given dose of such drugs as antidepressants and neuroleptics, the therapeutic response is highly variable. To a major extent, this is due to variation between patients in drug metabolism and pharmacokinetics. Many antidepressants and most neuroleptics are metabolised by the polymorphic CYP2D6. Seven percent of Caucasians are poor metabolisers (PM) mainly due to the presence of the *CYP2D6*4* allele encoding no CYP2D6 enzyme. In Asians and Africans there are two SNPs, *CYP2D6*10* and **17*, respectively coding for enzymes with decreased activity. Interestingly the *CYP2D6*17* allele encodes an enzyme with decreased rate of metabolism of debrisoquine and dextromethorphan, but not of the two other CYP2D6 substrates codeine or metoprolol. We discovered a new principle in drug metabolism, ultrarapid metabolism due to CYP2D6 gene duplication and multiduplication. There is pronounced inter - ethnic variation in the frequency of *CYP2D6* gene duplication: 1 % in Sweden, 10 % in Italy and Spain and as much as 29 % in Ethiopia. We recently showed that in nonresponders to antidepressant therapy in Sweden, 10 % had a *CYP2D6* gene duplication, which is a 10 - fold increase compared to the general Swedish population. Thus, ultrarapid metabolism seems to be one important factor for antidepressant non - response. Another polymorphic enzyme CYP2C19 catalyzes the N - demethylations of citalopram, clomipramine and diazepam. In Caucasians, 3 % are PM of CYP2C19 probe drugs e.g. omeprazole, while as many as 12 - 20 % of Asians are PM. This higher incidence is due to the presence of *CYP2C19*3* in addition to the *CYP2C19*2* present in all populations. Japanese patients had higher levels of clomipramine compared to Swedish patients due to the high incidence of PM and heterozygotes in the former compared to the later group. Most neuroleptics e.g. haloperidol, perphenazine and risperidone are metabolised by CYP2D6, but not clozapine. We found that the plasma concentration of clozapine increased several - fold when fluvoxamine was added to the treatment. Later, it has been confirmed that clozapine is metabolised by the tobacco inducible CYP1A2.

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Plenary Lecture 3

Pharmacogenetics Efforts by the Pharmacogenetics Research Group in Malaysia

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Many factors affect the pharmacology of drugs and the understandings of these factors continue to challenge us although

we have made great strides in our efforts. Over the last decades, the human genome has been discovered and it holds many keys in our understanding of the human organism including his interactions with his environment. Pharmacogenetics investigates the influence of genetics in the population response to drug therapy based on previous observations of population differences. Our group began by studying the genetic polymorphism of CYP2D6, a prototype in the study of pharmacogenetics in normal volunteers and in patient cohorts. We established its polymorphism in the major ethnic groups in Malaysia and we looked at the influence of the polymorphism on the pharmacokinetics of several index substrate drugs including metoprolol, methadone and tramadol. We subsequently expanded our investigations to other CYP's, NAT2, β_2 - adrenoreceptor and D_2 - dopamine receptor. We also studied their influences on several disease states and pharmacotherapies. Notable findings from our studies include the similarities and differences of our Malays, Chinese and Indians with other major populations in terms of the types and frequencies of the genetic polymorphism; the similarities and differences between patient cohorts and normal volunteers in terms of the types and frequencies of the genetic polymorphism and the influence of the polymorphism in the pharmacology of drugs including metoprolol, tramadol, warfarin and isoniazid. We conclude that the major ethnic groups in Malaysia are heterogeneous in relations to the types and frequencies of the genetic polymorphism with our Indians having more similarities with the Caucasians and our Malays and Chinese with the population in East Asia. We also conclude that the polymorphism may have impact on several diseases including their pharmacotherapies. Nevertheless, further studies are required to better define the relationships.

Plenary Lecture 4

A DNA Bank and Pharmacogenetics Database of African Populations - Tools for Molecular Medicine and Evolutionary Studies

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The idea of DNA Banks as a source of genetic information for a better understanding of disease susceptibility, behavioural differences and variability in drug response has been around for a while. It gathered international momentum with the initiation of the Human Genome Organization (HUGO) project in 1988 leading to the complete sequence of the human genome in 2003. Many countries, pharmaceutical companies and research institutes have various DNA banking programmes, with developed countries taking the lead. There are few DNA banks which include some African populations, of which all are at institutes in developed countries and none in Africa itself. The African Institute of Biomedical Science and Technology (AiBST) sponsored the initiation of a consortium of African countries in 2002 towards the establishment of a comprehensive DNA bank of Africa populations and Pharmacogenetics Database for genes known to influence people's response to medicines. Currently, there are 6 countries out of the 56 African countries that are participating in the DNA Bank and Pharmacogenetics Database. The countries are Nigeria, Kenya, Tanzania, Uganda, Zimbabwe and South Africa. More countries are being encouraged to join the consortium. Whereas other DNA Bank programmes focus on disease susceptible populations and family studies, the DNA Bank of African populations aims to sample from the major ethnic groups of the estimated over 2000 ethnic groups based on languages and historical records. Our current Bank has over 1000 DNA samples from 9 ethnic groups from the 6 countries. We aim to recruit an additional 5 countries each year with 300 - 400 samples (100 samples from each of the 3 - 4 major ethnic groups being collected from each country). Sample collection involves a 5 - 10 ml blood sample, which is then transported to AiBST laboratories. Part of the blood is used to prepare DNA, some of the blood is blotted and dried on filter paper, and the reminder stored at -80 ^{0}C. All samples are coded in such a way that one can identity their country and ethnic origin. These codes are entered into a computerised system, which also enable one to trace the storage location of the sample. Access to the samples is password protected. The DNA will be used for pharmacogenomics and pharmacogenetics characterisation of the populations with respect to: (a) evolutionary relatedness of African populations (b) mapping of genetic markers for efficacy & safety in the use of drugs (c) mapping the frequency of disease and/or ADRs susceptibility genes and (d) the selection and validation of target genes that will inter - individual and inter - ethnic applicability. Towards the above, we have started by establishing the pharmacogenetics of drug ADMET (absorption, distribution, metabolism, excretion and toxicology) genes. We are analysing the genotypes of 10 genes (*CYP2A6, 2C9, 2C19, 2D6, TPMT, GSTM1, GSTT1, UGT1A1, pGp, NAT2*) for over 30 SNPs (single nucleotide polymorphisms). We will discuss these results and their implications for inter - ethnic relatedness of African populations and the potential for variable therapeutic and toxicological response to drugs whose pharmacokinetics is influenced by these polymorphic genes.

Plenary Lecture 5

Metabolism Based Drug - Drug/Herb Interactions

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Pharmacokinetics describes the time course of what the body does to an administered drug, that is, its absorption, distribution, metabolism and excretion (ADME). When drugs are co - administered in the many clinical scenarios which

include; simultaneous treatment of several diseases states, use of combination therapy for increased efficacy and reduced emergence of drug resistance, and when conventional drugs are used together with traditional/herbal medicines, they can affect each other's pharmacokinetics. Interactions based on inhibition and induction of drug metabolising enzymes constitute the majority of clinically relevant cases hence will be the subject of this presentation. Because of our research interest in tropical medicine, we will highlight some examples in the treatment of infectious diseases. The major drug metabolising enzymes are CYP1A2, 2C9, 2C19, 2D6 and 3A4. Among them, they are responsible for the metabolism and elimination of over 90 % of drug on the market. It therefore implies that up - or down - regulation of expression or activity of these enzymes can alter the pharmacokinetics of a compound whose main route of elimination is by the affected enzyme. Towards predicting drug clearance and the potential of new chemical entities (NCE) to result in undesirable drug - drug interactions when co - administered with other drugs, the pharmaceutical industry has invested in pre - clinical studies that aim at identifying the CYPs responsible for the metabolism of NCE and to evaluate the inhibitory effects of the NCE on the CYPs. Should the properties that confer pharmacological activity be inseparable from those making the compound a potent inhibitor, there are also clinical protocols for evaluating the extent of drug - drug interactions in humans. *In vitro* pre - clinical studies use recombinantly expressed CYP450 with either CYP - specific marker reactions (monitoring requires HPLC - UV or LC - MS) or less substrates (monitored by fluorescence). When using human liver microsomes, hepatocytes and liver slices, CYP specific marker reactions are preferred since these cellular and sub - cellular fractions contain a mixture of all the CYPs. For inhibition studies, the most commonly used system in industry is the rCYPs with fluorescent markers for HTS and then rCYPs and HLM with marker substrates for detailed studies for compounds known to be inhibitors and still nominated as candidate drugs. Results from such studies help in the design of clinical drug - drug interaction studies. For induction studies, hepatocytes, reporter gene systems and tissues slices are used with hepatocytes being the most commonly used. For structure activity relationships of interactions with the regulatory elements, PXR, CAR and Ah receptors, the use of the reporter gene systems is suitable. *In vivo* clinical drug - drug interactions use CYP specific probes singly or in cocktail formats. Unlike the *in vitro* studies, the probe drugs used for each enzyme must be safe, specific and allow for easy sampling of urine or blood for substrate/metabolite analysis. Early clinical drug - drug interactions with probe drugs can then be followed by combinations of real drugs to be co - administered with the NCE. Information from these studies plus the mechanistic data on enzymes involved can then be included in the product insert to alert doctors and patients of the potential for drug - drug interactions when taking certain drug combinations.

Plenary Lecture6

Multiple Model Dosage Design and New Methods of Bayesian Updating of Individual PK/PD Models

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In developing dosage regimens of toxic drugs for patients, one wants to hit the desired therapeutic target with maximum precision. This is a problem with many current computer programmes, as they use parametric population PK/PD models and maximum aposteriori probability (MAP) Bayesian individualisation. These approaches provide only a single set of model parameter values and assume a normal or lognormal parameter distribution in the population. There is no way to calculate in advance the expected degree of precision with which the regimen will actually achieve the desired target goal. One simply assumes it will hit it exactly, and knows in his/her heart that this will not be so. Dosage regimens based on mean population parameter values may actually be dangerous. On the other hand, the multiple support points in a nonparametric population model provide multiple predictions of future serum concentrations resulting from a candidate regimen, each with a certain probability. Because of this, it is easy to compute the expected weighted squared error with which the regimen will fail to hit the target. One can then easily find the regimen which specifically minimizes this error, resulting in a regimen which, for the first time, hits the target with maximum precision. This is "multiple model" dosage design. Multiple model adaptive control is widely used in flight control and guidance systems for fixed wing aircraft and helicopters, and spacecraft, for example, and is well known in that field. The MM - USCPACK software now uses this approach for Bayesian adaptive control of drug dosage regimens. Bayesian updating of the nonparametric population models is done by simply recomputing the Bayesian poster probability of each support point in the population model. The parameter values are unchanged. Those that fit the patient's data well become much more likely. Those that fit it poorly become much less likely. Because of this, usually only a few points survive this multiple model (MM) Bayesian analysis and have significant posterior probability. A consequence of this is that the patient must be reasonably well represented by some support points in the population model, and must be within the range of the parameter values in the model. If this is not the case, then the posterior model may not find him/her. This can be a significant problem. Because of this, we have now developed a hybrid MAP - MM Bayesian procedure in which a MAP Bayesian analysis is done first. Then, extra support points are placed nearby, to "precondition" the population model for the data it will now receive. Then the MM Bayesian analysis is done on this augmented, preconditioned population model. This should greatly increase the precision and the safety of Bayesian analysis for unusual individual patients. Another problem is that of other unusual patients who clearly have changes in their parameter values during the period of data analysis. Up to now, all Bayesian updating procedures have assumed that there is one and only one set of parameter values (or distributions) that best fit the data. This is a significant problem in patients who clearly have changes, such as increasing or decreasing apparent volumes of distribution, in response to changes in their clinical status, as one changes from a

general medical patient, for example, to one in an intensive care unit, and *vice versa.* To deal with this problem, we have utilized the Interacting Sequential MM (IMM) Bayesian procedure, which is also widely used in the aerospace industry to better predict the position of hostile targets taking evasive action. This procedure has been shown to track the behaviour of a simulated patient with changing parameter values with slightly less than half the overall error of the MAP or MM Bayesian procedures. It has been incorporated into the MM - USCPACK software for use in such patients.

Plenary Lecture 7

Genotype to Phenotype Correlation in Health and Disease: Discovery and Application

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Human health and disease appear to result from the continuous interaction of endogenous (genetic) and exogenous (environmental) factors, the latter including chemical, biological, nutritional, pharmaceutical, physical and psychological exposures. Advances in understanding the differential impact of these manifold influences are likely to arise from the judicious use and careful analysis of animal models of the human condition. To increase understanding on the gene side, the U.S. National Institutes of Health (NIH) - National Institute of Environmental Health Sciences (NIEHS) developed a consortium of academic health centres to develop mouse models to determine the functional significance of human DNA polymorphism. This consortium, the Mouse Genomics Centres Consortium (CMGCC), includes scientists from University of Cincinnati; Harvard University; University of Texas Health Science Centre, San Antonio; University of Texas M.D. Anderson Cancer Centre, and University of Washington. To amplify knowledge of the role of environment, the NIH - NIEHS developed a Toxicogenomics Research Consortium (TRC), with membership from Duke University Medical Centre; Fred Hutchinson Cancer Research Centre and University of Washington; Massachusetts Institute of Technology; Microarray Group, NIEHS National Centre for Toxicogenomics; Oregon Health & Science University, and University of North Carolina at Chapel Hill. The TRC is designed to assess the reproducibility and reliability of *omics* technologies by carrying out TRC - wide experiments on mouse models of health and chemically induced disease. A wide variety of applications of toxicogenomics data has been proposed, including prediction of potential chemical pharmacology and toxicity, elucidation of mechanism of action, support of regulatory risk assessment, and as the successor to animal toxicity testing. The readiness of microarray technologies for use in these areas is addressed in a December 4 - 8, 2005 international conference and workshop that brings the TRC and CMGCC together with the NIEHS - supported non - U.S. governmental National Academies Committee on Emerging Issues and Data on Environmental Contaminants (http://www.ohsu.edu/croet/trc/trc2005/). A summary of the deliberations and conclusions of this high - level scientific discourse comprises the subject of this lecture.

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Plenary Lecture 8

Practical Experience of Clinical Pharmacogenetics

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We have developed a cocktail of 5 drugs as markers of the, what I consider, most important cytochrome P450 (CYP) enzymes in humans: caffeine for CYP1A2, losartan for CYP2C9, omeprazole for CYP2C19, debrisoquine for CYP2D6 and quinine for CYP3A4/5. This Karolinska cocktail may be used for different projects, for example to study which CYPs are induced or inhibited by a certain drug. The cocktail is given before and then during treatment with this special drug. We have also administered the cocktail to different populations to demonstrate inter - ethnic differences in drug metabolism. For drug metabolism in total, CYP3A4 is one of the most important enzymes. In the 1970 – ies, we were the first to study the disposition of the antiepileptic drug carbamazepine. The half - life of this drug after a single dose to healthy adult healthy subjects was about 35 hour. In newborns, infants to epileptic mothers treated with carbamazepine during pregnancy, the half - lives varied between 8 and 30 hour. This short half - life was surprising to us as newborns have a poorly developed drug metabolism. Now we know that CYP3A4 in the newborns was induced during the exposure to carbamazepine transferred from the mother to the fetus. Carbamazepine is today known as an inducer of its own metabolism and of other drugs metabolised by CYP3A4. CYP3A4 has a sister enzyme CYP3A5, which has similar substrates, inhibitors and inducers as CYP3A4. CYP3A5 is highly polymorphic. Among 136 healthy Swedes only 17 expressed CYP3A5 (1 with *CYP3A5*1/*1* and **16* with *CYP3A5*1/*3*). In 143 Tanzanians, however, as many as 38 and 68 subjects had 2 and 1 functional *CYP3A5* alleles, respectively, when genotyping for **3, *6* and **7*. In these Tanzanians there was a relationship between the metabolism of the CYP3A4/5 substrate quinine and the *CYP3A5* genotype. CYP3A5 seems to be an important enzyme for certain populations, for example black Africans, but not in others like Europeans. Together with Professor Ulf Diczfalusy, we have developed the endogenous 4 - beta - hydroxycholesterol as a marker of CYP3A4 and CYP3A5.

OP 1

C677T Polymorphism of Methylenetetrahydrofolate Reductase *Gene and Increased Plasma Homocysteine Concentration May Be an Independent Risk Factor for Ischaemic Stroke in Multi-Ethnic Malaysian Population*

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Hyperhomocysteinemia is associated with increased risk of atherosclerotic vascular diseases, like ischaemic stroke. Plasma total homocysteine levels are regulated mainly by 5, 10 -methylenetetrahydrofalate reductase (*MTHFR*) which is involved in the folate - dependent remethylation of homocysteine to methionine. The C677T polymorphism in the *MTHFR* gene has been suggested as a candidate risk factor for vascular disease in certain population groups. Our objective was to evaluate the prevalence of the $C_{677}T$ polymorphism of the *MTHFR* in subjects with and without ischaemic stroke in multi - ethnic Malaysian population and its correlation to homocysteine levels. Subjects recruited for this study were multi - ethnic ischaemic stroke patients from University Malaya Medical Centre, Kuala Lumpur, who gave informed consent and met inclusion criteria. *MTHFR* genotypes were analysed by polymerase chain reaction (PCR), followed by *Hinfl* digestion. One hundred and forty six control subjects and 91 stroke subjects were recruited for this study and were sex and age matched. The prevalence of CC, CT and TT genotype in the control group was 0.63, 0.36 and 0.06 while the stroke group had a prevalence of 0.46, 0.40 and 0.14, respectively. The CC and TT genotype distribution between the two groups were significant ($p < 0.025$). There was also a positive correlation between homocysteine levels and the genotype with mean concentration levels of 10.54 ± 3.16 mmol/L and 12.00 ± 3.46 mmol/L, respectively for CC and TT genotype. The difference between Hcy levels of CC and TT genotype was 1.46 mmol/L and was significant. TT genotype is associated with an increased plasma homocysteine level and was significantly more prevalent in patients with ischaemic stroke in our study. This may be an independent risk factor for ischaemic stroke and may help identify sub - groups that will benefit from homocysteine lowering therapy.

OP 2

A Report on the SNP in PGF_2 - alpha Receptor *Gene among Glaucomatous Patients*

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Prostaglandin analogues especially latanaprost has gained popularity and proven to be effective in reducing the intraocular pressure in glaucomatous patients. The pressure lowering effect of latanaprost which is achieved through the action on prostanoid receptor (FP) is believed to be responsible in increasing the uveosclereal outflow through unknown mechanism. Latanaprost though effective, has shown variation in achieving target pressure and side effects in difficult races. We postulated this variation is related to the polymorphism of the FP receptor. With the contemporary knowledge in pharmacogenetic and advances in the laboratory techniques, the success of the mutation detection will contribute to customize treatment to the glaucomatous patients. In this study, we wish to report the detection of the SNP located in the untranslated region of *PGF_2 - alpha receptor* gene, rs3766331, among the Malaysian population. Twenty - seven glaucomatous patients and 10 controls were recruited. Five millilitre of blood were taken and DNA extracted, and then screened for the *PGF_2 - alpha* gene by denaturing high performance liquid chromatography (DHPLC). Of these, 2 patients revealed a heteroduplex peak. Sequencing was performed to confirm the mutation and both the patients were proven to carry AG heterozygous genotype. To the best of our knowledge, this is the first reported detection of the SNP in *PGF_2 - alpha receptor* gene among the Malaysian population. The establishment of the screening method for this gene will be useful for those who are doing large scale patients screening. A larger study associating the responses to the drugs and the genotypes of the patients are underway.

OP 3

Dopamine D_2 Receptor *Gene Polymorphism among Drug Addicts in Malaysia*

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Several substances that share the potential for abuse also share the ability to enhance dopamine activity in mesolimbic/ mesocortical circuits thought to be responsible for behavioural and reinforcement causing addiction. It is reported that dopamine D_2 receptors (DRD_2) are importantly involved in drug abuse, as many abused drugs elevate limbic dopamine. Genes that are important in the mesolimbic/mesocortical dopaminergic pathways are thus strong candidate genes for possible contributions to inter - individual differences in substance abuse vulnerability. This study was approved by the Ethical Committee at Universiti Sains Malaysia in Kelantan. It investigated the genetic association between two functional polymorphisms (Ser_{311}Cys and *TaqIA*) in the *DRD_2* gene with drug addiction. Seventy - seven intravenous drug users (IVDU) that met the inclusion and exclusion criteria were enrolled after written informed consents were obtained. DNA from the subjects' whole blood were extracted using standard lysis methods and subjected to allele specific PCR. At the Ser_{311}Cys locus, 87 % were homozygous *wild - type* (Ser/Ser) with the rest found to be heterozygous (Ser/Cys). None were found with homozygous mutant (Cys/Cys) genotype. At the *TaqIA* locus, 31 % were homozygous *wild - type* (*A2/ A2*), 57 % were heterozygous (*A1/A2*) and 11 % homozygous mutant (*A1/A1*). Our result suggests that mutation at Ser_{311}Cys may not play a role in the development of drug addiction while the *A1* allele of the *Taq1A* receptor gene may. However, further studies are needed to confirm role of this polymorphism in drug addiction.

OP 4

Higher Occurrence of CYP2C8 *Variants at 3' UTR ($_{-241}$IVS) in Cardiovascular Patients Detected Using DHPLC*

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CYP2C8 belongs to the CYP2C subfamily and metabolises many endogenous compounds such as progesterone and arachidonic acid that have putative roles on the pathogenesis of cardiovascular diseases. CYP2C8 is also responsible for the metabolism of approximately 20 % of drugs including paclitaxel and omeprazole. *CYP2C8* is genetically polymorphic. The objective of the study was to develop a high throughput genotyping method using denaturing high performance liquid chromatography (DHPLC) and to unravel possible new variants of *CYP2C8* among local population. DHPLC method was developed by manipulating the denaturing temperature and buffer composition to allow elution of different DNA composition due to nucleotide changes. One hundred DNA samples form healthy volunteer and another 100 from cardiovascular patients were extracted from whole blood using alkali lysis method. PCR was performed to amplify all the 9 exons - introns of *CYP2C8* which was then screened using the validated DHPLC method. Reference *wild - type* and positive controls were used in each batch to categorise the samples according to similarity of peak profiles using Star Workstation". The samples were also screened using our previously published allele specific multiplex PCR (ASPCR). All the samples were successfully amplified by PCR. Nucleotide changes detected new by DHPLC included T_{4250}Del and C_{1232}T. In comparison to ASPCR, our DHPLC method was equally sensitive and specific. It has an added advantage of being able to detect new variants of *CYP2C8* that may be important in comparing healthy cohorts with disease cohorts. Our finding of the higher occurrence of 3' UTR ($_{-241}$IVS) variants in cardiovascular patients for instance suggest the possible role of the variant in relation to cardiovascular diseases. We conclude that the DHPLC method is useful in detecting novel variants of CYP2C8 for studies to compare normal populations with diseased cohorts.

OP 5

Genetic Polymorphism of* CYP2D6 *among Codeine Seekers

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Functional CYP2D6 is required to metabolise codeine to morphine and thus extensive metaboliser may be a pharmacogenetic risk factor in oral opiate dependence while PM is protective. In Malaysia, no study had been conducted to investigate the influence of CYP2D6 among individuals abusing codeine. Furthermore, no study had looked into the effect of *CYP2D6*10* which encode enzyme with reduced activity in codeine addiction. This study was designed as an initial attempt to characterise genetic polymorphism of CYP2D6 among codeine seekers and to investigate the possible correlation with codeine abuse. This study was approved by the Research and Ethic Committee of Universiti Sains Malaysia, Kelantan. Targeted subjects, comprising of codeine users, were recruited mostly by snow - ball recruitment methods from pharmacies and restaurants. Subjects were screened for substances abuse using established urine test and dependency using DSM IV instruments. Five ml of venous blood were sampled from each subject. DNA was extracted and subjected to PCR genotyping for alleles **1, *3, *4, *5, *6, *9, *10, *14, *17* and the duplicated gene. One hundred and four subjects were successfully recruited. The genotypes were then compared with a group of healthy

unrelated Malays. In comparison with the codeine users (COD), allele frequencies for **1* and **10* were significantly different between the COD and control groups. There was a higher prevalence of *CDYP2D6*1* in COD group than the control group whereas *CYP2D6*10* was more common in control group ($p = 0.014$, χ^2 analysis). There was also no genotype which predicts poor metabolisers (PM) being detected in the COD group; while 1.9% was predicted PM for control. More individuals in COD were found to have homozygous genotype predicting EM phenotype compared to controls. This suggested that *wild - type* allele encoding normal enzyme activity conferred the codeine users higher capabilities in transforming codeine to morphine and provide them stronger euphoric effects and thus a risk factor for codeine abuse.

OP 6

A Single Nucleotide Polymorphism (SNP) Detection in Mental Retardation Disease by High Density Oligonucleotide Array

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Single Nucleotide Polymorphisms (SNPs) are the common variations found in the DNA sequence of individuals. The identification and characterisation of naturally occurring variations in the human genome has become popular markers in molecular genetics because of its application in the identification of susceptibility to common diseases. We addressed the issue of SNPs in patients with mental retardation disorders using GeneChip" Human Mapping 10K Array *Xba* 131 (Mapping 10K Array, Affymetrix, Santa Clara, CA). We present here the results of the genome scan. A common SNP pattern was ia§tified in a total of 24 SNP arrays which are dupa)´ates. Samples from patients with confirmed and suspected Fragile X Syndrome were screened together with samples from normal patients. The reference DNA from Affymetrix GeneChip" Reagent was used as a control. Result for each of the allelic loci was determined by the GeneChip" DNA Analysis Software. The 10K Mapping Assay revealed genotype calls (AA, BB or AB) from 84 % to 97 % (average 91.73 ± 3.82 %) of the 11560 SNPs in the SNP array for DNA from all samples. This fulfilled the manufacturer requirement for identification of SNPs. We identified 194 differentials SNP loci among the samples. These variables sites are present with high density in the genome, making them powerful tools for mapping and diagnosing disease related alleles.

OP 7

Beta$_2$ - adrenergic Receptor (β_2AR) Polymorphisms and Risk of Asthma in Malays

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Bronchial asthma is a chronic inflammatory disease of the airway associated with high morbidity and mortality. It also involves frequent emergency room visits for acute exacerbations. A method to improve management approaches is therefore useful to overcome these. The study therefore aimed to determine β_2AR polymorphism in asthmatic patients and its association with risks for asthma exacerbations in a Malay cohort. One hundred and thirty two asthmatic patients presented to the emergency department of Hospital Universiti Sains Malaysia for acute exacerbation of bronchial asthma and fulfilled inclusion criteria were recruited during a study period of 15 months. Measurements of peak expiratory flow rate were performed before and after the patients were administered standard bronchodilator therapy. Five millilitres of venous blood was also taken for DNA extraction prior to allele specific multiplex PCR for β_2AR polymorphisms. Healthy unrelated Malays were recruited as control cohort. The outcome for prevalence and risk association were analysed using descriptive statistics. Substitution of amino acid for $Arg_{16}Gly$ and $Gly_{16}Gly$ were associated with acute exacerbations but variations at codon 27 had no association. Our results suggest that genetic variation of β_2AR at codon 16, may be useful to predict risk for asthmatic exacerbations.

OP 8

NAT2 *Polymorphisms in the Major Ethnic Groups in Malaysia*

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To date, *NAT2* is known to have more than 15 point mutations and 28 allelic variants. These variations result in amino acid changes causing altered enzyme activity that may alter cancer susceptibility, efficacy of drugs and the toxic potential of certain environmental pollutants. There are large inter - ethnic and geographical differences of *NAT2* variants. We hypothesize that due to mixed - marriages and cross - cultural dilutions of the many ethnic groups in Malaysia, the types and frequencies of *NAT2* variants among them are different. The objective of this study was therefore to investigate the types and frequencies of *NAT2* polymorphism in the three major ethnic groups in Malaysia. The protocols for this study were approved by the Research and Ethics Committee, USM. DNA from 212 Malays, 172 Chinese and 175 Indians were subjected to allele specific PCR to detect the polymorphism. The PCR method was to screen for variations at 111T>C, 190C>T, 191G>A, 341T>C, 434A>C, 481C>T, 499G>A, 590G>A, 759C>T, 803A>G, 845A>C and 857G>A. The distribution of the genotypes in our study was consistent with Hardy – Weinberg equilibrium in all the three major ethnic groups. The most common allelic variants among Malays and Chinese was *NAT2*4* at 43 % and 64 % respectively. This allele was found at a lower frequency among Indians at 23 %. On the other hand, the most common alleles among Indians were *NAT2*5* and *NAT2*6* with a frequency of 31 % for both. *NAT2*5* has the greatest reduction in acetylation followed by *NAT2*14* and *NAT2*6.* Our Indians were therefore predicted to have higher risks to side effects of drugs metabolised by NAT2 but would probably be at reduced risks for the development of cancers that are thought to result from the formation of carcinogens from pro - carcinogens in reactions mediated by NAT2. Our Indians had similarities to Caucasian and our Chinese had similarities to Koreans and Japanese. Our three major ethnic groups differed in their *NAT2* genotypes profiles. They would thus be predicted to have different profiles for susceptibility to cancers and side effects of NAT2 substrates. Further studies are however required to investigate the correlation between *NAT2* genotype and phenotype as well as genotype with side effects of NAT2 drugs and NAT2 induced cancers.

OP 9

Higher Occurrence of Homozygous $C_{3435}T$ of MDR_1 *among the Indians in Malaysia*

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P - glycoprotein (P - gp) is a member of the ATP - binding cassette transporters. It acts as an energy dependent pump that extrudes its substrate out of cells. The variability of P - gp expression between individuals has been associated with single nucleotide changes of the human multidrug resistance (*MDR_1; $ABCB_1$)* gene. This result in variable bioavailabilities and responses for cardiac glycosides, HIV - 1 protease inhibitors, anti - cancers, and other substrates of P - gp. Inter - ethnic and geographical differences in allele frequencies have been reported and these have implications in drug response. We thus developed a simple PCR method to detect single nucleotide change for $C_{3435}T$ variants in the local population. The protocols were approved by the local Research and Ethics Committee. Five ml of whole blood was taken from healthy unrelated individuals after written informed consent. DNA was extracted using alkaline lysis method and subjected to a 2 - step nested PCR. DNAs from 304 Malays, 288 Chinese, and 171 Indians were successfully amplified. $C_{3435}T$ variants were detected in all the 3 ethnic groups. Sample were selected randomly and sent for nucleotide confirmation by direct sequencing. Almost 50 % of all the Malaysian was heterozygous carriers of the C/T variant. The Indian had frequency of the homozygous T/T variant significantly higher than the Chinese and Malays; 39 % versus 14 % and 15 %, respectively. The Malaysians were different from the Africans with respect to the allele frequency. However, only the Malays and Chinese were found to be different compared to the Caucasian. The Indians resemble the Caucasians and are, thus at higher risk for adverse drug effects given the same dose of drugs compared to the Chinese and Malays. However, further studies are required to define its clinical significance.

OP 10

CYP2A6 *among Healthy Malays, Chinese and Indians in Malaysia*

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CYP2A6 is the major nicotine - oxidase in humans and is genetically polymorphic. The polymorphism may be important in nicotine dependence and susceptibility to cancer. The objective of this study was to investigate the types and frequencies of *CYP2A6* alleles in the three major ethnic groups in Malaysia. *CYP2A6*1A, CYP2A6*1B, CYP2A6*1x2, CYP2A6*2, CYP2A6*3, CYP2A6*4, CYP2A6*5, CYP2A6*7, CYP2A6*8* and *CYP2A6*10* were determined by multiplex PCR. Except for *CYP2A6*2* and **3* that were not detected in the Malays and Chinese, all the other variants were detected. Frequency for null *CYP2A6*4* allele was 7 %, 5 % and 2 %, respectively in Malays, Chinese and Indians. Among Malays and Chinese, the most common variant was *CYP2A6*1B* but it was CYP2A6**1A* among Indians. Since, CYP2A6 may have

effects on smoking; further studies are required to investigate the relationship between the CYP2A6 phenotype and genotype in the ethnic groups in Malaysia as well as their influence on diseases such as cancer and smoking behaviour.

OP 11

Cloning and Expression of CYP2C9 for Drug Metabolism Study

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Elucidation of the metabolic pathway of drugs or compounds is one of the processes routinely conducted during the development of a new chemical entity to therapeutic agent. An heterologous system that enables rapid screening of the principal routes of metabolism of these new chemical entities would be of enormous benefit in research and drug development. Cytochrome P450 (CYP) 2C9 is a principal enzyme involved in phase I drug metabolism. It is reported to metabolise many drugs including phenytoin, warfarin, tolbutamide, celecoxib, fluoxetine and losartan. We thus cloned and expressed both human *CYP* and *NADPH cytochrome P450 reductase* gene in *E.coli*. pCWori$^+$ containing the *CYP2C9* gene was transformed into *E. coli* cells together with *CYP - reductase*. Since bacterial cells lack suitable endogenous redox partner to catalyse efficiently the transfer of electron to CYP, the NADPH CYP - reductase would help to generate an active system. Yield was most optimum when the *E. coli* cells were incubated at 30 ºC and harvested after 48 hours. Immunoblotting using rabbit anti - human CYP2C9 antibody and rabbit anti - human CYP - reductase antibody as their primary antibodies demonstrated the presence of both CYP2C9 and reductase proteins with sizes approximately 55 kDa and 80 kDa. The presence of these proteins was determined spectrally at 450 nm after saturation with carbon monoxide. The concentrations of CYP2C9 and reductase obtained were 23.57 nmol/ml and 11.2 nmol/min/mg, respectively. In summary, we have successfully cloned and expressed a recombinant human CYP2C9 using *E. coli*. The availability of this *in vitro* enzyme is useful for enzyme kinetic and drug interaction study.

OP 12

The Effect of Grapefruit Juice, a P - Glycoprotein Inhibitor, on Organic Acid and Conjugates Urinary Profile in Healthy Human Subjects

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The purpose of the study was to investigate the effect of grapefruit juice, a P - glycoprotein inhibitor, on intestinal P - glycoprotein by elucidating the metabolic profiles of volunteers taking grapefruit juice; to compare these profiles with the metabolic markers of certain genetic disorders and to identify molecular markers for the screening of P - glycoprotein defects. Inhibition of intestinal P - glycoprotein was effected by administering grapefruit juice to healthy volunteers (children and adults). The organic acids in the urine samples were analysed after intake of the juice and compared with the controls (urine samples before grapefruit juice was taken). These acids were isolated from the urine, derivatized and determined by gas chromatography - mass spectrophotometry (GC - MS). The mass spectra obtained from the GC - MS analysis were qualitatively and quantitatively elucidated with the use of the automated mass spectral deconvolution and identification system (AMDIS) and Whiley's MS - library. The GC - MS spectra showed a remarkable increase in the organic acids present in urine samples after the administration of grapefruit juice as well as in their concentrations when compared with the controls. The organic acids from microbial origin were found to be significantly increased in the adults. There was no statistical significant increase in the children. This study confirmed that grapefruit juice inhibits P - glycoprotein in the intestine and this resulted in the presence of unusual organic acids from microbial origin in the urine of the adults. The presence of a number of these organic acids have been indicated in some metabolic disorders and are also known to give rise to toxic effects in the brain, liver, muscle and other tissues. These results emphasize the necessity to conduct further studies on P - glycoprotein expression in children so that its functional role and effect of the mutation of the gene encoding this protein can be established. It is interesting to note that most of the organic acids found to be increased by P - glycoprotein inhibition are phenolic compounds. Since the main purpose of this study was to establish a metabolic profile for people with p - glycoprotein inhibition, it could be suggested that phenolic compounds are substrates of P - glycoprotein and so, if there is a mutation or deficiency of P - glycoprotein, a rise in phenol and phenolic compounds could be expected in the urine of affected persons. A protocol for screening for P - glycoprotein deficiency is also suggested in the study.

P 13

An Approach to the **in vitro** *Evaluation of Potential Inhibition of* **Eurycoma longifolia***, Jack (Tongkat Ali) on*

Cytochromes P450IID6

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Eurycoma longifolia, Jack (Tongkat Ali), a local herb is used frequently in many local folks due to its aphrodisiac effect. Its metabolism pathway and thus possibility of inhibiting CYP2D6 is however not known. As CYP2D6 is involved in metabolism of more than 20 % of pharmaceutical, information on the inhibitory interaction of *Eurycoma longifolia*, Jack and pharmaceutical is thus crucial. We developed an *in vitro* heterologous enzyme system using *E coli* (DH5α) to study the inhibitory effect of Tongkat Ali. The *wild - type* recombinant CYP2D6 and NADPH - cytochrome P450 reductase cDNA were co - expressed from separate compatible plasmid with different antibiotic selection markers. Immunoblotting confirmed the presence of the proteins in bacterial membranes, where they were expressed at a level significantly higher than those found in human liver. Both proteins were able to couple to form an NADPH - dependent monooxygenase which metabolised the CYP2D6 specific substrate, bufuralol (V_{max} 6.85 nmol/min/nmol protein; K_M 9.17 µM) in isolated membrane fractions. Different concentrations of the herbal extract were incubated with recombinant CYP2D6 and bufuralol. The rate of formation of the product, hydroxyl - bufuralol was analysed using HPLC to determine the effect of *Eurycoma longifolia*, Jack. The production of metabolites was found to be reduced in the presence of the herb. The IC_{50} was 1 µg/ml of the extract of *Eurycoma longifolia*, Jack. The results suggested that concurrent use of *Eurycoma longifolia*, Jack and CYP2D6 substrates may cause increased plasma levels of the substrates and thus higher risk for serious drug adverse events in patients. This data contributes to our drug - herb interaction database to profile the metabolism pathways and inhibitory effects of local herbs.

OP 14

Effect of Ile_{264}Met and Ile_{264}Arg Mutations on CYP2C8 Activities

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The work described in the present project aimed to characterise the effect of mutation of amino acid isoleucine at location 264 of the CYP2C8 protein on its enzyme activities. Three mutations, Ile_{264}Met (I_{264}M), Ile_{264}Arg (I_{264}R) and Ile_{264}Asp (I_{264}D) were generated using *in vitro* site - directed mutagenesis technique. The mutant cDNAs, and the wild - type *CYP2C8* cDNA, were expressed separately in *Escheriachia coli* expression system together with the NADPH cytochrome P450 reductase (OxR). All four co - expression preparations gave a similar protein yield with amount of membrane fraction produced ranging from 18.47 to 23.57 mg per litre culture. Cytochrome c - reductase activities did not differ significantly among the four co - expressed protein preparations (means ranged from 1005.3 to 1314.7 nmol/min/mg). This indicated that the levels reductase protein produced were similar when it was co - expressed with different CYP2C8 proteins. The expressed wild - type and mutant CYP2C8s were subsequently examined and compared for their immunoprotein contents and spectral activities. Western blot analyses showed that the mutations caused reduced amount of the full - length CYP2C8 expressed in the bacterial membranes. Subsequent spectral analyses revealed significantly reduced spectral contents in the mutant enzymes when compared to that of the wild - type (i.e. in the range of 1.9 % to 3.5 % of the wild - type level). Substantial amount of expressed mutant proteins were in denatured forms as indicated by the presence of absorption peak at 420 nm. Examination of the catalytic activities of the expressed proteins using amodiaquine and tolbutamide are currently being investigated in our laboratory. This study indicated that mutation of Ile_{264} in CYP2C8 has resulted in production of mutant proteins with substantial reductions in spectral content. Substantial Soret peak at 420 nm seen in the mutants may indicate that Ile_{264} is likely to play a structural role in proper protein folding and haem incorporation in CYP2C8 enzyme.

OP 15

A Preliminary Report on Arg_{16}Gly and Gln_{27}Glu Polymorphisms of β_2 **- adrenergic Receptor** *Gene and Its Correlation with the Pressure Lowering Effect of Topical Timolol*

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We report the genotype frequencies of the two polymorphisms, $Arg_{16}Gly$ and $Gln_{27}Glu$ of the β_2 - *adrenergic receptor* (β_2*AR)* gene among glaucomatous patients and its correlation with pressure lowering effect of topical timolol. A total of 50 newly diagnosed glaucomatous patients with either POAG or OHT were enrolled from Hospital Universiti Sains Malaysia and Hospital Ipoh. All were planned for monotherapy treatment with topical Timolol XE 0.5 %. Baseline intraocular pressure (IOP) was taken. Repeated measurements were obtained at 1, 3, 6 and 12 months. Patients who obtained more than 30 % IOP reduction from baseline were catogerised as good responder and those less than 30 % IOP reduction as poor responder. Genomic DNA was extracted from 4 ml of blood. The $Arg_{16}Gly$ and $Gln_{27}Glu$ polymorphisms of the β_2 *AR* gene was simultaneously amplified from genomic DNA using single tube allele specific multiplex PCR followed by gel electrophoresis. Genotype frequency for glaucoma patients and control for allele 16 (Arg/Arg) was 44 % and 38 % and Arg/Gly was 56 % and 62% (p = 0.372); allele 27 (Gln/Gln) was 88 % and 66 %, Gln/Glu was 10 % and 30 % and Glu/Glu was 2 % and 4 % (p = 0.05) respectively. IOP reduction between good control and poor control for allele 16 (Arg/Arg) was 16 % and 28 % and Arg/Gly was 30 % and 26 % (p = 0.158); allele 27 (Gln/Gln) was 38 % and 50 %, Gln/Glu was 6 % and 4 % and Glu/Glu was 2% and 0 % (p = 0.250) respectively. The changes of the amino acid in this gene are believed to alter the responsiveness of the drug in the patients but larger sample size is needed to confirm this correlation.

OP 16

Ticlopidine and Metoprolol Interactions in Cardiovascular Subjects

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Ticlopidine is used as an anti - platelet in patients with ischaemic heart disease. An *in vitro* study suggested that ticlopidine inhibited CYP2D6 and the widely used antianginal metoprolol is metabolised by this polymorphic enzyme. The objective of this study was therefore to investigate the effect of the addition of ticlopidine to patients maintained on chronic metoprolol therapy. The study was approved by the Research and Ethics Committee of International Islamic University Malaysia (IIUM) and strictly adhered to Malaysian Good Clinical Practice (GCP) guidelines. This was an open labelled case controlled study where all the patients were screened for the inclusion/exclusion criteria. *CYP2D6* genotyping were performed for **3,*4,*5,*6,*9,*10, *14, *17* and duplication. Two weeks after the screening visit, blood samples for metoprolol were taken at timed intervals together with serial measurement on blood pressures and heart rates. Subsequently, the patients were given a standard dose of ticlopidine 250 mg twice daily for a period of one month. At the end of the study period, blood sampling for metoprolol were repeated together with serial measurement of blood pressures and heart rates. After 3 months, 15 patients completed the study. Here we report the interim results. One subject had a genotype that predicted poor metaboliser (PM) status (**4/*4*) and had asymptomatic bradycardia. He had reductions of both systolic and diastolic pressures after ticlopidine. The metoprolol dose was 25 mg twice daily. After ticlopidine, there was a neutrophil count reduction in 3 patients. One patient also had reduced platelet by almost 50 %. Concurrent use of ticlopidine with metoprolol may subject patients who are poor CYP2D6 metabolisers to have exaggerated response to beta blockade and blood dyscrasias may occur frequently.

OP 17

CYP2C9 ***Genotyping: Its Implication in the Management of Patients on Warfarin Therapy***

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Optimization of warfarin therapy has been difficult and pharmacogenomics has potential in offering clinically and economically useful interventions. We thus evaluated the distribution of *CYP2C9* genotypes, which encode enzymes responsible for the metabolism of warfarin among patients prescribed warfarin to bridge the importance of *CYP2C9* genotyping in anticoagulant management. A total of 189 patients on warfarin therapy in a local hospital were recruited after written informed consent. Their medical records were reviewed. Five millilitres of blood was taken from each subject and DNA was isolated and used for identification of *CYP2C9* allele **2, *3* and **4* using nested allele specific multiplex PCR. Half of the patients were Malays and the remaining were Chinese. Two different genotypes were detected among the patients, 93.7% had *CYP2C9*1/*1* and 6.3 % were *CYP2C9*1/*3.* With standard clinical practice, the warfarin doses prescribed ranged from 1 to 8 mg (mean = 3.31 mg) while the mean of international normalised ratio (INR)

achieved was 2.19 (SD 0.86; range 0.86 to 5.69). The mean dose prescribed was higher in patients with genotype of *CYP2C9*1/*1* (3.38 mg: SD 1.35 vs. 2.37 mg: SD 1.05; p = 0.007). Twelve subjects had INR level less than 1 and doses ranged from 1.5 to 6 mg (mean = 3.83). All of them had genotype *CYP2C9*1/*1*. Forty - eight percent of the patients with *wild - type* variant have INR value of 2 to 4 given mean dose of 3.38 (SD 1.34) while 2/3 of the patients with heterozygous **3* achieved desirable INR for a mean dose of 2.37 mg. Seven patients with *wild - type* variant had INR value of more than 4 (mean 4.95; range 4.15 to 5.69) givens dose of 1 to 5 mg of warfarin. The discrepancies observed are due to other factors including patients' compliances, drug interaction or patients having alleles not determined in this study. Even the mean for doses and INR between the 2 genotypes groups were similar, the standard error means were 3 times larger for patients with *CYP2C9*1/*3* compared to wild - type. Current dosing protocol for warfarin lacked efficiency and screening for *CYP2C9* may allow clinicians to develop protocols with increased therapeutic effectiveness.

OP 18

Simultaneous Detection of Tolbutamide and Its Metabolites in Human Plasma Using HPLC

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Tolbutamide is metabolised by CYP2C9 enzyme to 4' - hydroxytolbutamide and carboxytolbutamide by aldehyde dehydrogenase. The objective of this study was to develop an HPLC method to quantify tolbutamide and both its metabolites simultaneously for use in phenotyping. The drugs were extracted from plasma using diethyl ether. They were then injected onto a Gilson HPLC system controlled by Unipoint" software. Separation was achieved using Phenomenex" Luna C8 column with gradient programming. Detection was with UV set at 230 nm. Excellent peak separation was observed. Recoveries averaged from 74 to 108 %. Standard curves for each compound on three consecutive days were linear over the investigated concentration range. Limits of detection were 0.01 µg/ml for carboxytolbutamide, 0.02 µg/ml for hydroxytolbutamide and 0.1 µg/ml for tolbutamide in plasma, respectively. Limits of quantification were 0.03 µg/ml for carboxytolbutamide, 0.06 µg/ml for hydroxytolbutamide and 0.3 µg/ml for tolbutamide respectively. Average coefficients of variation (CV) and accuracy for inter - day determination of carboxytolbutamide, hydroxytolbutamide and tolbutamide were 5.67 ± 1.41, 5.62 ± 0.46, 5.62 ± 0.37 and - 0.07 ± 0.29, - 0.17 ± 0.67, 2.13 ± 5.33 respectively. Our method demonstrated good overall recovery, accuracy and precision and is suitable for CYP2C9 phenotyping study.

OP 19

A Simple High Performance Liquid Chromatography Method for the Determination of Amodiaquine and N - Desethylamodiaquine

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Amodiaquine metabolism to N - desethylamodiaquine is one of the major routes of its disposition. Amodiaquine was used as an *in vitro* probe drug for CYP2C8 activity as CYP2C8 showed selectivity for amodiaquine desethylation with high affinity and turnover number using human liver microsomes. The aim of the study was to develop a simple HPLC method to detect amodiaquine and N - desethylamodiaquine for an *in vitro* system. In this study, *CYP2C8* and *reductase* was cloned and expressed in *Escherichia coli* bacterial system which was then used in a 1 ml incubation medium containing phosphate buffer, NADPH and amodiaquine. Ice cold acetonitrile was used to stop the reaction before subjecting to high speed centrifugation to precipitate the protein. This method required drying of the supernatant under nitrogen gas and the dried salt was reconstituted in 125 ml of mobile phase prior to injecting 100 ml into the HPLC system. We used Gilson HPLC system consisting of 307 pump, 512 UV detector and auto injector with Waters" Sperisorb C8 column (150 x 4.6 mm, i.d. 5 mm). The compounds were eluted in 90 % 0.01 M ammonium acetate and 10 % acetonitrile, pH adjusted to 3.0 with formic acid with flow rate of 1.0 ml/min. Amodiaquine and N - desethylamodiaquine were detected at retention times, 13.8 and 18.8 minutes at 230 nm. Average coefficient of variation (CV) for desethylamodiaquine and amodiaquine were 5.03 % and 6.5 %, respectively. This method is adequately sensitive and specific for the two compounds of interest for *in vitro* studies.

OP 20

Detection of 16 Nucleotide Changes for 15 CYP2C19 *Alleles Using Allele Specific PCR*

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CYP2C19 plays a major role in the metabolism of omeprazole, diazepam, mephenytoin and proguanil. The gene is located on chromosome 10 and has a size of 90 kb with 9 exons. To date, more than 20 alleles have been reported with *CYP2C19**2, *CYP2C19*3* and *CYP2C19*4* associated with loss of enzyme activity. Our objective was to develop an allele specific multiplex PCR method to detect single nucleotide changes at 16 different sites for 15 respective *CYP2C19* alleles. The protocols for this study were approved by the Research and Ethics Committee of Universiti Sains Malaysia. DNA was extracted from blood using lysis method. Primers specific at the 3' end were designed to differentiate variants of each allele. First PCR was conducted in 2 sets for amplification of exons of interest. Set A amplified exon 1, 2, 5 and 9; Set B, exon 4, 7 and 8. The products were subsequently used as templates for second PCR that was divided into 8 sets to detect the mutation sites at 1A>G, 395G>A, 50T>C, 681G>A, 1297C>T, 55A>C, 1228C>T, 636G>A, 991A>G, 99C>T, 680C>T, 276G>C, 449G>A, 1252A>C, 1473A>C and 431G>A. Specific bands corresponding to the amplified products of interest were obtained. The methods were also validated by direct sequencing and tested against 188 DNA samples. Calculated allele frequencies were within expected figures. The methods were found to be specific, sensitive and reproducible and therefore suitable for the simultaneous detection of the *CYP2C19* alleles for used in large population genetic and clinical studies.

OP 21

Lyophilisation of PCR Master Mixes: An Alternative to Avoid Cold Chain for the Study of NAT2 Polymorphism

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N - acetyltransferase 2 (NAT2) is an enzyme that is genetically polymorphic and its variants are associated with differences in drug effectiveness and toxicities as well as susceptibility to certain cancers. Many genotyping methods have been developed to explore the clinical impact of genetic polymorphism of *NAT2* which include PCR - RFLP, SSCP, direct sequencing and allele specific multiplex PCR. Although sensitive and specific, most multiplex PCR methods require cold chain. Our objective was to develop a simple method based on lyophilised master mixes for the detection of 12 SNPs in the *NAT2* alleles. Steps we used for the development of lyophilised mixes included deglycerolisation of *Taq* DNA polymerase, preparation of the multiplex master mix and lyophilisation process. Subsequently, the lyophilised PCR mix was tested using a 2 stage PCR. The first PCR was to amplify the entire *NAT2* gene that contains all the SNPs of interest and the second PCR was to amplify the 12 SNPs. The SNPs detected are at 111T>C, 341T>C, 803A>G in 3 single reactions; 590G>A and 434A>C, 481C>T and 759C>T, 845A>C and 191G>A in 3 duplex reactions; and 190C>T, 499G>A and 857G>A in 1 triplex reaction. The methods were tested against a batch of 30 DNA samples previously genotyped for *NAT2*. For its robustness, the methods were tested against several operators in the laboratory. Our results showed that the method was able to reproduce the results of *NAT2* genotypes using conventional PCR. It was simple to perform as it required only to reconstitute the master mixes and to add DNA sample. The method minimised pipetting steps and reduced carry - over contamination. The lyophilised master mixes could now be transported without cold chain. This would allow the same assays to be carried out at different laboratories minimising assay errors and complication in data analysis.

OP22

A Modified Multiplex PCR Method for the Detection of DRD_2 *Polymorphism*

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Polymorphism of dopamine receptor has been implicated in the pathogenesis and response to drugs in patient with psychosis. It is also implicated in the pathogenesis of addiction. The previous method we developed was cumbersome and not reproducible. Our objective was therefore to modify our previous method to overcome these problems. The first PCR was conducted in 3 sets for amplification of exons of interest. Set A amplified exon 3 and 4; Set B, exon 7 and *TaqI* A RFLP site; Set C, promoter region of the gene. The products were used as templates for 2nd PCR that was divided into five sets to detect the mutation sites at $Ser_{311}Cys$, *TaqIA*, $Pro_{310}Ser$, $Leu_{141}Leu$, $Val_{96}Ala$, $Val_{154}Ile$, $_{-141}C$ Ins/Del and $A_{-214}G$.

Specific bands corresponding to the amplified product of interest were obtained. The methods were validated by direct sequencing and tested against 100 DNA samples from psychiatric patients. Calculated allele frequencies were within expected figures. The methods were found to be specific, sensitive and reproducible and therefore suitable for large population genetic and clinical studies.

OP 23

Elucidating Isoniazid Resistance: From Molecular Modelling Perspective

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The problem of tuberculosis drug resistance and the continuing rise in the disease incidence has generated a renewed interest in research on new drug as well as on increasing the understanding of the mechanism of tuberculosis drug resistance. Molecular docking and molecular dynamic simulations (MD) were performed to study the binding of isoniazid (INH; a first line tuberculosis drug) onto the active site of *Mycobacterium tuberculosis* enoyl - acyl carrier protein reductase (InhA) in an attempt to address the INH resistance of tuberculosis. The results support the theory that the activation of INH to isonicotinic acyl - NADH (INADH) by KatG enzyme for its ultimate activity. It is shown that INADH has tremendously high binding affinity towards InhA by forming more hydrogen bonds, *van der Waals,* electrostatic, hydrophobic interactions and p-p interactions, compared to the parent drug. InhA $S_{94}A$ mutation caused INADH to deviate from its crystal structure probably due to the poor contact between its highly polar NADH region with the hydrophobic Ala_{94}. The analysis of structural fluctuation, distances, hydrogen bonding, radial distribution function and μ_1 dihedral angle from MD trajectories showed that INADH bound more favourably to *wild - type* than to the $S_{94}A$ mutant type InhA. The molecular dynamic of INADH in InhA also revealed that water molecules mediated the formation of hydrogen bond between INADH and InhA. However, due to the mobility of the water molecules, there are no specific water molecules permanently mediated the bonding. The energetic differences from the simulations revealed that $S_{94}A$ mutation is a low level resistance compared to the high level resistance due to mutation or absence of KatG enzyme.

OP 24

The Advantageous Metabolic Effect of Angiotensin II Receptor Blockers

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Hypertensive patients have 2.5 times higher risk of developing type 2 diabetes mellitus. Patients treated with antihypertensive agents are prone to develop diabetes than those who are not treated. *In vivo* and *in vitro* studies were carried out to study the potential of diabetogenic effects of two angiotensin II receptor blockers (ARB), valsartan and irbesartan on insulin levels. Valsartan was studied to determine its effect on glucose - induced insulin secretion in isolated rat pancreas study. The isolated pancreases were perfused with Kreb's solution containing bovine albumin (200 mg/dl) with low glucose (60 mg/dl) followed by high glucose (300 mg/dl) at a rate of 4 ml/min. The dose of valsartan used was based on the peak plasma level achieved in human at standard single oral dose of 80 mg daily, which was 1.64 mg/ L. Five treatment groups were used: Control group, valsartan at 10 %, valsartan at 100 % and valsartan at 10 times of the 1.64 mg/L, and diazoxide 10 mg/ml group. Insulin levels in the perfusate were measured by radioimmunoassay. In a single blind and 3 months clinical study, 40 mild to moderate uncomplicated essential hypertensive patients were included (24 males and 16 females; age 50.9 ± 1.4 years; BMI 27.4 ± 0.7 kgm^{-2}; mean ± SEM). All patients were involved in plasma basal insulin test, and only 13 patients were involved in oral glucose tolerance test (OGTT). Plasma insulin levels were measured by enzyme immunoassay technique. Valsartan at all concentrations significantly increased glucose induced insulin secretion ($P < 0.05$). Valsartan at 10 %, valsartan at 100 % and valsartan at 10 times of the 1.64 mg/L, increased glucose induced insulin secretion by 226.4 %, 161.7 % and 156.3 %, respectively. Diazoxide, significantly inhibited glucose induced insulin secretion ($P < 0.05$). In clinical study, fasting basal plasma insulin was significantly increased after irbesartan therapy (7.53 ± 0.68 _IU/ml vs 11.63 ± 1.16 _IU/ml, $P < 0.001$). However, there was no significant difference in the plasma glucose noted (5.77 ± 0.31 mmol/l vs 6.16 ± 0.30 mmol/l, $P>0.05$). In OGTT, however, no significant differences in glucose and insulin levels were noted (34.50 ± 5.24 _IU/ml vs 31.93 ± 4.97 _IU/ml for mean insulin; 9.43 ± 0.57 mmol/l vs 9.68 ± 0.66 mmol/l for mean glucose, both $P > 0.05$). Both the systolic and diastolic blood pressures were significantly reduced after irbesartan therapy ($P < 0.001$). Valsartan at all concentrations stimulates glucose - induced insulin secretion in isolated rat pancreas technique. Irbesartan increased fasting basal plasma insulin and it had no effect on glucose levels. In conclusion, both studies consistently indicate that the insulin releases were stimulated. Both antihypertensive agents demonstrate no diabetogenic effects. This finding partly suggests that the ARB has advantageous metabolic effects.

OP 25

In Vitro *Selection of* Bacillus subtilis tyr*S T box Antiterminator mRNA: Identification of Novel tRNA - Bulge RNA Interaction*

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The T box transcription antitermination regulatory system, found in Gram - positive bacteria, is dependent on a complex set of interactions between uncharged tRNA and the 5' - untranslated mRNA leader region of the regulated gene. One of these interactions involves the base pairing of the acceptor end of cognate tRNA with four bases in a seven - nucleotide bulge of the antiterminator RNA. This latter tRNA - mRNA base pairing is presumed to stabilize the formation of the antiterminator over a competing, mutually exclusive, terminator stem–loop structure in order to effect antitermination. The specificity of the regulatory response is directed primarily by these two tRNA - mRNA interactions. We employed *in vitro* selection (also known as SELEX) to identify modes of interaction between *B. subtilis* tRNA (A73U) and antiterminator RNA. We randomised bulge of the model antiterminator RNA (AM1A) and used gel shift assay as a selection method. The selected bulge antiterminator sequences were divided into three groups. Groups 1 showed the consensus sequences 5' - UGGG - 3', complementary to four bases (5'UCCA3') of the acceptor end of tRNA (A73U). The consensus sequences for group 2 is 5' - AGGU - 3'. It might be parallel interaction. Whereas the Group 3 had sequences that complementary to T loop and anticodon loop. Both Group 1 and 2 showed high affinity binding to tRNA (A73U). The K_d values are tighter than that of the model antiterminator, AM1A. These studies suggest that the most probable mode of interaction is through complementary and parallel base pairing to the acceptor end of tRNA (A73U). SELEX method offered a way to identify key determinants of novel tRNA - bulge interactions and to generate high affinity antiterminator RNAs.

PP 1

IL - 1β Induces Airway - Remodelling in NHLF and BSMC

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IL-1β is a proinflammatory cytokine responsible for elevating several responsive genes during asthma attack. IL - 1β is known to alter the asthmatic airway and therefore induce an acute phase of protein production, increase in adhesion molecules, vasodilatation as well as release of matrix metalloproteinases. Normal Human Lung Firoblasts (NHLF) and Bronchial Smooth Muscle Cells (BSMC) were grown to confluent in 10 cm^2 culture dishes and then stimulated with 10 ng/ml of IL-1β. Stimulation was done at 2 time points; 1 and 24 hours. Microarray was done on HGU133A GeneChip" arrays (Affymetrix, Santa Clara, CA) that contain 22,000 probes of approximately 14,500 human genes. They were used to analyse genes that were up - and down - regulated in this stimulation process. Using multiple class Anova tools, significance analysis of the data was carried out. Inflammatory pathway was generated by Pathway Studio Central (Ariadne Genomics). Microarray data were then validated by Real - time PCR using IQ supermix SyBrGreen Kit (BioRad®). Overall analysis of stimulated NHLF gene expression data showed 34 and 98 genes were up - regulated by 4 - fold in 1 hour and 24 hours stimulation, respectively. For BSMC, gene expression data showed that 109 and 119 genes were up - regulated by 4 - fold in 1 and 24 hours stimulation, respectively. In 4 - fold down - regulation data, no gene was found in 1 hour stimulation whereas 23 genes were found in 24 hours stimulation. BSMC gene expression data showed that 196 and 262 genes were down - regulated in 1 and 24 hours stimulation, respectively. On the other hand, we also found that there were 28 common genes for both NHLF and BSMC being consistently up - regulated by 4 - fold. This set of genes consists of chemokine genes like *CCL5* and *CXCL6 (GCP - 2)*, inflammatory cytokines like *IL - 8* and *IL - 6*, cell adhesion genes like VCAM - 1, matrix - metalloproteinases like MMP3 and 12, tumour necrosis factor genes like *TNF - _* and many others. Pathway analysis based on the expression profile formed a unique inflammatory pathway consisting of IL - 6, IL - 8, VCAM, TNF, CCL5, SOD2 and others, suggesting similarity of action. Real - time PCR validation showed that a significant 2 to 3 fold amplification of the genes selected from the up - regulation data from both NHLF and BSMC. This study provides a profile of gene - expression for both NHLF and BSMC when challenged with IL - 1β. The induced genes are known to be candidates that may contribute to phenotypic features of asthma. However, this approach must be tested in *in vivo* models for confirmation.

PP 2

The Effects of Herbal Extracts on Human UGT Activity in vitro

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Glucuronidation, catalysed by the enzyme UDP - glucuronosyltransferase (UGT) is an important elimination pathway in humans for structurally diverse xenobiotics and endogenous compounds. Not much is known on the potential interactions of herbal preparations on human UGT isozymes. The aim of this study was to determine the effects of *Andrographis paniculata* (AP) and *Orthosiphon stamineus* (OS) on the *in vitro* glucuronidation of 4 - methyllumbelliferone (4MU) by recombinant human UGTs, UGT 1A1, 1A3, 1A6, 1A7, 1A8, 1A9, 1A10, 2B7 and 2B15. The potential inhibitory effects of AP and OS extracts on the activity of each of the UGT isoforms were investigated using 4MU as the substrate. Incubations contained UDP - glucuronic acid (UDPGA) as the cofactor, $MgCl_2$, cell lysate of respective isoform, and 4MU at the approximate apparent K_m or S_{50} value of each isoform. Final concentrations of AP and OS extracts used were 0, 0.025, 0.25, 2.5, 25 and 50 µg/ml and 0, 0.01, 0.10, 1.0, 10 and 50 µg/ml, respectively. All incubations were performed in duplicate; data points represent the mean (< 10 % variance) of the duplicate measurements. Both extracts variably inhibited the activity of most of the isoforms in a concentration dependent manner. AP inhibited all isoforms studied with strong inhibition on the activities of UGT 1A3 and 2B7 whereas OS inhibited most of the isoforms except UGT 2B7 and 2B15. Inhibition by AP ranged from 2 - 8 % at 0.025 µg/ml to 70 - 95 % at 50 µg/ ml whereas inhibition by OS ranged from 1 - 10 % at 0.01 µg/ml to 70 - 100 % at 50 µg/ml. The studies document the potential of AP and OS in inhibiting human UGT isoforms *in vitro.*

PP 3

Development of the β - agonists ELISA Kit

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The β - agonist group of drugs such as salbutamol and clenbuterol are proven repartitioning agents in meat producing animals, causing a reduction in fat content and improved production of lean meat. Illegal abuse of these drugs has resulted in muscle cramps and tremors, aggression, agitation, increased blood pressure, dizziness, and nausea in human beings. To fulfil the need for a rapid and inexpensive screening method, a β - agonist ELISA kit was developed for the detection and screening of β - agonists in urine and tissue samples. Antibodies were raised against salbutamol - bovine serum albumin (BSA) in New Zealand white rabbits (n = 9), with an immunisation interval of 6 - 8 weeks. The purified antibodies were coated on the 96 - well microtiter plate to emulate a competitive direct immunoassay format. The salbutamol - horseradish peroxidase (HRP) was used as a tracer and detected with colour formation by addition of a chromogen substrate, tetramethylbenzidine (TMB). The optimised kit yielded a detection limit (LOD) between 0.25 - 1 ppb depending on the sample matrix and sample preparation procedure. Studies showed that the cross - reactivity of the antibodies to similar group of drugs was high: salbutamol (100 %), terbutaline (84.59 %), and clenbuterol (20.34 %). Other drugs cross - reacted at low levels between 0.12 % - < 0.01 %. The kit is easy to use, sensitive, and enables quantification of β - agonists in various matrices.

PP 4

Renogenic' Ractopamine ELISA Kit

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Ractopamine is a phenolethanolamine b - agonist used as a growth promoter in meat producing farm animals which results in weight gain and carcass leanness. However misuse of these agents can be toxic and lead to human food poisoning. The development of the Renogenic Ractopamine ELISA kit allowed for the detection of this drug and its metabolites in urine and tissue samples. Both the ractopamine - BSA (bovine serum albumin) antigen and ractopamine - HRP (horseradish peroxidase) were synthesized by the carbodiamide method. Then, antibodies against ractopamine - BSA were raised in New Zealand white rabbits, with immunization every six weeks. Purified ractopamine antibodies from rabbit serum were coated on 96 - well plates. The assay was based on the competitive direct immunoassay format which involved the addition of samples and calibrators competing for the antibody binding sites, followed by a wash step, addition of tetramethylbenzidine and finally stopping the reaction with hydrochloric acid. Studies revealed that structurally similar drugs cross reacted at low levels between 0.08 - < 0.01 %. The limits of detection (LOD) depended on the sample matrix and sample preparation procedure. In general, the limits of detection achieved with this kit were between 0.25 - 1 ppb. Hence, this kit allowed a straightforward, sensitive and reliable assay for the quantification of ractopamine in various matrices.

PP 5

Renogenic' Chloramphenicol ELISA Kit

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Chloramphenicol is a broad - spectrum antibiotic against aerobic and anaerobic Gram - negative and Gram - positive bacteria with toxic effects on humans. Chloramphenicol is commonly associated with the depression of red blood cells in bone marrow resulting in aplastic anaemia that is lethal. There are concerns regarding the potential carcinogenic properties and antimicrobial resistance caused by chloramphenicol. Thus, chloramphenicol is banned for use in animals and livestock meant for food production. Since none of the ELISA kits available in the market were produced locally, therefore, a Malaysian made ELISA kit for chloramphenicol yielding detection limit (LOD) of 0.025 ng/g (n = 20), which is comparable with the existing commercial ELISA kit was developed. The Renogenic' Chloramphenicol ELISA kit is a competitive direct enzyme immunoassay for a quantitative analysis of chloramphenicol in various matrices. Antibodies against chloramphenicol were coated on the plates to compete for the free and enzyme labelled drugs. The assay was found to exhibit little cross - reactivity to other similar group of drugs (< 0.001 %). Due to the sensitivity of the assay, only simple sample extraction method was required for the analysis. Hence, the Renogenic' Chloramphenicol ELISA kit allowed a rapid, sensitive and reliable assay for the quantification of chloramphenicol and its metabolites in various matrices.

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PP 6

Potential Therapeutic Properties of Quercertin

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Flavonoids are polyphenols widely found in colourful pigment of fruits, vegetables and herbs. Recently, flavonoids have been widely investigated for their medical properties such as antioxidant and anticarcinogenic. Quercetin is one of the major components of flavonoids. The experiments carried out aimed to investigate the possible therapeutic properties of this flavonoid. Molecular geometries and stability of the complexes between quercetin and Ca^{2+} were studied *ab initio* using density functional theory (DFT). The results showed that the complexes with the lowest total energy (-1781.2064 hartree) was the one in which quercetin was complexed to Ca^{2+} through 3 - hydroxyl and 4' - oxo groups. Meanwhile, the total energy of calcium oxalate monohydrate (CaOxM) was -1125.4238 hartree. This showed that the formation of complex quercetin - Ca^{2+} was more favourable compared to that of calcium oxalate. It is known that the binding of flavonoids with Ca^{2+} could be a possible mechanism to inhibit the formation of calcium oxalate stones. By forming an energetically more favourable complex with Ca^{2+} compared to that of oxalate, quercetin could be a potential candidate in inhibiting kidney stone formation.

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PP 7

In Silico *Studies on Anticarcinogenic Properties of Quercetin*

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Quercetin is a flavonoid widely found in fruits, vegetables and herbs. It is thought to have antioxidant, anti - kidney stone formation and anticarcinogenic properties. To study the anticarcinogenic property of quercetin, the binding modes of quercetin with 15 different enzymes, including phosphoinositides - 3 - kinase (PI3K) receptor (PDB code = 2HCK), were assessed by using Autodock3.0. Grid parameter files and docking parameter files were employed to run autogrid and autodock. The inhibition constant, K_i value, of cyclooxygenase (1CX2) - quercetin binding was shown to be smaller compared to the one of 2HCK - quercetin binding, and the binding was more favourable compared to other enzyme - quercetin bindings *vis - à - vis* their K_i values. Hence, quercetin might be an irreversible inhibitor of 1CX2. Since cyclooxygenase, especially COX2 is known to be produced by precancerous tissues, its inhibition may help to treat and prevent cancer. In line with other findings, quercetin could therefore be an anticarcinogenic candidate.

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PP 8

Population Pharmacokinetics Modelling of Tramadol with Application of NPEM Algorithms

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Although the kinetic behaviour of tramadol has been described, the present study is the first to our knowledge, to report specifically on the population pharmacokinetic modelling of tramadol hydrochloride using NPEM algorithms. Our objective was to evaluate the influence of *CYP2D6* on pharmacokinetics of tramadol hydrochloride in patients. The parametric Iterative Two - stage Bayesian Population Model (IT2B) programme and the Nonparametric Expectation Maximisation Population Model (NPEM2) programme were used to determine population pharmacokinetic parameter values of tramadol in 138 postoperative orthopaedic patients. All patients received a 100 mg intravenous (IV) dose of tramadol, as their first postoperative analgesic. Blood was sampled at 0 min and subsequently at 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h, 20 h and 24 h for serum tramadol and analysed by HPLC. Patients were genotyped for *CYP2D6*1, *3, *4, *5, *9, *10, *17* and duplication of the gene by means of an allele - specific polymerase chain reaction. Overall, NPEM2 perceived more diversity in the population than did IT2B. The mean total clearance (CLT) was lower (19 L/h) and the half - life longer (5.9 h) than reported for the Western populations 28 L/h and 5.2 h, respectively. These differences could be due to the high frequency of the *CYP2D6*10* variants amongst Malaysian patients (40 %). The inter - individual coefficient of variation of CLT (49 %) was higher than that of Vol (38 %), indicating the presence of other possible influencing factors on tramadol's CLT such as *CYP2D6* polymorphism besides gender and age. The ultrarapid and extensive metabolisers had 2.6 and 1.3 times faster CLT respectively, than did the intermediate metabolisers. Tramadol's pharmacokinetics is influenced by *CYP2D6* polymorphism. Genotyping may be an important tool in determining tramadol's pharmacokinetics and drug dosages in future.

PP 9

Method Development and Validation of HPLC Method for Repaglinide in Human Plasma

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An analytical method using a simple liquid - liquid extraction and HPLC with UV detection has been validated to determine repaglinide concentration in human plasma. Indomethacin was used as internal standard. Chromatographic separations were performed on Purospher"STAR C - 18 analytical column (4.8 mm x 150 mm; 5 mm particle size). The mobile phase consisted of acetonitrile and 0.01 M ammonium formate buffer (60:40; v/v), pH 2.7. Its flow rate was 1 ml/min and the detection wavelength set at 244 nm. Run time for each analysis was 10 min. Calibration curves of repaglinide was linear in the concentration range of 30 - 200 ng/ml in plasma. Limits of detection and quantification in plasma were 10 and 30 ng/ml, respectively. The recovery of repaglinide when extracted by ethyl acetate at pH 7.4 is 92 %.Mean recovery was 99.4 %. Intra - and inter - day assay precision and accuracy fulfilled Food and Drug Administration (FDA) requirement. The precision for inter - day ranged from 5.42 % to 14.15% and for intra - day ranged from 2.04 % to 13.85 %; while inter - day accuracy ranged 93 % to 114 % and intra - day accuracy ranged 92 % to 109 %.This method was applied to determine repaglinide concentration in human plasma samples for a pharmacokinetic study.

PP 10

CYP2D6 *Screening among the Healthy Blood Donors in Kuala Lumpur: Pharmacogenetic Data Bank*

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CYP2D6 gene is highly polymorphic. Currently, more than 70 alleles have been described including the non - functional alleles caused by altered splicing sites *(*4, *11*), frame shift mutations *(*3, *6,*13,*14*) and deletion of the entire gene (**5*). Other alleles like **9, *10* and **17* are associated with a reduction of *in vivo* enzymatic activity. Given the interest in a more systemic study of *CYP* sequence divergence across populations, we therefore aimed to determine common

CYP2D6 alleles in Malaysia. Seven - hundred and sixteen healthy blood donors (276 Malays; 256 Chinese and 184 Indians) were recruited after written informed consents were obtained. Deoxyribonucleic acid (DNA) was extracted using standard lysis methods. Allele specific multiplex PCR was performed for determination of *CYP2D6*1, *3, *4, *5, *6 *9, *10, *14, *17 and* duplication gene. Almost 50 % of the population had genotype *CYP2D6*1/*1;* Indian (79.9 %) however have the highest frequency of this genotype followed by Malays (43.8 %) and Chinese (25.8 %). More than two - third of the Chinese had genotype *CYP2D6*1/*10* and *CYP2D6*10/*10,* confirming the "right shift" phenomena in Chinese. Genotypes that predicted poor metabolisers were detected in all the three races with Indians having the highest frequency. Indians while having the highest frequency of *wild - type* genotype also had about 2 % of the population with non - functional CYP2D6 which predispose them to adverse effects and CYP2D6 - related diseases. The results can also later provide basic information useful for pharmacotherapy with CYP2D6 - substrate drugs especially in clinical practice for personalised medicine.

PP 11

CYP2D6 *Genotypes of Schizophrenic Patients at Hospital Kuala Lumpur*

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Clinical screening for variations in CYP2D6 expression has been suggested as a potential mechanism for improving patient therapy especially in psychiatric patients. We conducted a study in Hospital Kuala Lumpur (HKL) between January to June 2005 to investigate *CYP2D6* genotypes among schizophrenic patients treated with haloperidol. Patients were recruited according to stipulated inclusion and exclusion criteria. Five millilitre of blood was drawn from the patients for DNA isolation. The ethical approval of this study was obtained from local Research and Ethics Committee. Sixty three eligible subjects were recruited and were successfully genotyped using the allele specific multiplex polymerase chain reaction method for *CYP2D6 *3,*4,*6,*9,*10,*14* and **17*. The patients' ages were between 18 to 56 years with mean and median age of 34.5 and 33 years respectively. The race distribution were Malays: 38 (60.3 %); Chinese: 16 (25.4 %); Indian: 8 (12.7 %) and others: 1 (1.6 % Burmese). Twenty seven subjects (41.5 %) were found to have the *wild - type* gene of whom 16 (59.3 %) were Malays, 4 (14.8 %) were Chinese, 7 (25.9 %) were Indians. Twelve subjects (19.0 %) were homozygous**10* of whom 8 (66.7 %) were Malays and 4 (33.3 %) were Chinese. Twenty one subjects (32.3 %) were heterozygous **1/*10* of whom 13 (61.9 %) were Malays, 7 (33.3 %) were Chinese and 1 (4.8 %) was a Burmese. Two (3 %) were heterozygous **1/*14*; a Malay and a Chinese. One subject (1.5 %) who was an Indian was heterozygous **1/*4*. The result showed heterogeneity of genotypes among patients. To further understand the contribution of the *CYP2D6* genotype to the clinical response and the occurrence of adverse effects, we are currently performing a prospective study in patients with an indication for treatment with haloperidol.

PP 12

Rapid Assessment of IVDUs' Status in a Private Clinic in Malaysia Using the WHO Drug Injecting Study Questionnaire: Implications on Pharmacogenetics

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Drug abuse does not only pose health hazards, especially with infections associated with intravenous drug use, but it also hinders the socioeconomic development of a country. Therefore, various rapid assessment methods on drug abuse has been developed to generate important public health information that can be used for the profiling of drug - related problems, mobilising HIV prevention efforts among injecting drug users as well as developing intervention programmes in general. This study used the WHO Drug Injecting Study Questionnaire to assess the IVDU status in a private clinic. Forty - nine male Malay subjects aged 21 to 54 who met the inclusion and exclusion criteria including the DSM - IV criteria for drug addiction were enrolled and were interviewed on a one to one basis. All the subjects admitted to be smokers and 61.2 % admitted to drink alcohol. Half started smoking at the age between 10 to 15 years old. Half started using drugs at the age of 16 to 20 years, the majority of whom started with heroine. Forty percent started injecting at the age between 16 to 20 and 31 % between 21 to 26 years old. Ninety percent injected heroin at that very first time. Reasons for starting injecting varied. Half started injection for a higher euphoric effect compared to 'Chasing the dragon' or sniffing. Half also obtained it as a gift (treat) while 41 % bought it themselves to inject for the first time. Before their first

injection, 84 % thought of trying it once or twice while only 16 % admitted to wanting to become regular injectors. Reasons for starting injecting varied and their proper understanding would be useful in efforts to reduce the behaviour. These factors may include genetic influences.

PP 13

Analytical Method of Metoprolol and Alpha - Hydroxymetoprolol for Pharmacogenetics Study

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Metoprolol is a beta - blocker used for the treatment of heart disease such as hypertension and also as a metabolic probe for CYP2D6 polymorphism. Therefore a high performance liquid chromatographic method using liquid - liquid extraction and measured with a fluorescence detector was developed for the analysis of metoprolol and its metabolite, α - hydroxymetoprolol in plasma samples. The plasma samples were extracted with MTBE and the chromatographic analysis performed using the eclipse XDB - Phenyl column with a particle size of 5 µm. A mixture of 0.1 % TEA in distilled water (pH 3.5) and acentonitrile in a ratio of 89:11 (v/v) was used as the mobile phase. Pindolol was chosen as the internal standard. Different wavelengths were applied for each drug namely metoprolol, α - hydroxymetoprolol and pindolol. This method was validated over a 4 inter - day run and 3 intra - day run within the concentration range of 10 - 200 ng/ml for the analytes. The total analysis time was 20 minutes per sample. Extraction recoveries were between 70 - 120 % for metoprolol, α - hydroxymetoprolol and pindolol. With spiked samples linear standard curves were obtained within the range of 10 - 200 ng/ml with coefficients of determination ≥ 0.995 (r^2 value). The inaccuracies for inter - assay and intra assay were found to be ≤ 14.73 %. This validated method was then applied to determine concentrations of metoprolol and its metabolite (α - hydroxymetoprolol) in human plasma from normal individuals.

PP 14

The Relevance of CYP2D6 Genetic Polymorphism on Chronic Metoprolol Therapy in Cardiovascular Patients

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CYP2D6 polymorphisms are well described in normal populations but data on its clinical significance in cardiovascular patients are lacking. We therefore investigated the influence of *CYP2D6* polymorphisms on steady - state plasma concentrations and apparent oral clearance of metoprolol in patients with cardiovascular diseases. Ninety - one patients on chronic metoprolol therapy were recruited. Plasma concentrations of metoprolol and a - hydroxymetoprolol were measured at 4 - hour post - dose. *CYP2D6* genotyping (**1, *3, *4, *5, *9, *8, *10, *17* and duplication) were performed on the DNA extracted. Ratios of plasma concentrations of metoprolol to a - hydroxymetoprolol and the apparent oral clearances of metoprolol were calculated. Plasma concentrations of metoprolol and a - hydroxymetoprolol were very variable and poorly correlated with metoprolol doses. A hundred - fold variation was noted for both plasma concentrations of metoprolol and a - hydroxymetoprolol. Plasma concentrations of metoprolol were found to be higher in patients with genotypes that predicted lower enzyme activity. One patient homozygous for *CYP2D6*4* had the highest metoprolol concentration per unit dose. With an antimode of 10, 2 patients were identified as PMs (2.1 %; 95 % CI: 2.9). The PMs who had a pMR of 37.8 was homozygous *CYP2D6*4* while the other with pMR 14.5 was genotyped *CYP2D6 *4/*10*. There was a 36 - fold difference between the highest and lowest clearance values. Large overlaps in the clearance values were noted between most of the genotypes. Although our data suggested a better prediction of metoprolol doses with the use of pharmacogenetic, further work is required before it can be used to design a more individualised regimen for metoprolol.

PP 15

Screening Assay for Time - Dependent Inhibition of CYP3A4 towards Predicting Metabolism Based Drug - Drug Interactions

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CYP3A4 is one of the major cytochrome P450s in the liver and is responsible for the metabolism and disposition of over 50 % of drugs on the market. The expression and activity of this enzyme is prone to modulation by environmental agents (inducers and inhibitors). CYP450 inhibition is one of the major causes of metabolism based drug - drug interactions. Inhibition of CYP3A4 can be by reversible, quasi - irreversible and irreversible mechanisms. Reversible mechanisms, which result in immediate effects on enzyme activity, are the most common and well - studied basis of drug - drug interactions. The quasi - irreversible and irreversible types are both time and concentration dependent and are difficult to predict since they can occur several days after drug administration. Several methods for the evaluation of new chemical entities (NCE) for the potential for time - dependent inhibition are being developed. Predicting the *in vivo* effects from the *in vitro* data is also still a challenging area. The effect of ketoconazole, indinavir, verapamil, erythromycin, ethynylestradiol, fluvoxamine, and troleadomycin in inactivating CYP3A4 with time were investigated using 6a - hydroxylation of testosterone as marker reaction. Human liver microsomes (HLM) where used in the studies. Two methods where used in evaluating the time - dependence of CYP3A4 inhibition. In the pre - incubation method, the test compound was incubated with HLM at 2 mg/ml protein concentrations and NADPH for 20 minutes. This incubation was then diluted 10 - fold into a new incubation mix with testosterone and NADPH. The CYP3A4 activity was measured and compared to effects of test compound in a non - pre - incubated mixture. In continuous sampling method, the test compound, testosterone, NADPH and HLM (0.2 mg/ml) where incubated together and 100 µl aliquots were sampled at varying time points. Any change in inhibitory effects with time could then be evaluated. In both methods, metabolite formation, detection and analysis were done by HPLC - UV. Both methods were able to identify known time dependent inhibitors. Fluvoxamine, ethynylestradiol and verapamil confirmed that ketoconazole and indinavir were not time - dependent inhibitors. In contrast to the continuous sampling method, the pre - incubation method was able to identify the low potency time dependent inhibitors, erythromycin and troleandomycin. For the potent inhibitors, the continuous sampling method gave more data, useful for the calculation of K_I and K_{inact} constants. Pre - incubation with high enzyme concentration is a rapid and reliable method to identify time dependent inhibitors especially the slow acting ones compared to the continuously sample method. For identified inhibitors, careful optimisation of the continuous sampling approach will give inhibition constants (K_I and K_{inact}) which are important for making *in vitro - in vivo* predictions of time dependent based drug - drug interactions.

PP 16

DNA Bank and ADMET Pharmacogenetic Database for African Populations

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DNA banks are fast becoming important in clinical research, forensic science as well as drug design and development. Some of the biggest beneficiaries of DNA banks are the pharmaceutical companies. Pharmacogenetics aims at understanding genetic factors describing absorption, distribution, metabolism, excretion and toxicology (ADMET) of drugs. Documentation of pharmacogenetics information for African populations is currently sparse and a few populations have been studied. A comprehensive database will allow easier access to pharmacogenetic information for researchers and pharmaceutical companies. African populations can therefore be better represented in the development of drugs by pharmaceutical companies. The Consortium for the Study of Pharmacogenetics of Drug Metabolism in African Populations currently consists of 6 members (Zimbabwe, South Africa, Nigeria, Uganda, Kenya and Tanzania). Blood samples have been collected from individuals of the major ethnic groups of Kenya, Nigeria, Zimbabwe and Tanzania. The blood and DNA bank has been formed with samples being encoded and entered into a database. Sequencing to identify new allelic variants and genotyping studies using PCR based techniques, were carried out for the major polymorphic Phase I (CYP2D6, CYP2C19, CYP2C9) and Phase II (GSTs, NAT2, TPMT, UGT1A1) drug metabolising enzymes and drug transporters (MDR_1). The blood/DNA bank so far contains 968 samples. New SNPs were found mostly in the *CYP2D6* but few in the *CYP2C19* and *CYP2C9* genes. The allele frequencies of *CYP2C19*2* was 15 - 21 %, *NAT2 *5* (30 %), *NAT2*6* (30 %). The results were entered into the ADMET Pharmacogenetics Database for African Populations which was being set up at AiBST. The fewer SNPs found in *CYP2C19* and *CYP2C9* suggests that there was less heterogeneity for these genes amongst studied Caucasians, Asians and Africans. The allele frequencies of *CYP2C19* and *NAT2* alleles fall within the range of other African populations that have been studied.
