

UNIVERSITY  
*of*  
OTAGO



*Te Whare Wānanga o Otāgo*

# The 4<sup>th</sup> Annual Carney Pharmacogenomics Symposium

*Christchurch  
Friday, 24 October 2008*

## Programme

University of Otago - Christchurch,  
7<sup>th</sup> Floor, Rooms 702 and 703  
Riccarton Road, Christchurch



## Origins of the Centre

The Carney Centre for Pharmacogenomics was established with a generous gift from the Jim and Mary Carney Charitable Trust, which was matched by the Government under the Partnerships for Excellence scheme. The Centre was opened in May 2005, and brings together several groups throughout the University of Otago and elsewhere. Together these groups span a wide range of clinical, pharmacological and generic expertise, and they are applying genetic and genomic techniques to the understanding of drug action and drug responses.

## Objectives of the Centre:

- To carry out excellent research into pharmacogenomics, from molecule to bedside
- To provide high quality postgraduate and medical training in pharmacogenomic areas
- To disseminate pharmacogenomics information in ways that inform and improve clinical practice

Contact for further information: [martin.kennedy@otago.ac.nz](mailto:martin.kennedy@otago.ac.nz)

Visit our website: [www.pgx.org.nz](http://www.pgx.org.nz)

The screenshot shows the homepage of the Carney Centre for Pharmacogenomics. At the top left is the University of Otago logo. The main header features the Carney Centre logo, which includes a stylized DNA helix and the text 'CARNEY CENTRE FOR PHARMACOGENOMICS'. Below the header is a navigation menu with links: HOME, ABOUT, RESEARCH, PUBLICATIONS, PEOPLE, INFORMATION, and CONTACT. The main content area is titled 'Home' and includes a 'CARNEY CENTRE NEWS' section. The primary news item is 'The 4th Annual Carney Pharmacogenomics Symposium' in Christchurch on 24th October, 2008, with a 'REGISTER NOW!' button. Below this is a 'RESEARCH NEWS' section with two items: 'New research from the Centre' and 'World pharmacogenomics news'. The website also features a 'Home' section with a graphic of three line graphs and a 'UOC > Departments > Carney Centre' breadcrumb.



- 8:45am Meet over coffee
- 9:15am Welcome - Martin Kennedy
- 9:20am **Cris Print**, *Auckland*  
"Mixing And Matching Data From Multiple Sources To Understand Cancer Pathology And Drug Response"
- 9:40am **Rod Lea**, *Wellington*  
"The Effects Of Vitamin Supplementation And MTHFR (C677T) Genotype On Migraine Disability"
- 9:55am **Patrick Gladding**, *Auckland*  
"A Simulation of Warfarin Maintenance Dose Requirement Using A Pharmacogenomic Algorithm In An Ethnically Diverse Cohort"
- 10:10am **Barry Palmer**, *Christchurch*  
"Cardiovascular Pharmacogenetics - Taking The Opportunities"
- 10:25am **Annette Gross**, *Sydney*  
"Evaluation Of Sulfasalazine & Nitrofurantoin As In Vivo Probes Of Breast Cancer Resistance Protein (Bcrp) Activity"
- 10:40am Morning Tea
- 11:15am **Dylan Glubb**, *Christchurch*  
"In Vitro Expression Analysis And Association Study Provide Evidence For Adrenomedullin Involvement In Response To The Antidepressant Paroxetine"
- 11:30am **Sarah Deng**, *Christchurch*,  
"Gene Regulation By Antidepressant Drugs"
- 11:45am **Richard Geary**, *Christchurch*,  
"Thiopurine Metabolites, Intermediate Steps On The Way To Complete Thiopurine Pharmacogenetics"
- 12:00pm **Rebecca Roberts**, *Christchurch*  
"Optimisation Of Methotrexate In Inflammatory Bowel Disease Patients"
- 12:15pm **Nuala Helsby**, *Auckland*  
"Cyclophosphamide Bioactivation Pharmacogenetics In Lupus Nephritis Patients"



12:30pm Lunch

1:30pm **Peter George**, *Christchurch*  
"Pharmacogenetics In The Clinical Testing Laboratory"

1:45pm **Scott Mead**, *Christchurch*  
"Genetic Analysis Of Cancer Patients With 5-Flurouricil Toxicity"

2:00pm **Lucy Dunbar**, *Auckland*  
"An Observational Study Of The Effect Of Knowing CYP450 Metaboliser Status On Clinician Prescribing Behaviour When Treating Psychosis With Risperidone"

2:15pm **Jane Vella-Brinact**, *Christchurch*  
"The Metabolism Of Cyclizine - Does It Involve CYP2D6?"

2:30pm **Evan Begg**, *Christchurch*  
"Pharmacogenetics - The Prodrug Hypothesis"

2:45pm **Martin Kennedy**, *Christchurch*  
"Is The CHRNA3-CHRNA5 Genomic Region Really A Susceptibility Locus For Nicotine Addiction?"

3:00pm Closing remarks

3:05pm Afternoon Tea

## **MIXING AND MATCHING DATA FROM MULTIPLE SOURCES TO UNDERSTAND CANCER PATHOLOGY AND DRUG RESPONSE**

**Cristin Print**

Department of Molecular Medicine and Pathology, University of Auckland,  
Private Bag 92019, Auckland, New Zealand

To understand how thousands of individual molecules work together to determine the response of tissues to drug treatment, it may be helpful to combine information from multiple sources. Constructively bringing together different types of clinical and 'omics information is one of the goals of systems biology. This process is likely to play an important role in developing a better understanding of anti-cancer drug action at a molecular level. This talk will describe experiments in our laboratories where we have combined genomic information from in vitro and in vivo microarray experiments, and where we have compared transcript abundance and pathway activation between epithelial cells and stromal cells in the same tumour. Bringing these different data types together has given us exciting insights into tumour biology, however it has also revealed some real challenges.

## **THE EFFECTS OF VITAMIN SUPPLEMENTATION AND MTHFR (C677T) GENOTYPE ON MIGRAINE DISABILITY**

**Rod Lea (PhD)<sup>1,2</sup>, Natalie Colson (PhD)<sup>2</sup>, Sharon Quinlan (MSc)<sup>2</sup>, John Macmillan (FRACP)<sup>3</sup> and Lyn Griffiths (PhD)<sup>2</sup>**

<sup>1</sup>The Institute of Environmental Science and Research Ltd. Wellington, New Zealand, <sup>2</sup>Genomics Research Centre, School of Health Science, Griffith University, Gold Coast, Queensland, Australia, <sup>3</sup>Queensland Clinical Genetics Service, Royal Children's Hospital Health Service District, Brisbane, Queensland, Australia.

### **Abstract**

**Background:** Migraine is a prevalent and debilitating disease that may, in part, arise due to disruption in neurovascular endothelia caused by elevated levels of homocysteine. The homocysteine metabolising gene variant MTHFR-C677T has previously been associated with migraine. This study examined the homocysteine-lowering effects of vitamin supplementation on migraine disability, frequency and severity and whether MTHFR-C677T genotype influenced treatment response.

**Methods:** This was a randomized, double-blind placebo, controlled trial of 6 months of daily vitamin supplementation (ie. 2mg of folic acid, 25mg vitamin B6, and 400µg of Vitamin B12) in 52 patients diagnosed with migraine with aura.

**Findings:** Vitamin supplementation reduced homocysteine by 39% (~4 µmol/L) compared to baseline, a reduction that was greater than placebo (P = 0.001). Vitamin supplementation also reduced the prevalence of migraine disability from 60% at baseline to 30% after 6 months (P=0.01), whereas no reduction was observed for the placebo group (P>0.1). Headache frequency and pain severity were also reduced (P<0.05), whereas there was no reduction in the placebo group (P>0.1). In this patient group the treatment effect on both homocysteine levels and migraine disability was associated with MTHFR-C677T genotype whereby carriers of the C allele experienced a greater response compared to TT genotypes (P<0.05).

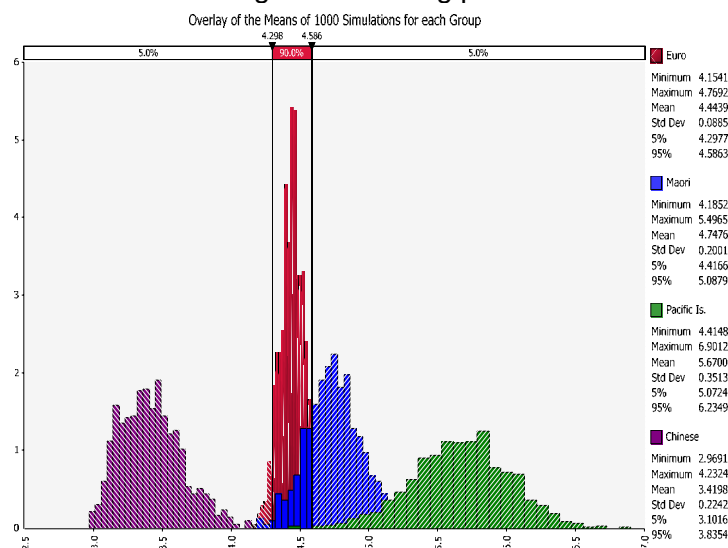
**Interpretation:** This study provides some early evidence that lowering homocysteine via vitamin supplementation reduces migraine disability in a subgroup of patients. Larger trials are now warranted to establish whether vitamin therapy is a safe, inexpensive and effective prophylactic option for treatment of migraine and whether efficacy is dependent on MTHFR-C677T genotype.

# A SIMULATION OF WARFARIN MAINTENANCE DOSE REQUIREMENT USING A PHARMACOGENOMIC ALGORITHM IN AN ETHNICALLY DIVERSE COHORT

**Patrick Gladding, Irene Zeng, Ralph Stewart, Mark Webster, Harvey White**

Green Lane Cardiovascular Service, Auckland City Hospital

Patient demographics and variant alleles in the CYP2C9 and VKORC1 genes account for 50% of the population variability in warfarin maintenance doses. These variant alleles occur in varying frequencies between racial groups and contribute to differences in mean dose requirements between these groups. 429 individuals with coronary disease, with self-reported ethnicity, were genotyped for the CYP2C9\*2 (rs1799853), \*3 (rs1057910) and VKORC1 \*2, -1639 G>A, (rs9923231) SNPs using the Sequenom® spectrometer. Body surface area, age, smoking status and genotype were entered into a modified pharmacogenetic algorithm with a target INR of 2.5. Bootstrap analysis using the @RISK software v5.0, (Palisade Co., NY) was performed to simulate a population of 1,000 for each ethnic group. Simulated warfarin doses were lower in Chinese than NZ Europeans ( 1.39mg, 95% CI 0.4 to 2.4, P = 0.006) due to the high prevalence of the VKORC1 \*2 allele in Chinese. Doses were higher in Pacific Islanders compared to NZ Europeans ( 1.26mg 95% CI = 0.6 to 1.9, P = 0.0002), due to the near absence of the CYP2C9 variant alleles. Simulated bootstrap results shown in Figure 1. Simulated warfarin doses differed between ethnic groups in this New Zealand cohort. This may be clinically significant when administering warfarin but traditional Bayesian methods may be sufficient to overcome this with INR monitoring in the loading period.



## **CARDIOVASCULAR PHARMACOGENETICS - TAKING THE OPPORTUNITIES**

**Barry R. Palmer<sup>1</sup>, Martin D. Jarvis<sup>1</sup>, Anna P. Pilbrow<sup>1</sup>, Chris M. Frampton<sup>1</sup>, Lorraine Skelton<sup>1</sup>, Mathew D. Littlejohn<sup>2</sup>, Martin A. Kennedy<sup>2</sup>, Rob N. Doughty<sup>3</sup>, Tim G. Yandle<sup>1</sup>, A. Mark Richards<sup>1</sup> and Vicky A. Cameron<sup>1</sup>**

<sup>1</sup>Christchurch Cardioendocrine Research Group, Department of Medicine, and <sup>2</sup>Carney Centre for Pharmacogenomics, University of Otago, Christchurch, and <sup>3</sup>Department of Medicine, University of Auckland, Auckland, New Zealand

Genetic association studies in separate cohorts of New Zealand post-Myocardial Infarction (PMI), Heart Failure (HF) and acute coronary syndromes (ACS) patients have led to the observation that genetic factors and pharmacological responses interact frequently in heart disease. Genotyping of 902 PMI patients for the CYP11B2 C-344T polymorphism demonstrated that only patients heterozygous for the C-344T polymorphism showed significantly better survival when treated with  $\beta$ -blockers compared to those without  $\beta$ -blocker treatment ( $p=0.009$ ). HF patients with the rare Ile164 variant of the ADRB2 gene, which renders the  $\beta_2$ -adrenoreceptor dysfunctional, showed a negative response to  $\beta$ -blocker treatment in terms of survival ( $p=0.019$ ). The cytochrome P-450 enzyme CYP1A1 plays a significant part in the detoxification of aromatic amines such as benzo[a]pyrene, one of the key toxic components produced during cigarette smoking. The CYP1A1 T6235C polymorphism (rs4646903) is a variant in the 3'-flanking region of the gene and has been linked to susceptibility to coronary heart disease and cigarette-smoking related lung cancer. In 1251 ACS patients the CYP1A1 6235CC genotype was associated with baseline characteristics of higher type-2 diabetes incidence ( $p=0.017$ ), elevated BMI ( $p=0.001$ ), and younger age ( $p=0.045$ ). The CC patients had significantly worse survival than TT/TC patients ( $p=0.020$ ), independent of established clinical risk factors and ethnicity. These examples suggest much more pharmacogenetic data might be mined from data collected on these large heart disease cohorts with extensive baseline characterisation and long follow-up.

## EVALUATION OF SULFASALAZINE & NITROFURANTOIN AS IN VIVO PROBES OF BREAST CANCER RESISTANCE PROTEIN (BCRP) ACTIVITY

**Annette S. Gross<sup>1</sup>, Kimberly K. Adkison<sup>2</sup>, Soniya S. Vaidya<sup>2</sup>, Daniel Y. Lee<sup>3</sup>, Seok Hwee Koo<sup>5</sup>, Linghui Li<sup>5</sup>, Amar A. Mehta<sup>2</sup>, Joseph W. Polli<sup>3</sup>, Yu Lou<sup>4</sup>, Edmund J.D. Lee<sup>5</sup>**

<sup>1</sup>Clinical Pharmacokinetics Modelling and Simulation (CPMS), GlaxoSmithKline, Sydney, Australia, <sup>2</sup>CPMS, <sup>3</sup>Drug Metabolism and Pharmacokinetics, <sup>4</sup>Discovery Biometrics, GlaxoSmithKline, Research Triangle Park, USA, <sup>5</sup>School of Medicine, National University of Singapore, Singapore.

**Introduction:** In order to define potential Breast Cancer Resistance Protein (BCRP, ABCG2) mediated drug-drug interactions during drug development, a specific, sensitive and safe in vivo BCRP probe is required. The ABCG2 C421A polymorphism is associated with reduced BCRP activity. Animal and in vitro studies indicate that nitrofurantoin and sulfasalazine are BCRP substrates. The utility of nitrofurantoin and sulfasalazine as in vivo probes of BCRP activity has therefore been studied by determining their pharmacokinetics in healthy subjects of known ABCG2 C421A genotype.

**Methods:** The pharmacokinetics of nitrofurantoin (100mg) and sulfasalazine (500mg) were each studied in 36 healthy male Chinese subjects of known ABCG2 421 CC, CA and AA genotypes (n=12 each). Plasma and urine concentrations of nitrofurantoin or sulfasalazine and its metabolite sulfapyridine were determined by LC/MS/MS or LC/UV. Genotype group pharmacokinetic parameters were compared using ANOVA.

**Results:** Nitrofurantoin (NF) pharmacokinetic parameters (AUC, C<sub>max</sub>, T<sub>1/2</sub>, f<sub>e</sub>) were similar among groups of ABCG2 CC, CA and AA subjects. Large interindividual variation in sulfasalazine pharmacokinetic parameters was observed. There were no significant differences in sulfasalazine (SFZ) or sulfapyridine (SP) pharmacokinetic parameters in ABCG2 421 AA or CA vs CC genotype groups.

Parameter Geometric Mean (95% CI)	ABCG2 421 Genotype		
	CC	CA	AA
NF AUC(0-∞); µg.h/mL	2.21 (2.00-2.45)	2.42 (2.11-2.78)	2.32 (1.99-2.70)
NF T <sub>1/2</sub> ; h	0.78 (0.59-1.02)	0.76 (0.64-0.89)	0.72 (0.62-0.84)
SFZ AUC(0-∞); µg.h/mL	32.1 (13.2-78.1)	16.8 (7.15-39.6)	62.7 (33.4-118)
SFZ C <sub>max</sub> ; µg/mL	4.01 (1.62-9.92)	1.70 (0.66-4.40)	6.86 (3.61-13.0)
SFZ T <sub>1/2</sub> ; h	6.80 (6.04-7.66)	7.62 (6.83-8.51)	8.76 (7.85-9.79)
SP AUC(0-t); µg.h/mL	40.1 (29.3-54.8)	34.2 (27.9-41.8)	25.2 (14.7-43.2)

**Conclusions:** The ABCG2 C421A polymorphism did not significantly influence the pharmacokinetics of nitrofurantoin or sulfasalazine. Sulfasalazine and nitrofurantoin are not sufficiently sensitive to BCRP activity in vivo to be useful clinical probes.

## **IN VITRO EXPRESSION ANALYSIS AND ASSOCIATION STUDY PROVIDE EVIDENCE FOR ADRENOMEDULLIN INVOLVEMENT IN RESPONSE TO THE ANTIDEPRESSANT PAROXETINE**

**Dylan Glubb<sup>1,3</sup>, Peter Joyce<sup>2,3</sup>, Patrick McHugh<sup>1,3</sup>, Sarah Deng<sup>1,3</sup> and Kennedy, M.A.<sup>1,3</sup>**

<sup>1</sup>Departments of Pathology and <sup>2</sup>Psychological Medicine, University of Otago, Christchurch, New Zealand; <sup>3</sup>Carney Centre for Pharmacogenomics, University of Otago, Christchurch, New Zealand

Genes which are regulated by antidepressants may be involved in mediating the action of these drugs. Therefore, we have examined acute antidepressant-induced gene expression changes in a serotonergic cell line to find novel candidate genes for association studies. Differentiated RN46A cells were exposed to the selective serotonin reuptake inhibitor paroxetine for 36 hours. RNA was taken for microarray analysis using Affymetrix rat 230 2.0 GeneChips and quantitative PCR (Q-PCR) assays were developed to validate transcriptional changes. The adrenomedullin gene (*Adm*) showed strong upregulation after exposure of the cells to drug, and as adrenomedullin levels have been previously implicated in mood disorders we focused our efforts on this gene. We screened human ADM for genetic variation and found one SNP, -1923 C>A (rs11042725) located in the 5' flanking region of the gene. Luciferase reporter gene assays in RN46A cells indicated the alleles had differential basal transcription rates, with -1923C showing lower expression than -1923A. Association studies were carried out with this SNP in a family study of depression, and the homozygous -1923C genotype was found to be associated with less likelihood of response to paroxetine in depressed individuals. This study needs to be replicated in an independent cohort before the association of ADM -1923 C/C with clinical response to paroxetine can be confirmed. However, this work has shown that a pharmacogenomic approach can identify gene variants which may be relevant to clinical responses to antidepressant drugs.

## **GENE REGULATION BY ANTIDEPRESSANT DRUGS**

**Xiaoyan Deng<sup>1</sup>, Patrick C. McHugh<sup>1</sup>, Kit Doudney<sup>1</sup>, Peter R. Joyce<sup>2,3</sup> & Martin A. Kennedy<sup>1,3</sup>**

<sup>1</sup>Departments of Pathology and <sup>2</sup>Psychological Medicine, University of Otago, Christchurch, New Zealand; <sup>3</sup>Carney Centre for Pharmacogenomics, University of Otago, Christchurch, New Zealand

Antidepressant drugs are used to treat several common mental disorders, but there is much about these drugs that is not well understood. Recent research has shown that antidepressants affect brain function in many ways beyond their known pharmacological effects on neurotransmitter systems, including the stimulation of a variety of cellular and molecular changes and the promotion of neuronal survival and growth. As one approach to better understanding antidepressant action, this study aims to examine chronic effects of antidepressant drugs on selected transcriptional promoter regions using reporter gene assays in transiently transfected neural cell cultures. Upstream regulatory regions for several candidate genes were amplified by PCR and introduced into a luciferase expression plasmid. The cell lines we are using are RN46A, an immortalised rat serotonergic precursor cell, and C6, a rat glioma cell line that shares many characteristics with astrocytes and oligodendrocytes. Each construct was transfected in parallel into cell lines cultured in the presence or absence of antidepressant paroxetine which is a selective serotonin reuptake inhibitor. Cells were processed 48 hours after transfection and the Dual-Luciferase™ Assay (Promega) was used to determine relative levels of promoter activity. Identification of promoters that show altered activity after exposure of transfected cells to antidepressants will provide a model system for exploring the regulatory pathways affected by these drugs.

## **THIOPURINE METABOLITES, INTERMEDIATE STEPS ON THE WAY TO COMPLETE THIOPURINE PHARMACOGENETICS**

**Richard B Gearry<sup>1,3,4</sup>, Rebecca L Roberts<sup>2,3</sup>, Martin A Kennedy<sup>2,3</sup>, Mei Zhang<sup>1,3,5</sup>, Murray L Barclay<sup>1,3,4</sup>**

Departments of Medicine<sup>1</sup> and Pathology<sup>2</sup> and the Carney Centre for Pharmacogenomics, University of Otago, Christchurch<sup>3</sup>, Department of Gastroenterology<sup>4</sup>, Christchurch Hospital, Canterbury Health Laboratories<sup>5</sup>, Christchurch, New Zealand

The thiopurine drugs azathioprine and 6-mercaptopurine remain the mainstay for the treatment of moderate to severe inflammatory bowel disease and are also used for other immune-mediated and inflammatory conditions. While thiopurine methyl transferase (TPMT) predicts many cases of life-threatening myelosuppression, and aids with dose estimation, much of the inter-individual variability in response to these drugs remains unexplained. The 3-6 month delay in the onset of action of thiopurine drugs makes them attractive targets for pharmacogenetic testing. The measurement of thiopurine metabolite concentrations provides clinicians with a surrogate marker of drug efficacy and may lead to therapeutic manoeuvres to maximise thiopurine therapy and minimise adverse effects. A recent study of 71 patients who were non-responsive to thiopurines revealed that 11% were non-compliant, 28% were under-dosed, 10% preferentially produced toxic metabolites and 51% did not respond despite adequate dosing. The metabolite profiles exhibited by these patients were stable after four weeks of thiopurine therapy. Such profiles provide an ideal starting point from which to identify genetic markers of thiopurine drug response. The search for genetic markers is an ongoing research focus of the Carney Centre. Validation of these markers in robustly phenotyped cohorts may lead to the development of pharmacogenetic tests which could in turn aid in the 'tailoring' of standard thiopurine therapy to the individual patient.

## **OPTIMISATION OF METHOTREXATE IN INFLAMMATORY BOWEL DISEASE PATIENTS**

**Rebecca L. Roberts**<sup>1,4</sup>, **Richard B. Gearry**<sup>2,3,4</sup>, **Mei Zhang**<sup>2,4</sup>, **Evan J. Begg**<sup>2,4</sup>, **Murray L. Barclay**<sup>2,3,4</sup>

Departments of Pathology<sup>1</sup> & Medicine<sup>2</sup>, University of Otago; Department of Gastroenterology<sup>3</sup>, Christchurch Hospital, Carney Centre for Pharmacogenomics<sup>4</sup>, University of Otago, NEW ZEALAND

Azathioprine and 6-mercaptopurine are the first-line immunosuppressants for inflammatory bowel disease (IBD). However, >40% of patients are intolerant or resistant to these thiopurine drugs and thus effective alternatives are essential. In the management of rheumatoid arthritis (RA), methotrexate (MTX-Glu1) is more commonly used than the thiopurines and gastroenterologists are now also increasingly using methotrexate in IBD. Methotrexate is rapidly transported into cells where glutamate groups are added to form polyglutamates (MTXGlu2-5) that inhibit enzymes in the folate pathway to produce an anti-inflammatory effect at low dose. MTX polyglutamate concentrations in the red blood cells (RBCs), as well as genetic variability in the folate pathway, have been found to be predictors of methotrexate efficacy in RA patients. However, data regarding methotrexate use in IBD is very limited, and current dose recommendations are based on a single randomised trial. To gain a greater understanding of the predictors of methotrexate response in IBD, we have performed a pilot study on 18 patients receiving methotrexate 15-25mg/week[1]. We detected significant correlations between RBC MTXGlu4&5 concentrations and toxicity, and also poor disease control. These findings contradict much of the rheumatology and oncology literature. To exclude a type I error due to small sample size, we have now commenced recruitment of 250-350 IBD patients on steady state methotrexate. The primary aims of this larger study are to a) test the correlations observed in our pilot study, and to b) establish both a therapeutic range of RBC MTX polyglutamate concentrations and a pharmacogenetic index to optimise methotrexate treatment.

Reference: Brooks AJ et al (2007). Red blood cell methotrexate polyglutamate concentrations in inflammatory bowel disease. *Therapeutic Drug Monitoring* 29(5):619-25.

## **CYCLOPHOSPHAMIDE BIOACTIVATION PHARMACOGENETICS IN LUPUS NEPHRITIS PATIENTS**

**Nuala Helsby<sup>1</sup>, Peter Gow<sup>2</sup>, Janak de Zoysa<sup>3</sup>, Malcolm Tingle<sup>4</sup>**

<sup>1</sup>Molecular Medicine and Pathology, University of Auckland; <sup>2</sup>Rheumatology, Middlemore Hospital; <sup>3</sup>Renal Medicine, Auckland City Hospital;

<sup>4</sup>Pharmacology and Clinical Pharmacology, University of Auckland

An association between CYP2C19 and CYP2B6 genotype and therapeutic failure in lupus nephritis patients treated with cyclophosphamide (CP) has been reported [1]. A clinical review of lupus nephritis patients at Middlemore hospital, indicated that treatment failure was higher in Polynesian patients compared with NZ European patients. This provided the impetus for a clinical study to determine the pharmacogenetics of cyclophosphamide bioactivation in NZ cohort of lupus nephritis patients.

Firstly, we have determined the relative importance of CYP2C19 and CYP2B6 variants in the bioactivation of cyclophosphamide in vitro. The results indicate that the presence of one variant allele (at either loci) results in significantly lower bioactivation of CP ( $P < 0.005$ ). We then determined the bioactivation ratio (4OH CP/CP) in lupus nephritis patients ( $n=13$ ) receiving iv CP ( $<1\text{g/m}^2$ ). The preliminary data indicate that there was  $>10$  fold variability in the bioactivation of CP in the patients. Moreover, one subject was a null bioactivator, with no detectable levels of the 4-OH CP metabolite. 60% of patients had at least one variant allele (CYP2C19 and/or CYP2B6) and bioactivation appeared to be lower in these patients. Further genotypic analysis is ongoing to determine the effect of the increased activity variants, CYP2C19\*17 and CYP2B6\*6, on the bioactivation ratio in these patients. However, preliminary data also indicate that drug-drug interactions may also play a part in variable bioactivation of this drug. These results may enhance the understanding of the complex factors which influence the variable bioactivation of cyclophosphamide in lupus nephritis patients.

Reference: [1] Takada et al 2004. Cytochrome P450 Pharmacogenetics as a Predictor of Toxicity and Clinical Response to Pulse Cyclophosphamide in Lupus Nephritis Arthritis and Rheumatism 50: 2202-2210.

## **PHARMACOGENETICS IN THE CLINICAL TESTING LABORATORY**

### **Peter George**

Biochemistry/Clinical Director, Biochemistry Department, Canterbury Health Laboratories

A large number of pharmacogenetic tests are now being performed in routine clinical laboratories. However, the clinical utility of these remains to be confirmed in routine clinical use. Current requests are received from Infectious Disease, Oncology, Rheumatology and Anaesthetics Specialists and samples are analysed using a variety of genotypic and phenotypic approaches. Current activities will be reviewed and illustrated.

## **GENETIC ANALYSIS OF CANCER PATIENTS WITH 5-FLUOROURICIL TOXICITY**

**Scott Mead<sup>1</sup>, Antony Chong<sup>1</sup>, Bridget Robinson<sup>2</sup>, Peter George<sup>1</sup>**

<sup>1</sup>Molecular Pathology, Canterbury Health Laboratories, Christchurch

<sup>2</sup>Oncology Service, Christchurch Hospital, Christchurch

Fluoropyrimidines, such as 5-Fluorouracil (5-FU) are a widely used chemotherapeutic agent for solid tumors. However approximately a third of patient administered 5-FU will experience dose-limiting toxicity, a major cause of mortality and morbidity in this group. Pharmacokinetic studies show there is a delicate balance between the anabolism and catabolism of 5-FU. Incorporation of 5-FU into anabolic pathways results in cytotoxicity, primarily by inhibition of thymidylate synthase (TS) - an essential enzyme in the de novo synthesis of dTMP. Several polymorphisms in the TS gene (TYMS) result in lower levels of TS expression, which has an increased risk of 5-FU toxicity. The initial rate-limiting step in the catabolism of 5-FU is catalysed by dihydropyrimidine dehydrogenase (DPD). Approximately 50% of patients who experience 5-FU toxicity have DPD deficiency. More than 30 mutations have been identified in the DPD gene (DPYD), some of which result in DPD activity levels associated with increased risk of 5-FU toxicity. Most people with altered TS expression or DPD activities are asymptomatic, raising the possibility of pre-dose testing for predisposition to 5-FU toxicity.

To determine if pre-dose testing would have predicted adverse reactions in cancer patients who had experienced severe 5-FU toxicity, we determined the genotype of putative 5-FU toxicity susceptibility genes in 12 patients requiring hospitalisation during 5-FU chemotherapy. DNA sequencing of the DPYD gene detected 9 sequence variations, including one patient who was homozygous for a known pathogenic DPYD mutation (IVS14+1G>A). The DPD enzyme activity was determined in two patients who had DPYD sequence variations of unknown pathogenicity. A combination of TYMS gene sequence variations associated with low TS expression were detected in 9 patients, an incidence higher than expected in the general population. The genotype for the dihydropyrimidase (DHP) and methylenetetrahydrofolate reductase (MTHFR) genes was also determined. Taken together, the results suggest that a targeted genetic analysis of the DPYD and TYMS genes, when combined with metabolite analysis, would provide a clinically useful pre-dose test for 5-FU toxicity.

## **AN OBSERVATIONAL STUDY OF THE EFFECT OF KNOWING CYP450 METABOLISER STATUS ON CLINICIAN PRESCRIBING BEHAVIOUR WHEN TREATING PSYCHOSIS WITH RISPERIDONE**

**Lucy Dunbar<sup>1</sup>, Rachael Butler<sup>1</sup>, Justin Pulford<sup>1</sup>, Amanda Wheeler<sup>1</sup>, Janie Sheridan<sup>2</sup>, Wayne Miles<sup>3</sup>**

<sup>1</sup>Clinical Research & Resource Centre (CRRC), Waitemata District Health Board, <sup>2</sup>Faculty of Medical & Health Sciences, University of Auckland,

<sup>3</sup>Knowledge Centre, Waitemata District Health Board

**Aims:** To identify the CYP450 metaboliser status of patients being treated with an antipsychotic medication as revealed by the AmpliChip CYP450 Test®; and to examine the impact of awareness of the metaboliser status on the prescribing behaviour of clinicians when prescribing antipsychotic medication.

**Method:** Test results and user experience were assessed via a retrospective review of clinical documentation of patients whose CYP450 status had been assessed using the AmpliChip test. Semi-structured interviews with doctors who ordered the tests were conducted to determine prescribers' perceptions of the test's utility.

**Results:** 42 doctors ordered tests for 93 patients. Ten patients were found to be 'poor' metabolisers, ten were 'intermediate', 68 were 'extensive', none were 'ultra-rapid', and results were inconclusive in five cases. In terms of the test's influence on prescribing behaviour, the study results were mixed. Statistical analysis of the clinical review data indicated that knowledge of a patient's metabolic status had minimal, if any, influence on prescription behaviour. Interview data, on the other hand, indicated that the test results may play a supporting role with regard to some dosing decisions, although there was little evidence that the test results were ever a primary influence on clinician behaviour.

**Conclusion:** The study presents initial data on the CYP450 metaboliser status of a New Zealand-based clinical population and highlights a gap between the perceived usefulness of the AmpliChip CYP450 test as a clinical decision support tool and the actual effect on the target clinical behaviour, namely the choice of dose of the drug.

## THE METABOLISM OF CYCLIZINE - DOES IT INVOLVE CYP2D6?

**Jane Vella-Brincat<sup>1,2</sup>, Evan Begg<sup>1</sup>, Berit Jensen<sup>1</sup>, Fiona Findlay<sup>1</sup>,  
Rebecca Roberts<sup>3</sup>, Mary Fairhall<sup>2</sup> and Sandy Macleod<sup>2</sup>**

<sup>1</sup>Department of Clinical Pharmacology, University of Otago/Christchurch Hospital, Christchurch, New Zealand and <sup>2</sup>Nurse Maude Hospice, Nurse Maude Association, Christchurch, New Zealand, <sup>3</sup>Carney Centre for Pharmacogenomics, University of Otago, Christchurch, New Zealand

Cyclizine is often used as an antiemetic in terminally ill patients. It is considered to be a long acting antihistamine (histamine-1 receptor antagonist), similar to hydroxyzine. Cyclizine was developed as an antiemetic (in 1952) and was used for this purpose during the first manned flight to the moon. It has a half life of 18 to 24 hours. Low concentrations are 30mcg/L, high 300mcg/L, toxic 1,500mcg/L and lethal 15,000mcg/L. Cyclizine is metabolised mainly to the inactive norcyclizine (N-demethylation). Although it is unclear whether the metabolism of cyclizine is primarily mediated by CYP2D6 this drug inhibits CYP2D6 and its close relative, hydroxyzine is metabolised by CYP2D6. Around 8% of Caucasians, 5% of Maoris and up to 3% of the Asians/ Pacific Islanders<sup>1</sup> do not produce functional CYP2D6 and these are known as Poor Metabolisers (PMs). Non-PMs are known as Extensive Metabolisers (EMs). Aims: To determine whether CYP2D6 status influences cyclizine:norcyclizine ratios, response and adverse effects in patients receiving continuous subcutaneous infusions of cyclizine. Method: 10 consecutive palliative care patients receiving continuous infusions of cyclizine had serial blood samples taken over 5 days for determination of CYP2D6 genotype and cyclizine/norcyclizine concentrations. Results: 5 patients have been recruited. One patient is inferred to be a PM based on genotype (CYP2D6\*4/\*4) while the remaining four are EMs (CYP2D6\*1/\*4, \*1/\*1). The PM has a cyclizine:norcyclizine ratio of around 10 while the EMs' ratios range from 2 to 5. Conclusion: Our preliminary results suggest that CYP2D6 may be involved in the metabolism of cyclizine.

1.Roberts RL, Begg EJ, Joyce PR, Kennedy MA. How the pharmacogenetics of cytochrome P450 enzymes may affect prescribing. New Zealand Medical Journal 2002; 115(1150): 137-40.

2.Benny K.A., Adithan C Genetic polymorphism of CYP2D6. Indian Journal of Pharmacology 2001; 33:147-169

## **PHARMACOGENETICS - THE PRODRUG HYPOTHESIS**

**Evan Begg**

University of Otago, Christchurch & Christchurch Hospital, Christchurch, New Zealand

In 2006 we proposed an algorithm that assessed the likelihood of genetics affecting drug/metabolising enzyme pairings sufficiently to have clinical utility (Gardiner & Begg, 2006). Very few pairings had 'definite' clinical relevance, and the list was short even for those with 'probable' and 'possible' relevance. A re-look at this total list and a few new contenders revealed that prodrugs featured disproportionately. This has some logic in relation to likely variance explained by genetically mediated 'production' versus 'elimination'. The 'prodrug hypothesis' will be illustrated with respect to established drug/enzyme pairings with genetic significance (azathioprine/TPMT, codeine/2D6) and potential newcomers (clopidogrel/2C19, tamoxifen/2D6, losartan/2C9). Perhaps, in the search for clinical relevance of pharmacogenetics, research efforts can be focussed more towards prodrugs.

Reference: Gardiner et al. (2006) Pharmacogenetics, Drug Metabolising Enzymes, and Clinical Practice. *Pharmac. Reviews* 58, 521-590

## **IS THE CHRNA3-CHRNA5 GENOMIC REGION REALLY A SUSCEPTIBILITY LOCUS FOR NICOTINE ADDICTION?**

**Martin A. Kennedy<sup>1,3</sup>, Joseph M. Boden<sup>2</sup>, Allison Miller<sup>1,3</sup>, L. John Horwood<sup>2</sup>, David M. Fergusson<sup>2</sup>**

<sup>1</sup>Departments of Pathology and <sup>2</sup>Psychological Medicine, University of Otago, Christchurch, New Zealand; <sup>3</sup>Carney Centre for Pharmacogenomics, University of Otago, Christchurch, New Zealand

**Objective:** To examine the relationships between the nicotinic acetylcholine receptor gene cluster polymorphism rs16969968, identified in several recent genome-wide association studies of smoking and lung cancer, and the development of smoking and nicotine dependence in a representative population sample.

**Method:** Data were obtained from the Christchurch Health and Development Study, a 30-year study of a longitudinal birth cohort. Outcome measures included data on cigarette smoking frequency and nicotine dependence obtained at assessments from ages 15 to 30 years. The associations between genotype and smoking outcomes were modelled using generalized estimating equation methods.

**Results:** Cohort members with the AA genotype had consistently lower levels of cigarette smoking outcomes than cohort members with either the GG or GA genotype. In particular, bivariate analyses revealed that those with the AA genotype smoked significantly ( $p < .05$ ) fewer cigarettes than those with either the GG or GA genotype. Adjustment for a range of potentially confounding factors related to family socio-economic background, individual factors, and peer and family smoking did not substantially alter the associations between CHRNA5 genotype and smoking outcomes.

**Conclusions:** The findings of the present study suggest that the CHRNA5 polymorphism did not influence cigarette smoking behaviours in the present cohort, and that the AA variant was associated with reduced risks of smoking related outcomes rather than increased risks.