CARNEY CENTRE FOR PHARMACOGENOMICS

UNIVERSITY of OTAGO

SAPERE – AUDE

Te Whare Wānanga o Otāgo

The 3rd Annual Carney Pharmacogenomics Symposium

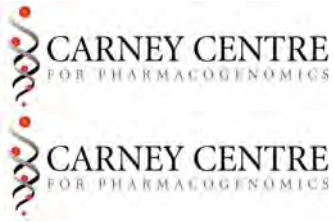
Christchurch
Tuesday, 11 September 2007

Programme

The Arts Centre of Christchurch,
Corner of Hereford Street & Rolleston Avenue, Christchurch

CARNEY CENTRE FOR PHARMACOGENOMICS

Symposium Notes:



	Meet over coffee
9:25am	Welcome - Martin Kennedy
9:30-11:45am	**Session 1 - 'IBD genetics and pharmacogenetics'**
9:30am	**Richard Gearry** 'IBD overview'
9:40am	**Graham Radford-Smith** **'**Genetics of IBD: a view from Brisbane'
10:20am	**Murray Barclay** 'IBD pharmacogenetics'
10:30am	**Rebecca Roberts** 'A molecular explanation for ultra-high TPMT activity'
10:45am	**Sharon Gardiner** 'Thiopurine methyltransferase (TPMT) and thiopurine dose in inflammatory bowel disease (IBD)'
11:00am	**Morning Tea**
11.30am	**Andrew Shelling** **'**Nutrigenomics and Crohn's disease'
11:45-12:35pm	**Session 2 - From the Laboratory to the Clinic**
11:45am	**Leslie Sheffield** 'Promoting use of pharmacogenomic testing by multicentre demonstration projects in teaching hospitals'
12:00 am	**Caroline Withington** 'CYP2D6 gene in metoprolol treatment of heart failure'
12:15pm	**Peter George** 'Putting PGx into practice: experience in a diagnostic laboratory'
12:35pm	**Lunch**
1:30-2:05pm	**Session 3 - Genomic technologies**
1:30pm	**Grant Montgomery** 'Genome wide association studies using DNA pools and dense SNP arrays'
1:50pm	**Rod Lea** 'Identifying genomic signatures for prediction of drug response'
2:05-3:15pm	**Session 4 - Pharmacogenomics of Mood disorders**
2:05pm	**Peter Joyce** 'Pharmacogenomics of antidepressants - overview'
2:20pm	**Patrick McHugh** 'Sepiapterin reductase: a candidate gene for antidepressant response and affective disorders?'
2:35pm	**Dylan Glubb** 'Antidepressant Pharmacogenomics of a Serotonergic Cell Line'
2:50pm	**Tony Harley** 'The Search for the Black Bile: Peripheral Biomarkers in Depression'
3:05pm	Overall closing comments - Evan Begg

3:15pm	Afternoon Tea

IBD OVERVIEW

Richard B Gearry[1,2]

[1]Department of Medicine, University of Otago, Christchurch, New Zealand; [2]Carney Centre for Pharmacogenomics, University of Otago, Christchurch, New Zealand

Inflammatory bowel disease (IBD) comprises Crohn's disease (CD) and ulcerative colitis (UC) and is characterised by inflammation of the intestine leading to symptoms such as abdominal pain, diarrhoea, rectal bleeding and weight loss. IBD are a group of complex diseases with both genetic and environmental factors playing a role in disease aetiology.

Polymorphisms in a wide range of genes encoding for proteins involved in the immune response and bacterial sensing have been shown to increase susceptibility to IBD although many genes remain to be identified. The major advances in IBD genetics have led to an improved molecular understanding of disease pathogenesis and while these genetic tests are not performed as part of routine clinical care, some have led to the identification of drug targets. Pharmacogenetics and genomics plays a vital role in the management of IBD patients particularly relating to the use of TPMT testing prior to the initiation of thiopurine drugs.

Members of the Carney Centre for Pharmacogenomics and their collaborators have played an important role in the corroboration of many genetic associations (including *NOD2, DLG5, TLR4, ATG16L, IL23R, Gli1* and others) with IBD in a unique population-based cohort and are well placed to develop this work further in the future.

GENETICS OF IBD: A VIEW FROM BRISBANE

Graham Radford-Smith

Royal Brisbane & Women' s Hospital, Brisbane, Australia

INFLAMMATORY BOWEL DISEASE PHARMACOGENETICS

Murray Barclay,[1,2] Rebecca Roberts,[3] Richard Gearry,[1,4] Martin Kennedy[3]

Departments of Gastroenterology and Clinical Pharmacology, Christchurch Hospital, and Departments of Pathology[3] and Medicine,[4] University of Otago - Christchurch, New Zealand

The incidence of inflammatory bowel disease is increasing sharply worldwide, especially Crohn's disease. Immunosuppressant medications are frequently required, including the thiopurines azathioprine and 6-mercaptopurine, and increasingly also methotrexate. Sixty-five percent of Crohn's disease patients and 25% of patients with ulcerative colitis in Canterbury have current or past use of one or more of these immunosuppressants.

However, response to these drugs is highly variable, so that approximately half of patients have either unacceptable adverse effects or lack of efficacy. Metabolism to the active and toxic metabolites of the thiopurines is complex and several enzymes in this pathway (including *TPMT*, *IMPDH1*, and *GMPS*) are subject to genetic polymorphisms that alter the ratios of these metabolites markedly. Risk of some thiopurine adverse effects is likely genetically determined. This includes an association of the *HLA-B* genotype with the flu-like hypersensitivity reaction.

In the case of methotrexate, it is likely that there is a therapeutic range for the active methotrexate polyglutamates (MTXPG), and that this range might be utilised clinically. It is also probable that MTXPG concentrations are subject to genetic polymorphisms and this is to be explored further.

TRINUCLEOTIDE REPEAT VARIANTS IN INFLAMMATORY BOWEL DISEASE PATIENTS EXHIBITING ULTRA-HIGH THIOPURINE S-METHYLTRANSFERASE ACTIVITY

Rebecca L. Roberts[1], Richard B. Gearry[2 & 3], Michael V. Bland[1], Christiaan W. Sies[4], Peter M. George[4], Michael Burt[2], Martin A. Kennedy[1], Murray L. Barclay[2 & 3]

Departments of Pathology[1] & Medicine[2], University of Otago, Christchurch; [3]Department of Gastroenterology, Christchurch Hospital, Christchurch; [4]Clinical Biochemistry, Canterbury Health Laboratories, Christchurch, NEW ZEALAND

Thiopurine S-methyl transferase (TPMT) is a cytosolic enzyme that catalyses the S-methylation of the thiopurine immunosuppressants. TPMT deficiency has been studied for over 25 years and is one of the few examples of pharmacogenetics making the transition from research to diagnostics. To date, 21 mutations have been identified that are predictive of decreased TPMT activity. In contrast, no molecular explanation has been found for the 1-2% of Caucasians that exhibit ultra-high TPMT activity. Here we report the identification and functional characterization of polymorphisms within a trinucleotide (GCC) repeat element of the *TPMT* promoter in two patients with inflammatory bowel disease (IBD). The first patient had a TPMT enzyme activity reading of 25.6units/ml red blood cells (RBC) (normal range of activity: 9.3 to 17.6 units/ml RBC) and was heterozygous for a variant allele carrying seven GCC repeats (GCC_7). The second patient had a TPMT enzyme activity reading of 65 nmol 6-MTG x g^{-1} Hb x h^{-1}(normal range of activity: 20-50 nmol 6-MTG x g^{-1} Hb x h^{-1}) and was heterozygous for a variant allele containing five GCC repeats (GCC_5). Fifty IBD patients with normal TPMT activity were all homozygous for six GCC repeats. *In vitro* reporter gene assays demonstrated that the GCC_5 and GCC_7 alleles increased mean basal transcription from 7.90 to 12.30 and 13.40 (p-value = <0.001) respectively, strongly suggesting that these variants are responsible for the ultra-high TPMT activity observed in these patients. To our knowledge, this is the first time that sequence variants has been associated with a significant increase in *TPMT* gene expression.

THIOPURINE METHYLTRANSFERASE (TPMT) AND THIOPURINE DOSE IN INFLAMMATORY BOWEL DISEASE (IBD)

Sharon J Gardiner[1], Richard B Gearry[1,2], Evan J Begg[1], Murray L Barclay[1,2].

[1]Departments of Medicine and [2]Gastroenterology, University of Otago, Christchurch and Christchurch Hospital, Christchurch, New Zealand

TPMT deficiency (*TPMT*3/*3*) occurs in ~0.6% of Caucasians and necessitates a decrease in thiopurine dose (~10% of normal) to avoid severe myelosuppression. Intermediate (*TPMT*1/*3*) activity occurs in 10% of individuals and may lead to altered thiopurine dose requirements. **Aim**: To correlate TPMT genotype and phenotype with thiopurine dose requirements in patients with IBD who have intermediate or normal TPMT status. **Methods**: Seventy-seven (77) patients initiated on a thiopurine (mainly azathioprine) were followed for nine months. Dose was adjusted according to usual practice and through measurement of active 6-thioguanine nucleotide (6-TGN) concentrations. **Results**: One, 7 (9.5%) and 67 (90.5%) of 75 subjects who were both genotyped and phenotyped for TPMT had the *TPMT*3/*3, *1/*3* and **1/*1* genotypes, respectively. The mean initial thiopurine dose (as azathioprine equivalents) was similar (~1mg/kg/day) in the *TPMT*1/*1* and **1/*3* genotypes, but after nine months the dose was ~50% lower in the *TPMT*1/*3* group (0.9 versus 1.8 mg/kg/day, p<0.0001). Despite dose adjustment, median 6-TGN concentrations remained higher in the *TPMT*1/*3* group (424 versus 270 pmol/8x10^8 RBC per mg/kg/day, p=0.02). Similar findings were seen for TPMT phenotype. **Discussion**: These results suggest that individuals with the intermediate TPMT metaboliser status require half the dose of 'normal' metabolisers.

NUTRIGENOMICS AND CROHN'S DISEASE

Ivonne Petermann, Claudia Hübner, Martin Philpott, Brian Browning, Lynn Ferguson and Andrew Shelling

Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

Inflammatory bowel disease (IBD) is a complex disorder characterised by chronic inflammation of the gastrointestinal tract. There are two main clinical subtypes, Crohn's disease and ulcerative colitis. Crohn's disease can affect any part of the intestine, and is associated with discontinuous, transmural lesions of the gut wall. Ulcerative colitis inflammation is confined to the colon and rectum, and lesions are continuous and superficial. Most studies support a polygenic model of inheritance, and it is clear that environmental factors contribute significantly to the development of the disease. Single nucleotide polymorphisms (SNPs) within the CARD15/NOD2, DLG5, SLC22A4 and SLC22A5 genes have been associated with the development of Crohn's disease. However, other candidate susceptibility genes must exist and be associated with predisposition to Crohn's disease. Recent genome-wide association studies have confirmed some of these genes as strong candidates, but have also identified a number of new associations, including SNPS within the IL23R and ATG16L1 genes. Genes that are most strongly associated with Crohn's disease appear to fall into two overlapping pathways, defects in autophagy and the processing of phagocytosed bacteria. Identifying SNPs involved in the development of Crohn's desease is an important first step in the Nutrigenomics research programme, and are currently being used to design innovative functional assays to characterise foods and food components.

This research was funded through Nutrigenomics New Zealand, a collaboration between AgResearch Limited, Crop & Food Research, HortResearch and The University of Auckland

PROMOTING USE OF PHARMACOGENOMIC TESTING BY MULTICENTRE DEMONSTRATION PROJECTS IN TEACHING HOSPITALS

Leslie Sheffield[1], Miles Sparrow[2], Peter Irving[3], Peter Gibson[3], Tony Catto-Smith[4], Finlay Macrae[5], Arun Gupta[5], Keith Byron[6], Richard Gearry[7].

[1]Murdoch Childrens Research Institute, [2]Department of Gastroenterology, Box Hill Hospital Royal Children's Hospital, [3]Department of Gastroenterology, [4]Royal Children's Hospital, [5]Royal Melbourne Hospital, [6]Healthscope Molecular , Melbourne, Australia [7]Department of Gastroenterology, Christchurch, New Zealand

There are a number of recent studies showing that pharmacogenomic testing is often not considered to be used from Australia, New Zealand and Europe. There are many reasons given including cost, availability, lack of clear interpretation instructions or advice from a knowledgeable colleague. In order to familiarise clinicians with availability and interpretation we plan a series of multicentre studies to study specific groups of patients, using newly available molecular pharmacogenetic test. The first test is a molecular test for common variants of the thiopurine methyltransferase gene (*TPMT*). A single base extension method was used to test for *TPMT 3A, TPMT3C* and *TPMT*2*. using blood samples and cheek brush samples. We combined this with a study measuring 6-thioguanine levels (6-TGN) and 6-MMP. We are enrolling IBD patients taking a thiopurine drug for at least 3 months and being on a stable dose for at least 4 weeks. We aim to compare thiopurine drug dose between wild type and heterozygotes for *TPMT* and compare 6-TGN levels. We are also are documenting the number of *TPMT* deficient patients amongst a group of IBD patients who have a history of myelosuppression. This study is expected to familiarise clinicians with the concept of *TPMT* testing within a relatively short period of time. It may lead to preliminary data that could be used to mount a more widespread prospective study of the health value of TPMT testing. We plan further studies in the future for other tests and planning is under way for a warfarin dosage test.

CYP2D6 GENOTYPE AND ITS RELATION TO METOPROLOL DOSE, CONCENTRATIONS AND EFFECTS IN PATIENTS WITH SYSTOLIC HEART FAILURE.

CF Sharp[1], EJ Begg[2], SJ Gardiner[2], RL Roberts[3], BP Jensen[2], JG Lainchbury[4] & RW Troughton[1,4].

Departments of Pharmacy[1], Medicine[2], Pathology[3] and Cardiology[4], University of Otago and Christchurch Hospital, Christchurch.

Metoprolol has been promoted as an example of a cytochrome P450 2D6 (CYP2D6) substrate for which polymorphism may be important in the clinical setting, because the AUC of metoprolol is 3- to 6-fold higher in poor metabolisers (PMs) than in extensive metabolisers (EMs). However, most clinical studies have failed to demonstrate a relationship between CYP2D6 activity and the beneficial or adverse effects of metoprolol. A detailed study of the genotypes, individual enantiomers (S- and R-metoprolol) and clinical effects may demonstrate a clinical correlate. **Aims**: To examine the relationships between *CYP2D6* genotype and metoprolol dose, concentrations, and clinical effects in patients with NYHA class II-IV systolic heart failure. **Methods**: 62 patients stabilised on their maximum tolerated dose underwent blood sampling for *CYP2D6* genotype, and R- and S-metoprolol concentrations. Clinical and laboratory end-points of desired and adverse effects were observed. **Results**: Eight subjects were excluded from analysis due to protocol violation and non-availability of genotype. Twenty-eight (50%) of 54 subjects were EM-high activity, 22 (41%) EM-low activity, 1 (1.9%) intermediate metaboliser (IM) and 3 (5.6%) PMs. Median tolerated doses were 1.1, 0.6, 1.3 and 0.7 mg/kg/day, respectively (NS). Median S-metoprolol concentrations were 3.4- and 6.4-fold higher in EM-low activity and PMs versus EM-high activity (p=0.0007) and R-metoprolol concentrations were 3.8- and 10.6-fold higher, respectively (p=0.0002). There was no correlation between *CYP2D6* genotype, or concentrations of R- and S-metoprolol, and any indices of clinical effect. **Conclusions**: We confirmed gene-concentration relationships with metoprolol. However, we were not able to demonstrate that these correlated with variations in clinical indices.

PUTTING PGX INTO PRACTICE: EXPERIENCE IN A DIAGNOSTIC LABORATORY

Peter George

Canterbury Health Laboratories, Christchurch

GENOME WIDE ASSOCIATION STUDIES USING DNA POOLS AND DENSE SNP ARRAYS

Grant W. Montgomery[1], Stuart Macgregor[2], Zhen Zhen Zhao[1], Anjali Henders[1], Nicholas G. Martin[2], Peter M. Visscher[2],

[1]Molecular Epidemiology and [2]Genetic Epidemiology Laboratories, Queensland Institute of Medical Research, Brisbane, Australia

Recent advances in large scale genotyping have made genome-wide association (GWA) possible. However, the major limiting factor in many GWA studies is cost. Typing individual samples is often prohibitively expensive and genome scans of suitable size (hundreds/thousands of cases and controls, hundreds of thousands of markers) typically cost over US$1 million. We have evaluated alternative approaches which reduce the genotyping cost. We demonstrate that DNA pooling offers a means of dramatically reducing the cost of GWA studies. Building on previous work on Affymetrix arrays, we have developed new methodology for statistical analysis of data from the Illumina platform. The method is based upon contrasting case and control pools and hence does not require independent estimates of rates of unequal amplification of alleles. The same pools were typed on Illumina and Affymetrix arrays. Illumina arrays were found to offer an order of magnitude decrease in pooling error variance compared with Affymetrix arrays. With Illumina arrays concordance with individual genotyping data is excellent; in terms of effective sample size it is possible to extract >80% of the information available with individual genotyping. After taking into account pooling error, one stage scans can be performed for >100 fold reduced cost compared with individual genotyping. With appropriately designed two stage studies, individual genotyping can provide confirmation of pooling results whilst still providing ~20 fold reduction in total cost compared with individual genotyping based alternatives. The large cost savings with Illumina based pooling imply that GWA studies are possible for an extended range of phenotypes were suitable samples are available.

IDENTIFYING GENOMIC SIGNATURES FOR PREDICTION OF DRUG RESPONSE

Rod A Lea (PhD)[1], David Hall[1], Donia Macartney[1], Lisa McCallum[1], Geoff Chambers[2] from Victoria University of Wellington

[1]Institute: Environmental Science and Research Ltd, [2] Victoria University of Wellington

The utility of pharmacogenomics in clinical medicine depends on how accurately a DNA-based test can predict a patient's response to a particular drug. The development of useful pharmacogenomic tests has been hampered by the fact that many drug response outcomes are complex traits, which are explained by the synergistic effects of environmental factors and multi-gene networks. In an effort to address the latter we have employed the case-control design with SNP chip technology and bioinformatic algorithms to develop a method for identifying a characteristic multi-SNP set that best predicts a complex trait (genomic signature). This presentation will show some preliminarily results supporting the application of our method in pharmacogenomics.

PHARMACOGENOMICS OF ANTIDEPRESSANTS - OVERVIEW

Peter Joyce

University of Otago, Christchurch

SEPIAPTERIN REDUCTASE (SPR): A NOVEL CANDIDATE GENE FOR ANTIDEPRESSANT RESPONSE AND AFFECTIVE DISORDERS?

Patrick C McHugh[1], Peter R. Joyce[2,3] and Martin A. Kennedy[1,3],

[1]Departments of Pathology and [2]Psychological Medicine, University of Otago, Christchurch, New Zealand; [3]Carney Centre for Pharmacogenomics, University of Otago, Christchurch, New Zealand

In a previous study we examined the effects of chronic exposure to the selective serotonin re-uptake inhibitor paroxetine on the proteome of cultured neural cells, and identified several proteins with altered expression (McHugh *et al.* 2007). In particular, we observed a three-fold increase in the level of the enzyme sepiapterin reductase (SPR), which catalyzes the final step in synthesis of tetrahydrobiopterin (BH_4). BH_4 is an essential co-factor for synthesis of many neurotransmitters including serotonin. Given this important role the *SPR* gene is an intriguing candidate for mediating response to antidepressant drugs. We hypothesized that genetic variability in *SPR* will impact on an individual's response to antidepressant drugs. To search for polymorphisms we carried out DNA sequencing of *SPR* in 24 unrelated individuals (12 depressed, 12 non-depressed) and identified two promoter SNPs, -196C>A (MAF 0.34) and -1,095A>G (MAF 0.08). Effects of these SNPs on basal gene expression were assessed by cloning the promoter variants into pGL3-basic and transfecting into COS-7 cells. After 48 hours the cells were harvested and expression levels measured using the Dual-Luciferase® Reporter Assay System (Promega). We observed a 1.4-fold decrease in the transcription rate of the promoter carrying the -196A allele. It is conceivable that this reduced transcription rate may affect the abundance and availability of SPR, which in turn may modulate BH_4 synthesis and thus impact on neurotransmitter production. We are currently exploring the association of these alleles with phenotypes relevant to antidepressant treatment.

Reference: McHugh et al. (2007) Proteomic analysis of embryonic stem cell - derived neural cells exposed to the antidepressant paroxetine J. Neurosci. Res. (In press).

ANTIDEPRESANT PHARMACOGENOMICS OF A SEROTONERGIC CELL LINE

Dylan Glubb[1], Geraldine Rogers, Peter R. Joyce[2,3] and Martin A. Kennedy[1,3],

[1]Departments of Pathology and [2]Psychological Medicine, University of Otago, Christchurch, New Zealand; [3]Carney Centre for Pharmacogenomics, University of Otago, Christchurch, New Zealand

A rat serotonergic cell line, RN46A, which expresses the serotonin transporter (5-HTT), the molecular target of selective serotonin re-uptake inhibitors (SSRIs), was used as a model for examining the acute effects of the SSRI paroxetine on gene expression. RN46A cells were differentiated and then incubated in the presence of paroxetine for either 30 min or 36 hr. RNA was taken for microarray analysis using Affymetrix rat 230 2.0 GeneChips and a list of differentially regulated genes was generated for each time point. 92 genes had significant expression changes after 30 min exposure to paroxetine whilst 253 expression changes were found after 36 hr exposure. Quantitative real-time PCR (Q-PCR) assays were developed to validate the transcriptional changes. Two genes (*Id2* and *Ucn2*) from the 36 hr experiments were found to have significant transcriptional changes and three other genes that exhibited expression changes at this time point were close to conventional statistical significance. The five candidate genes have biological functions which may be relevant to antidepressant response. We observed that three of these candidate genes, and many other genes that shared expression changes from the microarray experiments, are also differentially expressed in hypoxia. Mild hypoxia activates neuroprotective pathways and has even been shown to have antidepressant-like effects in animal models. It is therefore possible that the molecular and behavioural effects of paroxetine may be mediated through hypoxia-related mechanisms.

THE SEARCH FOR THE BLACK BILE: PERIPHERAL BIOMARKERS IN DEPRESSION.

James A Harley[1,2,3], Peter R. Joyce[2,3] and Martin A. Kennedy[1,3]

[1]Departments of Pathology and [2]Psychological Medicine, University of Otago, Christchurch, New Zealand; [3]Carney Centre for Pharmacogenomics, University of Otago, Christchurch, New Zealand

Successful treatment of depression can be achieved with pharmacotherapy, however it is difficult to predict which patients are likely to respond. The development of predictive biomarkers of response is essential to improve the outcome of depressed patients.

The actions of antidepressants require the stimulation of neurogenesis and the modulation of synaptic plasticity, events that require modification of gene expression. There is potential that the mRNA changes in the brain are reflected by expression in a more readily sampled tissue, the blood. With the development of advanced techniques we have the capability for screening for new candidate biomarkers by investigating transcription changes in the periphery.

Peripheral Blood Mononuclear Cells (PBMCs) are a population of leukocytes that are directly exposed to antidepressants in plasma. They have previously been shown to express the serotonin transporter, the target of most antidepressants, and expression pattern changes have been observed in studies of many other disorders.

We plan to identify new biomarkers of antidepressant response by performing a whole genome expression analysis using mRNA from rat PBMCs treated with either citalopram or fluoxetine. Strong candidates from the arrays will be confirmed by quantitative PCR. The ultimate aim is to transfer assays of PBMC gene expression to cohorts of human patients at initiation of antidepressant therapy to determine if these putative biomarkers have significant predictive ability.

Symposium Notes:

The Carney Centre for Pharmacogenomics

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 research groups throughout the University of Otago and elsewhere. Together these groups span a wide range of clinical, pharmacological and genetic 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@chmeds.ac.nz

Visit out website: www.pgx.org.nz