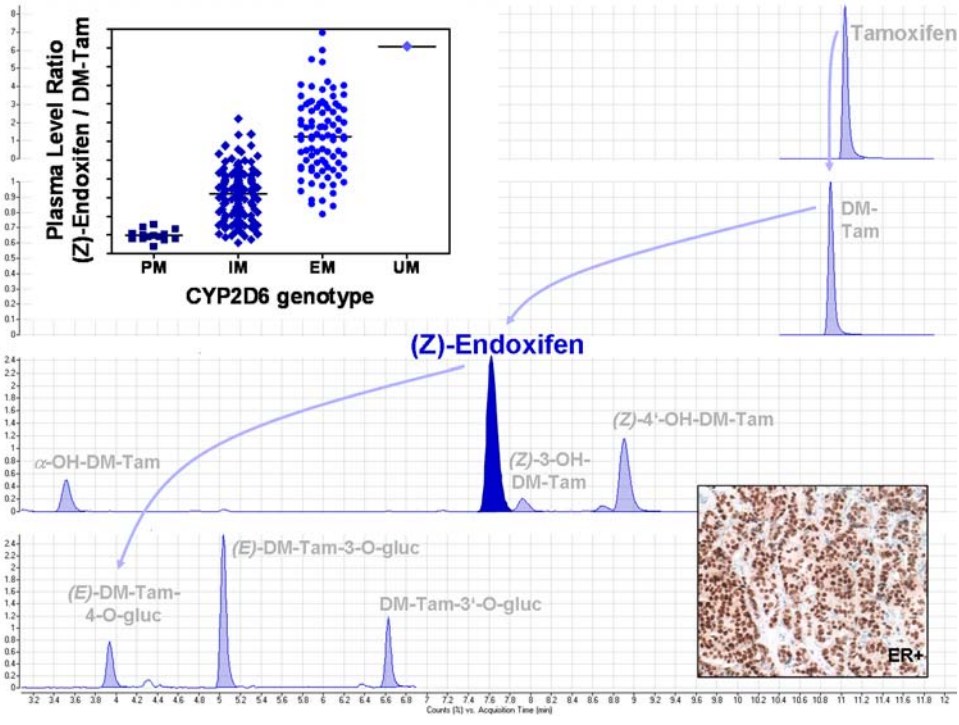
Abstracts of papers presented
at the 2010 meeting on

PHARMACOGENOMICS & PERSONALIZED THERAPY

November 17–November 21, 2010



Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

PHARMACOGENOMICS & PERSONALIZED THERAPY

November 17–November 21, 2010

Arranged by

Hiltrud Brauch, *IKP Stuttgart, Germany*
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Cold Spring Harbor Laboratory
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Cover: CYP2D6 Tamoxifen Pharmacogenetics in Breast Cancer. Tamoxifen is a standard of care for patients whose tumors stain positive for the estrogen receptor (ER) by immunohistochemstry (lower right panel). The background figure shows an LC/MS/MS analytical profile of steady-state plasma levels of tamoxifen and its metabolites in a patient treated with a daily dose of 20 mg tamoxifen. Levels of (Z)-Endoxifen, one of the active metabolites (center) follow a CYP2D6 gene dose effect (upper left panel, > 230 patients). Patients with no or low function alleles (PM) have lowest, patients with impaired enzyme activity (IM) have intermediate, and patients with two and more full function alleles (EM and UM, respectively) have highest Endoxifen levels (expressed as metabolic ratio of the precursor metabolite). Image provided by Thomas Mürdter and Hiltrud Brauch, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University Tübingen, Germany.

PHARMACOGENOMICS & PERSONALIZED THERAPY
Wednesday, November 17 – Sunday, November 21, 2010

Wednesday	7:30 pm	**1** Opening Session
Thursday	9:00 am	**2** Translational Bioinformatics
Thursday	2:00 pm	**3** Poster Session I
Thursday	4:30 pm	Wine and Cheese Party
Thursday	7:30 pm	**4** Next Generation Sequencing Approaches in Pharmacogenomics
Friday	9:00 am	**5** Genome-wide Analyses and CNVs
Friday	2:00 pm	**6** Genetic Control of Expression
Friday	7:30 pm	**7** Poster Session II
Saturday	9:00 am	**8** Epigenetics and Functional Genomics
Saturday	2:00 pm	**9** ELSI, Pharmacoeconomic Issues and Population-based Pharmacogenetics
Saturday	6:00 pm	Banquet

Poster sessions are located in *Bush Lecture Hall*

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm
Bar is open from 5:00 pm until late

Printed on 100% recycled paper.

PROGRAM

WEDNESDAY, November 17—7:30 PM

SESSION 1 OPENING SESSION

THURSDAY, November 18—9:00 AM

SESSION 2 TRANSLATIONAL BIOINFORMATICS

Chairperson: **E. Schadt,** Pacific Biosciences, Menlo Park, California

THURSDAY, November 18—2:00 PM

SESSION 3 POSTER SESSION I

THURSDAY, November 18—4:30 PM

Wine and Cheese Party

THURSDAY, November 18—7:30 PM

SESSION 4 NEXT GENERATION SEQUENCING APPROACHES IN PHARMACOGENOMICS

Chairperson: **D. Nickerson,** University of Washington, Seattle

Genetic risk profile predicting anthracycline-induced cardiotoxicity in children
Henk Visscher, Colin J. Ross, Rod Rassekh, Amina Barhdadi, Marie-Pierre Dubé, Hesham al-Saloos, George S. Sandor, Andrew M. Brown, Paul C. Rogers, Michael S. Phillips, Michael J. Rieder, Bruce C. Carleton, Michael R. Hayden.
Presenter affiliation: University of British Columbia, Vancouver, Canada. 46

FRIDAY, November 19—9:00 AM

SESSION 5 GENOME-WIDE ANALYSES AND CNVs

Chairperson: **P. Donnelly,** Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom

CNVs in association studies
Peter J. Donnelly.
Presenter affiliation: Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom. 47

Prognostic impact of inherited genetic variations in SRD5A and androgen inactivating UGT2B genes in prostate cancer after prostatectomy
Étienne Audet-Walsh, Judith Bellemare, Geneviève Nadeau, Louis Lacombe, Yves Fradet, Pierre Douville, Hugo Girard, Chantal Guillemette, Éric Lévesque.
Presenter affiliation: Centre Hospitalier Universitaire de Québec (CHUQ) Research Center and Laval University, Québec, Canada. 48

Inherited genetic variation in *FZD3* , *EPHA5* and *FGD4* and Paclitaxel (P) induced peripheral neuropathy (PN)—Results from a genome-wide association study (GWAS) in CALGB 40101
Michael Baldwin, Kouros Owzar, Chen Jiang, Dee Watson, Joel Mefford, John S. Witte, Eric Jorgenson, Hitoshi Zembutsu, Michiaki Kubo, Howard L. McLeod, Clifford A. Hudis, Eric P. Winer, Yusuke Nakamura, Larry N. Shulman, Mark J. Ratain, Deanna L. Kroetz.
Presenter affiliation: UCSF, San Francisco, California. 49

FRIDAY, November 19—2:00 PM

SESSION 6 GENETIC CONTROL OF EXPRESSION

Chairperson: **S. Brenner,** University of California, Berkeley

FRIDAY, November 19—7:30 PM

SESSION 7 POSTER SESSION II

See Poster Session I for list of posters.

SATURDAY, November 20—9:00 AM

SESSION 8 EPIGENETICS AND FUNCTIONAL GENOMICS

Chairperson: **M. Esteller,** Bellvitge Biomedical Research Institute, Barcelona, Spain

SESSION 9 ELSI, PHARMACOECONOMIC ISSUES AND POPULATION-BASED PHARMACOGENETICS

Chairperson: **D. Veenstra,** University of Washington, Seattle

SATURDAY, November 20

BANQUET

Cocktails 6:00 PM Dinner 6:45 PM

EN ROUTE TO THE ERA OF GENOMIC MEDICINE

Eric D Green

National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, 20892

The Human Genome Project's completion of the human genome sequence in 2003 was a landmark scientific achievement of historic significance. It also signified a critical transition for the field of genomics, as the new foundation of genomic knowledge started to be used in powerful ways by researchers and clinicians to tackle increasingly complex problems in biomedicine. To exploit the opportunities provided by the human genome sequence and to ensure the productive growth of genomics as one of the most vital biomedical disciplines of the 21st century, the National Human Genome Research Institute (NHGRI) is pursuing a broad vision for genomics research beyond the Human Genome Project. This vision includes facilitating and supporting the highest-priority research areas that interconnect genomics to biology, to health, and to society.Current efforts in genomics research are focused on using genomic data, technologies, and insights to acquire a deeper understanding of biology and to uncover the genetic basis of human disease. Some of the most profound advances are being catalyzed by revolutionary new DNA sequencing technologies; these methods are already producing prodigious amounts of DNA sequence data, including from large numbers of individual patients. Such a capability, coupled with better associations between genetic diseases and specific regions of the human genome, are accelerating our understanding of the genetic basis for complex genetic disorders and for drug response. Together, these developments will usher in the era of genomic medicine.

PHARMACOGENETICS / GENOMICS: PAST, PRESENT AND FUTURE

Michel Eichelbaum

Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, 70376, Germany

Genetic factors have been suggested depending on the drug to account for 20 to 95 % of the variability in drug disposition and effects. The best recognized examples of genetic polymorphisms that influence drug response in humans are highly penetrant monogenic traits of drug metabolizing enzymes (DME). Inherited difference in a single gene of DME has such a profound effect on the pharmacokinetics of a drug resulting in more than a 100 fold difference in systemic drug exposure with clinically important effect on drug response. Loss of function of DME genes have been identified as mechanisms of severe and life-threatening toxicity. Examples are CYP P450 and thiopurine-S-methyltransferase deficiency where case report and case control studies have demonstrated that the high systematic drug exposure associated with loss of function allele translate into differences in response and toxicity. Gene duplication as it has been demonstrated for CYP2D6 has been identified as a mechanism of poor treatment response. In the case of prodrugs loss of function alleles are predictive for non-response. Examples include tamoxifen, a drug used for the treatment breast cancer, where carriers of loss of function alleles have a poorer clinical response with respect to time to recurrence and disease free survival. In the case of clopidogrel the antiplatelet effect is mediated by a metabolite which is formed by the polymorphic CYP2C19. A major limitation in implementing pharmacogenetic testing in the clinical setting has been the lack of clinical trial demonstrating that such testing can improve drug therapy by reducing toxicity and increasing efficacy.

AN INTEGRATIVE GENOMICS STRATEGY TO ELUCIDATING THE COMPLEXITY OF DRUG RESPONSE

Eric E Schadt

Pacific Biosciences, Chief Scientific Officer, Menlo Park, CA, 94025

Drug response can be considered as a complex traits like obesity, diabetes, and heart disease, where such traits are driven by constellations of genetic and environmental (both micro and macro) factors interacting in complex ways. However, the genetic and environmental perturbations do not directly lead to variations in drug response, but rather impact molecular processes that underlie physiological states associated with such responses. Therefore, in order to develop a complete understanding of drug response, biological systems must be queried in a comprehensive fashion in multiple dimensions, and then appropriately integrated so that we understand how these different pieces inter-relate to one another to define the molecular networks that define physiological states associated with drug response. Focusing on a single dimension, even if done in a comprehensive fashion, will not be sufficient to get at a complete understanding of drug response. Here I present network-based approaches for integrating large-scale, high dimensional data generated in mouse and human populations to construct the underlying molecular drivers of disease. By projecting molecular signatures from different disease models onto these networks, I demonstrate how different subtypes of disease emerge. By matching the networks underlying these subtypes to drugs that perturb such networks, we can achieve a better understanding of response both in terms of efficacy as well as adverse events.

DEVELOPMENT OF MULTI-SNP PREDICTIVE MODELS AND CHARACTERIZATION OF PERFORMANCE METRICS

Scott L Marshall, Jared R Kohler, Laura B Gillis, Christina M Bromley

BioStat Solutions, Inc., 114 South Main Street, Suite 002, Mt. Airy, MD, 21771

Development of predictive DNA biomarkers in pharmacogenomic (PGx) studies presents a challenge in not only how to construct a classifier under real-life limitations but also how to assess the performance of the classifier under these settings. Consider a genome-wide association study that yields multiple findings of interest. The predictive ability of any single nucleotide polymorphism (SNP) alone is driven by three main factors: (1) response rate (2) effect size and (3) variant allele frequency. In traditional classification problems the sensitivity and specificity are primarily driven by the underlying performance of the diagnostic tool (which in theory can be improved); however, development of a SNP classifier is primarily limited by the frequency of the variant in the population. Given a moderate effect size for a treatment with reasonable efficacy and irrespective of variant allele frequency, it can be demonstrated that jointly maximizing the sensitivity and specificity at levels deemed clinically meaningful (e.g. > 75%) is impractical. Alternatively, positive predictive value (PPV) and negative predictive value (NPV) are two performance metrics that are less impacted by the variant frequency and under similar conditions may provide a more meaningful assessment of clinical utility. While these performance metrics in general are commonly accepted, there is no consensus best approach for the construction and characterization of the predictive model. Here, we suggest an approach to overcome the limitations of single SNP predictors by combining the information across multiple SNPs with small to moderate effects using supervised principal components analysis (PCA) which may improve performance metrics.

SYSTEMS (PATHWAY) BIOLOGY IN PHARMACOGENOMICS RESEARCH: A HIGHLY SCALABLE BAYESIAN NETWORK MODELING APPROACH

Andrei S Rodin[1], Grigoriy Gogoshin[1], Stephen T Turner[2], Julie A Johnson[3], Arlene Chapman[4], Eric Boerwinkle[1]

[1]University of Texas - Houston, Human Genetics Center, Houston, TX, 77225, [2]Mayo Clinic, Nephrology and Hypertension, Rochester, MN, 55905, [3]University of Florida - Gainesville, Pharmacotherapy and Translational Research, Gainesville, FL, 32611, [4]Emory University, Renal Medicine, Atlanta, GA, 30322

"Systems biology" data analysis and modeling methods (primarily the Bayesian Network (BN) modeling) are becoming increasingly important for reverse-engineering the interplay of gene variation and other variables contributing to inter-individual differences in response to drug treatment. We will present a novel BN modeling strategy that takes advantage of (1) highly optimized numerical libraries, (2) efficient memory utilization to prevent possible memory overflows (into "virtual" memory), (3) iterative search algorithms that allow for easy parallelization, (4) cumulative bootstrap technique to evaluate the robustness, and (5) a prototype Bayesian Dirichlet metric evaluation engine. The main advantages of this new strategy are drastically increased scalability (up to 100,000s single nucleotide polymorphisms (SNPs) and other variables), robustness (via improved search convergence) and ability to incorporate different metrics, search algorithm variations, and prior expert knowledge "on the fly". We will then present the results of the application of this novel BN modeling methodology to both the candidate gene and GWAS pharmacogenomics datasets (blood pressure response to a thiazide diuretic). We will close by discussing the issue of the trade-off between scalability and model expressiveness in general terms, and how it applies to the systems biology approach to the analysis and interpretation of the pharmacogenomics data.

SYSTEMS CHARACTERIZATION OF DRUG COMBINATION THERAPIES IN A MOUSE MODEL OF METABOLIC SYNDROME

Marijana Radonjic[1], Marjan van Erk[1], Robert Kleemann[2], Teake Kooistra[2], Lars Verschuren[1], Peter Y Wielinga[2], Ben van Ommen[1]

[1]TNO Biosciences, Physiological Genomics, Zeist, 3704 HE, Netherlands, [2]TNO Biosciences, Vascular and Metabolic Disease, Leiden, 2333 CK, Netherlands

The multifactorial nature of metabolic syndrome and associated pathologies represents a major challenge for designing optimal treatment strategies. To experience health benefit, patients often require a drug combination therapy which targets multiple disease endpoints. In addition to beneficial effects of such drug combinations, polypharmacy may also increase a risk of adverse side effects.

To explore the global effects of single cholesterol-lowering drugs and to test if effects of drug combinations may be predicted from the effects of the single compounds in the drug mix, we analysed hepatic transcriptome of ApoE3Leiden mice that were fed high-cholesterol atherogenic diet supplemented either with rosuvastatin, ezetimibe or a combination of these two compounds. Understanding the global genome-wide effects of single drugs and ability to predict efficacy and safety of their combinations will provide evidence-based rationales for designing optimal polypharmacological treatments, tailored to meet individual patient's needs.

GENETIC VARIATION IN THE TGF-B SIGNALING PATHWAY IS ASSOCIATED WITH RESPONSE TO PLATINUM-BASED CHEMOTHERAPY AND OVERALL SURVIVAL IN OVARIAN CANCER PATIENTS

Michelle A.T. Hildebrandt[1], Xia Pu[1], Larissa Meyer[2], Carol Etzel[1], Jie Lin[1], Dong Liang[3], Karen Lu[2], Xifeng Wu[1]

[1]University of Texas MD Anderson Cancer Center, Dept. of Epidemiology, Houston, TX, 77030, [2]University of Texas MD Anderson Cancer Center, Dept. of Gynecologic Oncology, Houston, TX, 77030, [3]Texas Southern University, Department of Pharmacy and Health Sciences, Houston, TX, 77004

The TGF-β signaling pathway has been shown to play a role in the development and progression of ovarian cancer, as well as patients' response to chemotherapy. However, there is little information regarding the effect of common, germline genetic variation within this pathway on clinical outcomes for ovarian cancer patients. In this study, we took a pharmacogenetics approach and genotyped 218 single nucleotide polymorphisms (SNPs) in TGF-β pathway genes in 319 non-Hispanic Caucasian ovarian cancer patients. These genotypes were then analyzed for response to platinum-based chemotherapy and overall survival. Fourteen variants were associated with response, of which eight predicted a favorable response for those carrying variant genotypes. The most significant finding was for *SMAD9*:rs9576129 associated with a 55% decreased risk of a poor response to therapy (OR:0.45, 95%CI:0.26-0.80). For overall survival, 15 significant associations were identified among seven pathway genes. This included two SNPs in *BMP1* that were also associated with response to therapy: rs3857979 and rs4075478. Individuals carrying two variant alleles of these SNPs were at increased risk for both poor response (OR:1.90, 95%CI:1.04-3.48 and OR:2.39, 95%CI:1.17-4.89, respectively) and survival (HR:1.80, 95%CI:1.23-2.91 and HR:1.89, 95%CI:1.22-2.91, respectively). Significant gene-gene interactions were also observed for overall survival which identified subgroups of patients with dramatically different risks of dying (P for trend:$5.4x10^{-5}$) and median survival times (P-value:$1.5x10^{-5}$). A significant dose response between number of pathway risk genotypes and survival was evident (P for trend:$3.40x10^{-10}$). Patients with 8 to 13 risk genotypes had a median survival time of only 26.8 months compared to over 97 months for those with 1 to 5 risk genotypes (P-value:$5.3x10^{-8}$). These results provide evidence that genetic variation within this important pathway modulates clinical outcomes in ovarian cancer patients and may help to individualize treatment to optimize response and prognosis.

IDENTIFYING QUANTITATIVE TRAIT GENES FOR *IN VITRO* DRUG TOXICITY USING HIGH-CONTENT SCREENING

Oscar Suzuki[1], Natasha Butz[1], Brian Steffy[1], Bethany Parks[2], O. Joseph Trask[2], David Scoville[1], Amber Frick[1], Daniel Crona[1], Russell Thomas[2], Tim Wiltshire[1]

[1]UNC Eshelman School of Pharmacy, Pharmacotherapy and Experimental Therapeutics, Chapel Hill, NC, 27599-7361, [2]The Hamner Institutes for Health Sciences, Center for Genomic Biology and Bioinformatics, Research Triangle Park, NC, 27709-2137

Pharmacogenetic studies have successfully identified genetic variants that contribute to variation in drug response, but it is still a complex and challenging task to identify the genetic components of response to drugs across the human genome. Despite limitations, there is a pressing need to identify genetic components that contribute to the efficacy and toxicity of drugs across a wide spectrum of agents. We have proposed that using an alternative model population approach will provide many underlying mechanisms and pathways that are implicated in drug activity and responses. We have developed a platform of in-vitro assays using embryonic fibroblasts from genetically well-defined mouse strains which enables us to evaluate cellular toxicity effects of current and novel drug therapy agents. Here, we present results obtained using this strategy to identify genes and gene pathways that underlie susceptibility to cellular-level adverse drug reactions. We have screened 75 drugs and environmental toxins using high-content imaging to assess specific cell-health status phenotypes (nuclear changes, membrane permeability, mitochondrial membrane potential and cytochrome c localization). The cellular responses from 32 inbred mouse strains were used in genome-wide association analyses to identify quantitative trait loci (QTL) underlying the toxicity variations. This strategy has led to reproducible identification of QTLs, allowing a hypothesis-free identification of candidate genes. Although no targets have been fully validated yet, we discovered a number of genes that potentially affect the measured drug toxicity phenotypes. Gene-set enrichment analysis of the candidate loci suggests an over-representation of genes involved in cell-cycle regulation. Genes were selected and prioritized for validation based on network analysis, expression in mouse embryonic fibroblasts and statistical significance of the observed association.

VARIATION IN SEX STEROID METHYL TRANSFERASES PREDICTS EFFICACY TO ANDROGEN DEPRIVATION THERAPY (ADT) IN PROSTATE CANCER

Manish Kohli[1], Douglas Mahoney[2], High Chai[2], David Hillman[2], James Cerhan[3]

[1]Mayo Clinic, Oncology, Rochester, MN, 55905, [2]Mayo Clinic, Biomedical Statistics & Informatics, Rochester, MN, 55905, [3]Mayo Clinic, Epidemiology, Rochester, MN, 55905

Background: We evaluated variation in hormonal pathway genes in a homogenous population of prostate cancer patients receiving ADT, with the overall goal of identifying single nucleotide polymorphisms (SNPs) associated with treatment response duration. TagSNPs in androgen biosynthesis genes (belonging to the C4 Δ pathway, C5 Δ pathway, C21 CYP pathway and the alternate "Backdoor pathway" for androgen synthesis) and androgen metabolism pathway genes were identified using HapMap phase I+II, NIEHS and Seattle SNP databases. A total of 84 genes were included.

Methods: To capture common genetic variation, tagSNPs with predetermined minor allele frequency (MAF) >5% were targeted using Illumina GoldenGate assay. Germline DNA from 338 advanced prostate cancer patients were submitted for genotyping. All patients had received and failed ADT. Time to ADT failure was the primary endpoint. Patient samples with <98% call rate, incomplete clinic data were excluded, leaving 304 samples for subsequent analysis. After filtering out SNPs with <5% MAF, <98% call rate and <0.0001 Hardy-Weinberg chi-square p-value, 747 SNPs were used towards final analysis with response to ADT.

Results: Of the genes analyzed for association with duration of response to ADT, *TRMT11 (tRNA methyltransferase 11 homologue)* was strongly associated with ADT response ($p<0.0008$; adjusted p-value for FDR-0.068). This remained significant after evaluating the gene without adjustment for age and Gleason score (p-value = 0.001264 and FDR=0.014045). Of four *TRMT11* SNPs analyzed (rs1268121, rs2326215, rs6569442, rs6900796) with progression time on ADT, two (rs1268121, rs6900796) were highly significant for duration of ADT response. An overall protective effect was observed in the presence of 0,1 or 2 alleles for these SNPs with response duration ranging between 3.08 years to 5.86 years. Four additional genes showed moderate association with ADT response ($0.025 \leq$ p-value ≤ 0.07) including another methyltransferase, *PRMT3* with 2 of the 20 SNPs analyzed significantly associated with ADT response each having a protective effect (rs11025588 (p=0.0127) and rs11025592 (p=0.0009)). Additionally, three other genes (*WBSCR22, SLC7A6OS*, and *LOC390956* with significant association to ADT response (p=0.0648, 0.0545, and 0.0312 respectively).

Conclusions: Variation in methyl transferase metabolic activity in hormonal pathway genes are strongly associated with ADT response duration time. Validation of these findings is being attempted in independent datasets of prostate cancer patients. Since ADT is a commonly used treatment in prostate cancer, the use of predictive biomarkers has a large public health impact in choosing treatments.

GENDEP: RECENT PHARMACOGENETIC AND PHARMACOGENOMIC OUTPUT

K J Aitchison[1], R Keers[1], R Uher[1], N Perroud[1], R Smith[1], K Malki[1], J Paya-Cano[1], M Rietschel[2], O Mors[3], N Henigsberg[4], W Maier[5], J Hauser[6], D Kozel[7], D Souery[8], L Schalkwyk[1], C Lewis[1], I W Craig[1], P McGuffin[1]

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Background: GENDEP, a European multicentre integrated pharmacogenomic study, aimed to identify genomic correlates of antidepressant response including ADRs.
Methods: White European subjects with major depression were treated with escitalopram (ESC) or nortriptyline (NOR), in a part-randomised potential crossover design, and prospectively rated for clinical response and ADRs (Uher et al, 2008; 2009).
Results: Polymorphisms in *SLC6A4* (*5-HTTLPR* and STin4 not previously investigated) interact with stressful life events (SLEs) occurring in the 6 months prior to treatment to predict response to ESC but not to NOR (Keers et al, 2010). Such SLEs were also associated with a higher cognitive symptom score and with a greater reduction in this on ESC but not with NOR (Keers et al, in press). Converging results from our genetic association and genome-wide association analysis (GWAS) indicate that *NTRK2* is associated with suicidality (Perroud et al, in press). The *CYP2D6* ultrarapid metaboliser (UM) genotype was associated with a lower dose of both antidepressants over the 12-week trial (β=-0.56, 95% CI -0.88 to -0.25 $P < 0.001$; Keers et al, 2010). Rodent gene expression analysis and clinical trial transcriptomics data point to a role for *PPM1A* in response to antidepressants (Malki et al, under review). GWAS analysis reveals an association between rs2500535 in the uronyl 2-sulphotransferase gene and response to NOR, while response to ESC was predicted by a marker in (*IL11)* (Uher et al, 2010). Conclusion: Findings which replicate other data are emerging, of potential biomarker utility. Funding: GENDEP was fundded by the European Commission, Framework 6 Programme (LSHB-CT-2003-503428), supplemented by the UK NIHR (Biomedical Research Centre for Mental Health) and an MRC-GSK tag-on project for GWAS.

IDENTIFICATION OF COMPOUNDS ACTIVE AGAINST MITOCHONDRIAL DISEASE USING A NOVEL YEAST-BASED ASSAY

Elodie Couplan[1], Raeka S Aiyar[2], Roza Kucharczyk[3], Nahia Ezcurdia[4], Julien Gagneur[2], Robert P St. Onge[5], Benedicte Salin[4], Marie Le Cann[1], Lars M Steinmetz[2], Jean-Paul di Rago[4], Marc Blondel[1]

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Mitochondrial diseases are a class of serious, prevalent, phenotypically diverse and genetically complex disorders for which few effective treatments have been discovered. We present a simple and powerful yeast-based assay to screen for drugs with the potential to treat human mitochondrial diseases that affect ATP synthase. Our method exploits the high conservation of mitochondrial function between yeast and human, as well as the amenability of the yeast mitochondrial genome to site-directed mutagenesis. The latter possibility has enabled the creation of yeast mutants corresponding to several point mutations in the *ATP6* gene associated with NARP (neuropathy, ataxia, and retinosis pigmentosa) syndrome. The severity of the phenotypes in yeast and human correlate, demonstrating the suitability of yeast as a model of NARP. Our screen identified several compounds, each of which are effective only in particular mutants, thus providing a good platform to investigate the role of genetic variation in the treatment of ATP synthase disorders. One of these compounds rescues, to varying extents, virtually every mitochondrial defect measured in one yeast model of NARP and is also effective in human cybrids derived from NARP patients. These results validate our method as a useful high-throughput approach to identify drugs potentially active in the treatment of ATP synthase disorders and offer prospects for the development of personalized therapeutics for these disorders.

AN ASSOCIATION BETWEEN CARBAMAZEPINE-INDUCED MACULOPAPULAR EXANTHEMA AND THE HLA-C REGION IN CAUCASIANS

Ana Alfirevic[1], Stephane Bourgeois[2], Andrea Jorgensen[1], Graeme Sills[3], Tony Marson[3], Michael Johnson[4], B Kevin Park[1], Munir Pirmohamed[1]

[1]University of Liverpool, Pharmacology, Liverpool, L69 3GL, United Kingdom, [2]Sanger Institute, Human Genetics, Cambridge, CB10 1SA , United Kingdom, [3]Wolton Centre for Neurology, Neurology, Liverpool, L9 7LJ, United Kingdom, [4]Imperial College, Medicine, London, SW7 2AZ, United Kingdom

Maculopapular exanthema (MPE) is the most common form of hypersensitivity reaction to the antiepileptic drug carbamazepine (CBZ). It requires drug withdrawal and re-challenge is not recommended as it may lead to more severe reactions. Previous pharmacogenetic studies conducted in patients with CBZ-induced MPE did not demonstrate any strong associations which could be replicated by other groups. Given the immune etiology of MPE and the strong association between drug-induced hypersensitivity and several genes in the MHC region, we have investigated genetic polymorphisms in the entire MHC region on Chromosome 6 in two cohorts (discovery and replication cohorts) of Caucasian patients with CBZ-induced MPE.

In total 2961 patients and controls were included into the study. The discovery cohort comprised thirty nine patients who experienced CBZ-induced MPE and two control groups- CBZ-tolerant patients (n=43) and WTCCC healthy individuals (n=2849). Our replication cohort comprised 30 patients identified from a randomised controlled trial (SANAD). All patients and controls were Caucasians. Genotyping for 1293 SNPs was undertaken using the Illumina MHC SNP mapping panel and BeadArray technology. We compared genotype frequencies in our patients with GWAS data (using the 1.2 million Illumina chip) available at the Wellcome Trust Sanger Institute for all healthy volunteers. Replication study genotyping was performed using TaqMan assays and KBiosciences genotyping services. SNP genotype frequencies in patients and controls were compared with frequencies in the HapMap, dbSNP and WTCCC databases. Data analysis was performed using the BeadStudio, Haploview and PLINK software.

Minor allele frequencies (MAF) across the MHC region in our patients were similar to frequencies in dbSNP in Caucasian population. We found an association between maculopapular exanthema and a SNP in the coding region of HLA-C (P=2.2E-9). We confirmed this finding in our replication study and performed meta-analysis (pooled odds ratio OR10 (95% CI 5.5 to 18.6).

In this study we have identified novel candidate genes and validated our findings in a carefully selected replication cohort. Whether this SNP is causal for CBZ exanthema or merely reflects linkage disequilirium with a causal variant is unclear, and will need to be investigated using functional studies.

DIFFERENTIAL EFFECT OF THE RS4149056 VARIANT IN SLCO1B1 ON MYOPATHY ASSOCIATED WITH SIMVASTATIN AND ATORVASTATIN

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Statins reduce cardiovascular morbidity and mortality in appropriately selected patients. However, statin-associated myopathy is a significant risk associated with these agents. Recently, variation in the SLCO1B1 gene was reported to predict simvastatin-associated myopathy. The aim of this study was to replicate association of the rs4149056 variant in SLCO1B1 with severe statin-associated myopathy in a cohort of patients using a variety of statin medications and to investigate the association with specific statin types. We identified 25 cases of severe statin-associated myopathy and 84 controls matched for age, gender, statin type and dose. The rs4149056 variant in SLCO1B1 was not significantly associated with myopathy in this group as a whole. However, when subjects were stratified by statin type, the SLCO1B1 rs4149056 genotype was significantly associated with myopathy in patients who received simvastatin, but not in patients who received atorvastatin. Our findings provide further support for a role for SLCO1B1 genotype in simvastatin-associated myopathy, and suggest that this association may be stronger for simvastatin compared to atorvastatin.

ANALYSIS OF CYTOCHROME P450 2B6 HAPLOTYPES AND ASSOCIATIONS WITH EFAVIRENZ PLASMA CONCENTRATIONS IN A CHILEAN HIV COHORT

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Cytochrome P450 2B6 (*CYP2B6*) is highly polymorphic and a number of functional single nucleotide polymorphisms (SNPs) and haplotypes have been reported in different populations. To date little information is available about the frequencies and functional consequences of CYP2B6 polymorphisms in Latin American populations. We report the frequency and functional consequences of CYP2B6 SNPs for the known CYP2B6 substrate, efavirenz (EFV), in 219 HIV+ patients recruited at Fundación Arriarán, Chile between Sep. and Dec. 2008. EFV plasma concentrations were determined using a validated LC-MS/MS assay. All individuals were genotyped for 30 SNPs with a minor allele frequency (MAF)>0.05 in the HapMap CEU population at intervals of ~1kB across the CYP2B6 locus using the Sequenom iPLEX MALDI-TOF-based genotyping platform. SNPs with a MAF<0.01 and Hardy Weinberg disequilibrium p-value<0.01 were omitted from subsequent analysis. Thirteen SNPs passed QC and were carried forward for further analysis. One-way analysis of variance (ANOVA) of EFV plasma concentration with genotype for individual SNPs indicated statistical significance ($p<0.001$) for 11/13 SNPs within the CYP2B6 gene. The exceptions were rs4802100 (5' upstream) ($p=0.08$) and rs34083050 (Intron 4) ($p=0.91$). The statistical significance of the genotype associations with EFV plasma concentrations ranged from $p=4.4x10^{-8}$ (rs227344) to $p=3.6x10^{-22}$ (rs8192719). Linkage disequilibrium analysis of the 13 *CYP2B6* polymorphisms showed a significant degree of LD, similar to the Caucasian population. Pair-wise tagging SNP analysis (R2>0.8) identified 3 SNPs (rs10403955, rs2279345 and rs8192719) which were representative of the 11 plasma EFV concentration-associated SNPs. A composite genetic model of these 3 high EFV concentration associated alleles was constructed. An association between carriers of 4-6 of these alleles and risk of EFV plasma concentration > an upper limit for Ctrough of 4µg/ml was identified. 22/38 individuals with EFV > 4µg/ml possessed 4-6 associated alleles compared to 4/140 of those with EFV plasma concentration < 4µg/ml. This represents an odds ratio of 48.1 (95%CI: 13.5-207.7). The positive predictive value was 84.6% and negative predictive value was 89.8% with a sensitivity of 57.9% and specificity of 97.2%. Our data suggest that the haplotype structure in the Chilean Hispanic population is similar to that in Caucasians. Furthermore, a number of polymorphisms in the *CYP2B6* gene are associated with increased plasma EFV concentrations. Further investigation of the functional basis of this association is needed.

THE INFLUENCE OF MULTIDRUG AND TOXIN EXTRUSION 1 (MATE1) TRANSPORTER GENOTYPES ON THE PHARMACOKINETICS OF METFOMIN

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Background
The biguanide derivative metformin is the first-line oral hypoglycemic drug for the treatment of type 2 diabetes. Metformin is a substrate of organic cation transporters (OCTs), which mediate drug absorption and elimination. The result of studies regarding the effect of polymorphism of OCT2 on metformin pharmacokinetic is different by different ethnics. This result gives a clue that other transporters' polymorphism may play an important role. This study is aimed to evaluate the associate between Multidrug and Toxin Extrusion 1 (MATE1) transporter and metformin pharmacokinetics.

Methods
Sixteen healthy Koreans were enrolled in a pharmacokinetic study and genotyped for SNP of MATE1, rs2289669 and SNP of OCT2, rs316019. Subjects received 1000mg metformin on the 1st day and 750mg metformin on the 2nd day. Blood for metformin concentration was collected at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12, and 24 h after 2nd metformin dose. Urine collection for metformin concentration was done following time intervals: 0–4, 4–8, 8–12, and 12–24 h. The pharmacokinetics of metformin were compared between the MATE1 genotype groups (GG=3, GA=8, and AA=5). OCT2 genotype distribution was not significantly different in each group by Fisher's exact test (p=0.330). Metformin concentrations in plasma and urine were determined by LC/MS/MS.

Results
AUC and Cmax of metformin carrying rs2289669 GA genotype increased 11% for Cmax (GG vs GA, 1347±21 vs 1500±425 ng/ml, p=0.053) and 26% for AUC (GG vs GA, 7790±637 vs 9243±2758 ng/ml*hr , p=0.065), carrying AA genotype significantly increased 27% for Cmax (GG vs AA, 1347±21 vs 1706±209 ng/ml , p=0.031) and 37% for AUC (GG vs AA, 7790±637 vs 10641±2086 ng/ml*hr , p=0.095) compared to those of GG genotype. Renal clearance and secretion clearance of metformin was not significantly different by MATE1 genotype.

Conclusion
Our results suggested the association between the genetic polymorphisms of MATE1 rs2289669 and the pharmacokinetics of metformin.

THE SEQUENCING ERA: ARE PHARMACOGENETIC PROFILES BECOMING A REALITY?

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With the rapid development of sequencing technologies, vast amounts of data are being generated at affordable prices. In order for the field of pharmacogenomics to reap the benefits of this sequencing era, this excess of data will require appropriate analyses. Thus, careful analyses of the variation detected in genes of interest is necessary to allow for the possibility of personalised pharmacogenetic profiles to become a reality. To demonstrate this, we have taken data from the "bushman" data library (http://main.g2.bx.psu.edu/library), which contains the genetic variation observed in the genomes/exomes of thirteen individuals (four KhoiSan individuals, South African Archbishop Desmond Tutu, two Yoruba individuals, four Caucasians, including Craig Venter and James Watson, a Chinese individual and a Korean individual). From these data, we identified the variation present in the top ten PharmGKB pharmacogenetic genes by extracting a region corresponding to the "GP Gene Boundary", as specified by the database. Known functional variation was subsequently identified through the use of the *CYP* and *UGT1A1* allele nomenclature websites, in combination with data obtained from PharmGKB. Additionally, we performed Sorting Intolerant from Tolerant (SIFT) analyses to identify non-synonymous SNPs that were not described in the aforementioned websites. Together, these data were utilised to create pharmacogenetic profiles for all thirteen individuals.

From the results generated, we observed that each of the individuals possessed a unique combination of variation that has been reported to influence pharmacogenetic applications. When comparing the population groups, we noted that although there was consistently more variation present in African populations, certain variants appear to be population specific. Moreover, African individuals, specifically the Southern African individuals, exhibited far more novel/uncharacterised non-synonymous SNPs than non-African individuals.

In conclusion, the exciting developments in sequencing technologies need to be accompanied with careful analyses. It is important that the poorly characterised African populations, especially those residing in Southern Africa, benefit from these technologies which are uniquely suited to detect the high levels of sequence variation present in these populations. Lastly, due to the extensive nature of the sequencing data, these analyses can be extended to create a more complete picture for drug response.

ETHNICALLY DIVERSE INDUCED PLURIPOTENT STEM CELLS FOR PHARMACOGENOMICS

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There is extensive evidence showing that genomic variations affect drug toxicity and efficacy, but there is currently no practical means to screen candidate drugs in vitro for genome-associated toxicity early in the drug development pipeline. We are building an ethnically diverse panel of human induced pluripotent stem cell (hiPSC) lines that could be useful for early stage toxicology screens. To date, we have derived 25 iPSC lines from a multiethnic cohort of healthy individuals, including several Caucasians, African Americans, a Sudanese and a Yoruba individual. The Yoruba, from sub-Saharan Africa, is one of the original four ethnic groups analyzed by the worldwide haplotype mapping (HapMap) consortium.

We derived hIPSCs from fibroblasts and keratinocytes obtained from skin biopsies and hair samples (outer root sheath of hairs). These primary cultures were expanded for cryopreservation and transduced to induce pluripotency using four transcription factors. The iPSCs were subjected to a standard set of assays to confirm that they were typical pluripotent stem cells and that the exogenous reprogramming factors had been silenced. These assays included immunocytochemistry for pluripotency-associated proteins such as OCT4/POU5F1, RTPCR for the exogenous genes, and genome-wide gene expression profiling. SNP genotyping was used to examine genomic integrity, confirm the ethnicity of the cell lines, and identify the
CYP variants and HLA haplotype of the cells.

Since ninety percent of drug metabolism occurs in the liver, we are developing methods for directing differentiation of iPSCs into hepatocyte-like cells that can be incorporated into toxicity assays. We have differentiated iPSCs by a multi-step protocol, and have obtained cells that express typical hepatocyte markers, including ASGR, albumin and CYP1A2. Our goal is to test the potential of these cells for predictive toxicology screens.

TOWARDS INDIVIDUALIZATION OF PROSTATE CANCER TREATMENT: CHARACTERIZATION OF A MOTILITY-RELATED BIOMARKER

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We have shown that the tetraspanin CD151 has prognostic value in prostate cancer (PCa); patients whose cancer has low expression of CD151 have better prognosis than those with high levels1. We are now interested in CD151's role in PCa as a motility and metastasis promoter and whether inhibition of CD151 results in reduced progression of PCa. Human PCa cell lines LNCaP and PC3 were used in cell migration and invasion assays. The motility and invasiveness of wild-type LNCaP (low endogenous level of CD151) vs. CD151 transfected LNCaP cells and PC3 (high endogenous CD151) vs. CD151 knock-down PC3 cells (KD PC3) was analyzed. LNCaPs transfected with CD151 showed increased motility and invasion compared to control LNCaPs ($P<0.05$), while KD PC3 cells demonstrated reduced motility and invasion compared to control PC3s ($P<0.05$). Paired primary and secondary PCa generated using a SCID mouse model bearing implanted human PCa cell lines are also being examined for expression of CD151. More recently we have conducted in silico screening with compounds predicted to bind the large extracellular domain of CD151, and found that a number of these compounds possess in vitro and vivo properties in inhibiting PCa progression. Stratification of PCa patients with higher levels of CD151 may thus enable more tailored approaches to anti-metastatic therapeutics of this common and often unpredictable malignancy.

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GENETIC FINDINGS OF *P2Y12* SINGLE-NUCLEOTIDE POLYMORPHISMS AND THEIR INFLUENCES ON THE VARIATION IN ADP-INDUCED PLATELET AGGREGATION

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Although *P2Y12* has a significant role in normal hemostasis and thrombosis, no genetic study has been described about the association between *P2Y12* variants and the extent of ADP-induced platelet activation in the Korean population. The expression levels of two reference sequences of *P2Y12* mRNA transcripts (variants 1 and 2) were examined in the whole blood before direct DNA sequencing. The subjects were screened for single-nucleotide polymorphisms (SNPs) in *P2Y12* by direct DNA sequencing (n=50). Frequencies of *P2Y12* SNPs, linkage disequilibrium blocks, haplotype structures, and haplotype-tagging SNPs were determined. The effects of genetic variation in the *P2Y12* gene on the extent of ADP-induced platelet aggregation were studied in healthy Korean men (n=40). Variant 2 (NM 176876.1) was the predominantly expressed form in all subjects, but variant 1 was also weakly expressed in all cases (n=10). A total of 20 SNPs were identified: 2 in exons, 5 in introns, and 8 and 5 in the 5'-untranslated regions of the known *P2Y12* RNA variants 1 and 2, respectively. Genetic analysis of the P2Y12 SNPs and haplotypes revealed a statistically significant association between *P2Y12* haplotype, denoted H3, and an increase in the ADP-induced platelet aggregation response relative to that for the reference haplotype H1 ($P = 0.01$). Application of these findings to the development of a multivariate model might be useful in explaining the variable outcome of antiplatelet drug therapy in Asian populations.

INTERNAL AND EXTERNAL EVALUATION OF A PHARMACOMETRIC MODEL FOR WARFARIN USING PREDICTION CORRECTED VISUAL PREDICTIVE CHECK (PC-VPC)

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Objective: To evaluate the performance of a pharmacometric model for warfarin using PC-VPCs in an internal [1] and an external [2] dataset.
Background: Variability in both pharmacodynamics and pharmacokinetics contribute to the pronounced variability in individual warfarin dose requirement. We have developed a pharmacometric model for the relationship between warfarin dose and anticoagulant response (INR) based on data from 1426 Swedish warfarin patients [1]. Visual predictive check (VPC) is becoming an important diagnostic tool for pharmacometric model evaluation [3]. For data collected in studies with adaptive design as for warfarin, prediction corrected VPC (PC-VPC) is more suitable [4].
Method: The final model and parameter estimates obtained from analyses of the Swedish study [1] were used in the internal and external model evaluation. PC-VPCs were constructed for the median, 5th and 95th percentile of the observed data. Model predictions were based on 100 simulated datasets and presented with non-parametric 95% confidence intervals. The procedure was repeated on an external dataset derived from a British prospective warfarin study [2].
Results: The PC-VPC of the internal data did not indicate any major differences between observations and model predictions. The PC-VPC of the external data showed some evidence of model misspecification with signs of under prediction of INR, especially at INR > 3.
Conclusion: The external evaluation showed some evidence of under prediction of INR. The reason for this is currently unknown. Possible reasons for this finding include a difference in INR methods (Owren vs. Quick) and/or a difference in *in vivo* behaviour of warfarin tablets from Sweden and UK.
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POLYMORPHISMS OF SENSIBILITY GENES AND THE RISK OF RELAPSE OR SURVIVAL TO CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN KOREA

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Objective
In our previous genome-wide association study of childhood acute lymphoblastic leukemia (ALL), six single nucleotide polymorphisms (SNPs) in 4 genes (i.e., HAO1 rs6140264, EPB41L2 rs9388856, rs9388857, rs1360756, C2orf3 12105972, MAN2A1 rs3776932) were strongly associated with childhood ALL risk. In a subsequent study, we evaluated whether those SNPs are associated with recurrence or death of ALL among Korean children.
Methods
Incident childhood ALL cases (n=50) and non-cancer controls (n=50) frequency-matched by age and sex, were recruited from three teaching hospitals in Seoul between 2003 and 2008. The blood samples were genotyped using Affymetrix SNP Array 6.0 platform. ALL risks were estimated as odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression analysis adjusted for age and birth weight. The false discovery rate (FDR) was used for adjusting multiple tests.
For the selected SNPs that were strongly associated with childhood ALL risk, risk of ALL was estimated for the cases with or without recurrence or death, respectively. The difference of survival function between the two groups was evaluated by logrank sum and Wilcoxon-Gehan test.
Results
When we estimated the risk about the selected SNPs, the results showed decreased risk about SNP in MAN2A (OR=0.03, 95% CI = 0.00-0.35; OR = 0.15, 95% CI = 0.05-0.48; with and without, respectively), and increased risk in HAO1 (OR = 5.28, 95% CI = 0.93-30.11; OR = 8.14, 95% CI = 2.57-25.81; with and without, respectively) in both groups of cases. But SNP in C2orf3 showed decreased (OR = 0.11, 95% CI = 0.03-0.35) and SNPs in EPB41L2 showed increased risk (OR = 28.97, 95% CI = 5.34-157.20) in only cases that without recurrence or death. However, when the difference of survival function between two groups was estimated, the results were not statistically significant (P > 0.05).
Conclusion
Although we conducted subsequent study to indentify that whether previously selected SNPs would modify the recurrence or death of childhod ALL, the results were not statistically significant.
Keywords
childhood acute lymphoblastic leukemia (ALL), single nucleotide polymorphism (SNP), relapse, survival

BRAIN-DERIVED NEUROTROPHIC FACTOR GENE POLYMORPHISMS AND ESCITALOPRAM RESPONSES IN PATIENTS WITH MAJOR DEPRESSION

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Brain-derived neurotrophic factor (BDNF) is a candidate molecule for influencing the clinical response to antidepressant treatment, especially to citalopram, an antidepressant of selective reuptake inhibitors (SSRI) class. The aims of this study were to determine the relationship between the Val66Met polymorphism in the BDNF gene and the response to escitalopram, another SSRI antidepressant, in patients with major depressive disorder (MDD). Fifty seven Korean patients with MDD were enrolled and had treated with escitalopram at dose of 5–40 mg/day. Clinical symptoms were evaluated using the HAM-D scale at baseline and after 1, 2, 4, and 8 weeks of treatment. The genotype and allele frequencies were compared according to the response and remission status using multiple logistic regression and the difference of % decline of HAMD scores by treatment between genotypes was analyzed using type III generalized linear model. The proportions of M allele carriers were 81.6% and 83.8% in responders at 4 weeks and at 8 weeks, respectively, which were higher in non-responders at each corresponding time points (P=0.005 and P = 0.013). The percentile decrease of HAMD scores was significantly larger in M allele carriers than those in patients possessing VV genotype at 4 weeks of treatment (P = 0.019). In addition, the proportion of M allele carriers was 84.6% in remitter at 4 weeks, which was significantly higher than 64.5% in non-remitter (P = 0.038). These results suggest that BDNF V66M affect the therapeutic action of esciatlopram in MDD, and that genotypes of BDNF V66M may be a genetic marker for predicting the response to escitalopram treatment.

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DEVELOPMENT AND VALIDATION OF REAL-TIME PCR-BASED TPMT GENOTYPING METHOD FOR DOSE ADJUSTMENT OF THIOPURINE COMPOUNDS

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Background: TPMT (thiopurine S-methyltransferase) methylates and thus inactivates thiopurine drugs such as azathioprine, 6-mercaptopurine and 6-thioguanine, which are widely used to treat inflammatory bowel disease and childhood acute lymphoblastic leukemia. Genetic alterations in *TPMT* gene directly affect the metabolism of such drugs, causing side effects including bone marrow toxicity, hepatotoxicity and pancreatitis. PCR-direct sequencing method is a gold standard for *TPMT* genotyping, but it is time consuming and expensive. In Korean population, mostly *3C variants (c.719A>G) have been reported with incidence of about 0.3-7.6%, therefore full sequencing is not ecconomical for *TPMT* genotyping. Herein, we developed and validated a real-time PCR-based method for *TPMT* genotyping.
Methods: SNP genotyping methods were developed under the real-time PCR platform using allele specific primers and 5' nuclease probes. Three important SNPs for *TPMT* gene (c.238G>C, c.460G>C, and c.719A>G) were genotyped based on the differences in Ct (cycle threshold) in the real-time PCR system. Each reaction was confirmed by PCR-direct sequencing method. In addition, automatic interpretation algorithm was developed to display genotypes at each site.
Results: The validity of amplification reactions was assessed based on the difference in Ct (ΔCt) for the reactions targeting two different alleles at each SNP site. For each SNP site, homozygote specimens showed ΔCt greater than 2.0 and heterozygote specimens showed ΔCt smaller than 2.0. All these results were concordant with those confirmed by PCR-direct sequencing method. Overall turnaround time was around 2 hours and 24 genomic DNA specimens could be analyzed simultaneously.
Conclusions: We developed a real-time PCR based genotyping kit for *TPMT* using allele-specific primers and probes, which produced consistent results with those by direct sequencing method. This method is expected to be practically applied in the clinical practice to determine the individualized dose of thiopurine compounds.

CLINICAL CHARACTERISTICS AND HLA ANALYSIS OF ACUTE GENERALIZED EXANTHEMATOUS PUSTULOSIS

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Background: Acute generalized exanthematous pustulosis (AGEP) is an acute pustular eruption occurring usually after drug ingestion and resolves spontaneously after discontinuation. Drug-specific CD4+/CD8+ lymphocytes are known to play roles in skin lesions of AGEP, however, little is known about pathogenesis or genetic susceptibility of AGEP. Objectives: To characterize of clinical disease features of AGEP in Korean people and evaluate its association with HLA types. Methods: We reviewed AGEP cases retrospectively from the database of the Korean Pharmacogenetic Adverse Drug Reaction Research Network. Results: Twenty five patients were included with approximately equal gender distribution (male 52%) and a mean age of 46.8 years (18-83 years). The most common drugs were antimicrobials (50%), NSAIDs (21%) and anti-tuberculosis drug (18.4%), respectively. In 17 patients, mean latency was 5.2 days. Most patient recovered without any complication (81.2%). Systemic corticosteroid was administered in 70% of patients. HLA analysis were done in 20 patients and HLA-B*4402 and C*0501 alleles were found in 15% of AGEP patients, which was significantly frequent compared with Korean general population (2.5% and 0.6%, respectively) (Odd ratio 6.5 and 7.8, respectively, both P value< 0.05). Conclusion: Our data suggests HLA-B*4402 and C*0501 could contribute to the genetic susceltibility to AGEP.

IDENTIFICATION OF A NOVEL VARIANT OF CYP2C8, CYP2C8*11, IN ASIAN POPULATIONS AND ITS EFFECT ON THE ROSIGLITAZONE DISPOSITION IN VIVO

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The objectives of this study were to identify the genetic variants of CYP2C8, analyze CYP2C8 single nucleotide polymorphisms (SNPs), and characterize their functional consequences in the CYP2C8 substrate drug rosiglitazone in humans. The direct full sequencing of CYP2C8 genomic DNA was performed in a Korean population (n=50). A total of seventeen CYP2C8 variants including a novel coding variant (E274Stop) were identified. The novel CYP2C8 E274Stop variant was assigned as CYP2C8*11 by the Cytochrome P450 Nomenclature Committee. Seventeen SNPs were used to characterize linkage disequilibrium, haplotype structures, and haplotype tagging SNPs. Genotyping for CYP2C8*11 in an extended set of Koreans (n=400), Caucasians (n=100), Han-Chinese (n=348), Vietnamese (n=100), and African-American (n=93) was performed by a newly developed pyrosequencing method. The frequency of CYP2C8*11 was 0.3% in Koreans, 1% in Vietnamese, and 0.14% in Chinese. However, none of the Caucasians and African-Americans contained the CYP2C8*11 allele. Subjects with the CYP2C8*1/*11 exhibited a higher plasma concentration-time profiles of rosiglitazone compared to those of 9 control subjects carrying CYP2C8*1/*1. The AUC and Cmax of rosiglitazone in individuals carrying CYP2C8*1/*11 (n=5) were 54% and 34% higher compared to the mean values observed in the control subjects carrying CYP2C8*1/*1, respectively (P=0.015 and P=0.025, respectively). In summary, this is the first report to characterize the allele frequency and haplotype distribution of CYP2C8 in a Korean population, and provides functional analysis of a new variant CYP2C8*11. Our findings suggest that individuals carrying CYP2C8*11, a null allele found in Asians only, would have lower activity for metabolizing CYP2C8 substrate drugs.

ASSOCIATION OF GENETIC VARIATION IN TAMOXIFEN-METABOLIZING ENZYMES AND PLASMA CONCENTRATION OF TAMOXIFEN METABOLTIES

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Background: Tamoxifen metabolism is complex and involve N-demethylation, aromatic hydrocylation, side chain alpha-hydroxylation and N-oxidation. The cytochrome P450 enzymes (CYPs) are involved in the metabolism. Tamoxifen is further inactivated by sulphotransferase (SULT) 1A1 and UDP-glucuronosyltransferases (UGTs). N-Desmethyltamoxifen (NDM), a major primary metabolite of tamoxifen, is hydroxylated by CYP2D6 to yield endoxifen. Because of its high antiestrogenic potency, endoxifen may play an important role in the clinical activity of tamoxifen.
Method: We conducted a prospective trial in 65 patients with breast cancer who were treated with 20mg/d tamoxifen to further understand the the relationship between genotypes of these enzyme-encoding genes and the plasma concentration of tamoxifen metabolites. Genotyping for *CYP2D6*, *CYP3A4*, *CYP3A5*, *CYP2C9*, *CYP2C19*, *SULT1A1*, *UGT2B15* and *UGT2B7* were performed using DMET plus array (Affymetrix, CA, USA). Steady state plasma concentrations of tamoxifen and its metabolites were determined after 8 weeks of tamoxifen treatment, using liquid chromatography-tandem mass spectrometry.
Results: The median and range of plasma concentrations of tamoxifen, NDM, 4-hydroxytamoxifen, and endoxifen were 128.0 ng/ml (range, 56.8 - 289.0), 231.0 ng/ml (range, 97.4 - 623.0), 9.6 ng/ml (range, 2.6 - 19.9), and 25.5 ng/ml (range, 14.1 - 58.6), respectively. The mean endoxifen plasma concentration was significantly lower in *CYP2D6* IM genotype group than in those of EM genotype group (26.8 ng/ml versus 20.9 ng/ml, P = 0.01). Endoxifen/NDM plasma ratios were significantly different between these groups (P = 0.0015). NDM levels were affected by *CYP2C19* genotype groups. Homozygotes with *CYP2C19**2 or *3 and patients with *CYP2C19**2/*3 showed significantly higher NDM levels than wild type or heterozygotes (P = 0.01 and 0.02, respectively). Genetic polymorphisms of the other genes were not associated with tamoxifen metabolism.
Conclusion: *CYP2D6* genotype are highly associated with endoxifen plasma concentration and endoxifen/NDM ratios and may have an impact on the response to tamoxifen therapy. *CYP2C19* genotype may partially explain the wide inter-individual variations in the plasma level tamoxifen metabolite.

AUTOMATIC INTERPRETATION PROGRAM CAN FACILITATE WARFARIN GENOTYPING METHOD, MAKING FASTER & EASIER TO APPLY IN CLINICAL LABORATORY

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Background: Polymorphisms of the*VKORC1* (vitamin K epoxide reductase complex subunit 1) and *CYP2C9* (cytochrome P450 2C9) genes are the most important genetic factors related to dose adjustment of oral anticoagulant warfarin. Fast and reliable test method is essential for prompt dose adjustment of warfarin to prevent serious hemorrhagic or thrombotic complications. Previously, we developed a real-time PCR method for *VKORC1* and *CYP2C9* genotyping. However, interpretation of the real-time PCR results is not easy. The aim of this study was to develop and validate an automatic interpretation program for real-time PCR results.

Methods: Automatic interpretation logic was devised based on the difference in Ct (cycle threshold) and display tabs were added to visualize the interpreted results and all the reaction curves of each specimen. Validity of real-time PCR and automatic interpretation program was confirmed with the 28 clinical specimens whose genotypes had been previously confirmed by PCR-direct sequencing method. Real-time PCR was performed with previously developed kit (AccuPower® warfarin genotyping kit, Bioneer, Daejeon, Korea). Automatic interpretation results were compared with those determined by visual inspection of reaction curves and with those confirmed by PCR-direct sequencing method.

Results: The algorithm was devised in that difference in Ct smaller than 2 designates heterozygote and greater than 2 for homozygote. Also all the genotyping results were described in standard nomenclature and summarized in the reaction summary tab. In addition all the reaction curves for six SNP sites (*VKORC1* 3673A>G, 6484C>T, 6853C>G, 9041G>A; *CYP2C9* 430C>T, 1075A>C) were displayed in one screen, making visual confirmation of all the results possible. For all the specimens, automatic interpretation, visual inspection of real-time PCR and PCR-direct sequencing showed consistent results.

Conclusion: Automatic interpretation program can be a useful component of the real-time PCR genotyping system, making the genotyping for warfarin dose adjustment faster and more convenient.

ASSOCIATION BETWEEN MDR1 AND SIDE EFFECTS OF METHYLPHENIDATE IN KOREAN ADHD PATIENTS.

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Objectives: MDR1 plays an important role in the clearance of psychotropic drugs and their metabolites from brain tissues. This study aimed to discover the association between MDR1 genetic variations and OROS-methylphenidate (MPH) effects in Korean attention deficit hyperactive disorder (ADHD) patients.

Methods: Korean ADHD patients completed four week trial of OROS-MPH treatment. They were divided into two groups which are mild and severe side effect groups using Barkley stimulant drug side effects rating scale (SERS). SNaPShot analysis was performed to genotype several tag SNPs of MDR1 from ADHD patient subjects. Association with MPH taking ADHD patients was studied using statistical analysis. After that, MDR1 membrane vesicle ATPase assay was experimented for comparing rs******* allelic differences of MPH transporting activity.

Results: MDR1 tag SNPs were analyzed between mild and severe side effect groups of MPH taking ADHD patient subjects. Among tag SNPs, specific genotype of rs******* showed significant association with severe side effect of MPH (p=0.0028, OR=10.74) in the logistic regression analysis. Rs******* specific allele of MDR1 showed the least activity to transport MPH than other alleles by vesicle ATPase assay.

Conclusion: The specific genotype of rs******* in human MDR1 gene is associated with severe adverse reactions to OROS-MPH treatment in Korean ADHD patients.

PATHWAY-TARGETED PHARMACOGENOMICS OF CYP1A2 IN HUMAN LIVER

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The human drug metabolizing cytochrome P450 (CYP) 1A2, is one of the major P450 isoforms contributing by about 5-20% to the hepatic P450 pool and catalyzing oxidative biotransformation of up to 10% of clinically relevant drugs including clozapine and caffeine. CYP1A2 activity is interindividually highly variable and although twin studies have suggested a high heritability, underlying genetic factors are still unknown. Here we adopted a pathway-oriented approach using a large human liver bank (n=150) to elucidate whether variants in candidate genes of constitutive, ligand-inducible, and pathophysiological inhibitory regulatory pathways may explain different hepatic CYP1A2 phenotypes. Samples were phenotyped for phenacetin *O*-deethylase activity, and the expression of CYP1A2 protein and mRNA was determined. CYP1A2 expression and function was increased in smokers and decreased in patients with inflammation and cholestasis. Of 169 SNPs in 17 candidate genes including the CYP1A locus, 136 non-redundant SNPs with minor allele frequency >5% were analyzed by univariate and multivariate methods. A total of 13 strong significant associations were identified, of which 10 SNPs in the *ARNT*, *AhRR*, *HNF1α*, *IL1β*, *SRC-1*, and *VDR* genes showed consistent changes for at least two phenotypes by univariate analysis. Multivariate linear modelling indicated that the polymorphisms and non-genetic factors together explained 42%, 38% and 33% of CYP1A2 variation at activity, protein and mRNA levels, respectively. In conclusion, we identified novel trans-associations between regulatory genes and hepatic CYP1A2 function and expression, but additional genetic factors must be assumed to explain the full extent of CYP1A2 heritability.

GENERATING TETRACYCLINE INDUCIBLE MRP2 CELL-LINES

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Introduction: A novel technology termed "Screen and Insert" (ScIn) provide convenient and reproducible ways to isolate stable cell clones in which transgene expression is stringently regulated by tetracycline (tet). The key to ScIn is a powerful d2EGFP-based screen to identify optimal chromosomal loci combined with site specific recombination (SSR) to target the insertion of transgenes (Brough et al. 2007).
Methods and Material: We used multidrug resistance-associated protein 2 (MRP2), an efflux pump involved in the terminal excretion and detoxification of endogenous and xenobiotic organic anions, to evaluate this approach.
Results: Tet-transactivator expressing human fibrosarcoma HT1080 cells were transfected with a target construct expressing reporter gene (*d2EGFP*) from a tet-responsive promoter (TRP = TRE + CMVmin) using a *lox71* site positioned between the TRP and the reporter gene (Brough et al. 2007). A clone (Rht14-10) with stringently tet-regulated d2EGFP expression was selected for co-transfection with a Cre-expression plasmid and a promoterless MRP2-EGFP insertion construct which was linked to a *lox66* site positioned upstream of MRP2 (lox66-MRP2-EGFP.pEGFP-N1). Cre-mediated recombination between the insertion construct and the integrated target construct placed the MRP2-EGFP successfully under the control of the TRP. G418 resistant colonies were selected. For further analyses clones that had lost cytosolic d2EGFP expression showed a clear MRP2-EGFP fluorescence in the plasmamembrane with stringently tet-regulated MRP2-EGFP expression analysed by FACS and Western Blot. Clones of high purity as judged by PCR for correct insertion events were positive in a CMFDA and mBCl assay for functional MRP2-EGFP expression.
Conclusions: The ScIn approach described a highly effective way to achieve stringent and reproducible tet-regulated MRP2 gene expression in an HT1080 background. The method is particularly attractive for functional comparison of gene-variants.
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A CYP2C9 HAPLOTYPE EXHIBITED A POSSIBLE ASSOCIATION WITH INCREASED WARFARIN SENSITIVITY IN MECHANICAL HEART VALVE REPLACEMENT PATIENTS

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CYP2C9 single nucleotide polymorphisms (SNPs) are important in safe and effective oral anticoagulation with warfarin use.The objectives of this study were to determine the distribution of CYP2C9 variants in Koreans and investigate their association with warfarin dose requirements in patients who received mechanical heart valve replacements (MHVRs). All 9 exons, intron–exon junction, and promoter region of CYP2C9 were amplified and the amplified fragments were directly sequenced in 50 healthy normal Koreans. Additional direct DNA sequencing of the CYP2C9 gene was conducted in 36 of the 267 MHVR patients who required low maintenance warfarin doses without carrying CYP2C9*3 and VKORC1 1173T mutations. The effects of CYP2C9 genetics on warfarin maintenance dose was assessed in 267 MHVR patients. Thirty-nine SNPs including seven previously unidentified SNPs were identified in 50 Koreans by direct DNA sequencing. One of the CYP2C9 haplotypes exhibited an association with warfarin low dose requirement. This haplotype consisting of -1565C>T, -1188T>C, IVS3+197G>A, IVS3-334C>T, IVS3-65G>C, IVS4-115A>G, and IVS5-73A>G was found in 15% of 36 MHVR patients who required low warfarin doses, while 4% of 50 normal healthy subjects exhibited this haplotype. One of the SNPs comprising this haplotype, -1565C>T, apparently changed a protein binding pattern as observed in electrophoretic mobility shift assay. The haplotype including -1565C>T, -1188T>C, IVS3+197G>A, IVS3-334C>T, IVS3-65G>C, IVS4-115A>G, and IVS5-73A>G seems to be associated with lower warfarin dose requirement and this haplotype could be considered in the development of warfarin dose prediction model for Asian populations.

RESPIRATORY DEPRESSION FOLLOWING THERAPEUTIC ADMINISTRATION OF OPIOIDS IN THE OPERATING ROOM: AN OPIOID PATHWAY PHARMACOGENETIC ANALYSIS

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Background: Systemic approaches are needed to understand how variations in the genes associated with absorption, distribution, metabolism, elimination and response to opioids can be used to predict clinical outcome. We present 2 cases of life threatening opioid-induced respiratory depression in the operating room. *Case One:* The patient had severe respiratory depression following 2 mg of subcutaneous morphine on top of intrathecal morphine administered for a Cesarean section. The patient had a history of near apnea with one dose of codeine/acetaminophen (30mg/500mg respectively), but tolerated hydromorphone. *Case Two:* Life threatening respiratory depression occurred following epidural morphine given at standard doses for surgical removal of tumor. Post-operatively, the patient needed only 0.6mg total of IV hydromorphone over 4 days for pain management.
Methods: Functional candidate polymorphisms in genes involved in opioid metabolism and action pathway (*CYP2D6, UGT2B7, ABCB1, OPRM1, COMT*) were genotyped by using SNaPshot® and TaqMan® Drug Metabolism Genotyping assays or by amplifying and re-sequencing the corresponding genomic regions.
Results: *Case One:* Genotype results revealed this patient had an increased propensity to generate active metabolites from both codeine (extensive CYP2D6 activity) and morphine (increased UGT2B7 activity) while having a functional μ-opioid receptor system. These active metabolites are not generated with hydromorphone. *Case Two:* Collectively, this patient appeared to have increased exposure and overall sensitivity to morphine and hydromorphone. Decreased ABCB1 efflux transporter activity, in combination with low COMT activity associated with increased sensitivity of the μ-opioid receptor system may have predisposed the patient to this adverse outcome.
Conclusions: An opioid pathway pharmacogenetic approach along with clinical history may provide insight into severe respiratory depressive events in patients who received therapeutic doses of opioids and may be useful information to mitigate future adverse events.

ADIPONECTIN AND PPAR-Γ GENE VARIABILITY AND RISK OF CORONARY HEART DISEASE IN NORTH INDIAN POPULATION-A CASE-CONTROL STUDY

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Adiponectin (*Apm1*) is an adipocyte-secreted protein and its serum levels are decreased in obesity, type II diabetes, and coronary heart disease (CHD). Peroxisome proliferator-activated receptor-γ (*PPAR-γ*), a member of the nuclear hormone receptor family is involved in the differentiation of adipose tissue. Activation of the *PPAR-γ* improves insulin sensitivity and exerts anti-atherogenic effects. In the present study, we have examined the association of SNP 45 T/G of the *Apm1* and SNP P12A (C/G) of *PPAR-γ* gene in coronary heart disease patients (n=300) and their age matched controls (n=300). Genotyping was done by PCR and RFLP methods.
Results- For *Apm1*-45 T/G, the frequency of G allele was 14.6 in cases and 12.0 in controls, whereas for T allele 88.0 in controls and 85.3 in cases. 5.1 folds elevated risk of CHD was observed with the GG genotype (OR-5.1, 95% CI-1.45-17.81;p=0.002). With various risk factors, G allele of Apm1 was associated with increased risk of developing CHD in patients with smoking (OR-2.89, 95% CI-1.52-5.52;p<0.001), alcohol intake (OR-2.14, 95% CI-1.26-3.63;p<0.01), diabetes (OR-7.72, 95% CI-3.80-15.72;p<0.001), hypertension (OR-9.50, 95% CI-4.92-18.35;p<0.001), mental stress (OR-6.54, 95% CI -3.35-12.77;p<0.001) and familial history of CHD (OR-7.47, 95% CI 3.89-14.34;p<0.001). There was marginal association of G allele as a risk for CHD in patients with positive central obesity (OR-2.08, 95% CI-1.04-4.14;p=0.01).
For *PPAR-γ* P12A (C/G) SNP G allele frequency was 50.0 in controls and 37.5 in cases whereas for C allele 62.5 in cases and 50.0 in controls. The GG genotype of PPAR-γ was associated with the lower risk of development of CHD hence, has a protective effect against the CHD (OR-0.35, 95% CI-0.22-0.55;p<0.001). PPAR-γ A12 (G) allele also decrease the risk of CHD in patients with risk factors like smoking, diabetes, hypertension, mental stress and familial history of CHD. Significant protective effect of GG genotype was associated with central obesity (OR-0.35, 95% CI-0.15-0.82; p<0.01).
Conclusion- Our study showed that G allele of *Apm1*- 45T/G polymorphism is associated with elevated risk of development of CHD in North Indian population whereas,
G allele (alanine) of P12A (C/G) polymorphism in *PPAR-γ* gene is associated with low risk of CHD in north Indian population independently as well as along with various risk factors.
Key words SNP; atherosclerosis; CHD; adiponectin; - PPAR-γ

PERIOPERATIVE PHARMACOGENOMICS - A NEW FRONTIER FOR PERSONALIZED MEDICINE

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Adverse cardiovascular outcomes after surgery are common and a serious public health concern. Myocardial infarction (MI) is one of the leading causes of postoperative morbidity and mortality. Between 5-6% of patients with preexisting coronary artery disease (CAD) suffer from perioperative MI, which is associated with a 30-40% mortality rate.

One of the most widely accepted strategies to reduce perioperative MI in high-risk patients is the use of β-blockers (class I recommendation ACC/AHA 2007 guidelines). Beta-blockers block the adrenergic response and have been shown to reduce cardiovascular risk in many clinical trials. However, recent placebo-controlled trials questioned the safety of this intervention. The PeriOperative ISchemic Evaluation (POISE) trial found that perioperative β-blocker use prevented myocardial infarctions but at the expense of a 2-fold increased risk of death and nonfatal stroke.

Ample evidence exists that the efficacy and safety of β-blocker treatment is influenced by genetic factors, most prominently in the adrenergic signaling pathway and the cytochrome P450 2D6 (CYP2D6) enzyme which metabolizes most clinically used β-blockers.

Here, we present GENE-VISION, a substudy of the VISION (Vascular Events In Noncardiac Surgery Patients Cohort Evaluation) study [clinicaltrials.gov: NCT00512109]. VISION is an international, multicenter prospective observational cohort study with a targeted enrollment of 40,000 patients. As of Sept. 2010, 18,000 patients have been enrolled.

GENE-VISION will investigate of the association between β-blocker use, perioperative MI and genetic variation in the adrenergic signaling pathway and CYP2D6 enzyme, but more importantly establish a platform for future perioperative (pharmaco-)genomic research.

AGGREGATE EFFECTS OF GENETIC VARIANTS WITHIN PATHWAYS ON FLUCLOXACILLIN AND CO-AMOXICLAV INDUCED LIVER INJURY.

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Flucloxacillin and co-amoxiclav (a combination of amoxicillin and clavulanic acid, commonly known as Augmentin) are beta-lactam antibiotics. Despite their efficacy, and in the case of co-amoxiclav broad use, both can cause severe drug-induced liver injury (DILI), although with rare incidence. Recent genome-wide association studies (GWASs) have demonstrated an important role played by genetic risk factors in drug-specific predisposition of both flucloxacillin and co-amoxiclav-induced liver injury. However, the rarity of these adverse drug reactions limits the sample sizes that can be collected. This results in studies that are not adequately powered to discover genetic risk factors with moderate-to-low risk. If such variants are present in functionally related genes, for example belonging to a common biological pathway, they might have a detectable cumulative effect, allowing the identification of pathways enriched in SNPs with nominally significant association to DILI. We describe a novel approach to combine the GWAS results with gene pathway information to identify aggregated effects of genetic variations. We used data publicly available through the International Serious Adverse Events Consortium (flucloxacillin: 77 cases / 288 genetically-matched controls; and co-amoxiclav 212/561). Subjects were genotyped using Illumina 1M or 1M-Duo BeadChips. Tests of association were carried out for each SNP by logistic regression conditioned on known associated SNPs and adjusting for the top four principal components to account for population structure. Then, we paired SNPs to one or more neighboring genes on the basis of linkage disequilibrium (LD) with SNPs within those genes. After sorting genes based on the minimum p-value of their paired SNP(s), we evaluated the enrichment in higher-ranked genes for 200 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways using Gene Set Enrichment Analysis (GSEA). We found 4 and 22 pathways being significantly enriched (p-value < 0.05) for flucloxacillin and co-amoxiclav, respectively. The heparin sulfate biosynthesis pathway was shared in both analyses with consistent large odds ratios, suggesting a common genetic susceptibility to liver injuries caused by both drugs.

DEEP SEQUENCING *APOE* EXPOSES INADEQUACIES OF GENETIC TESTING

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Genotype to phenotype association studies provide one common approach to detect genetic variants associated with increased risk to disease or specific response to drug's efficacy or toxicity. While these studies are published in the thousands annually, inconsistent results among the studies are common. Discrepancies between association studies could be attributed to different confounding factors which include issues with sample size, statistical power, allelic heterogeneity and population structure.

APOE is associated with cardiovascular and Alzheimer disease and is a target for clinical genetic testing. Our analysis of novel deep DNA sequence data argues against the use of current *APOE* tests because it inadequately predicts disease risks. The present study deep sequences 3805 bases of the *APOE* gene containing 909 bases of promoter, 2755 bases of the structural gene and 141 bases of the 3'flanking region in an Oklahoma population of African descent. Included in these data are known functional variations within the Apolipoprotein E (*APOE*) coding region and its regulatory elements. Our study reveals previously undetected population structure at *APOE* between American communities of African descent, which questions previous treatment of this ethnic group as a single population in genetic association studies.

This study concludes that 1) admixed populations, such as African Americans, should not be characterized as a single genetic population, 2) the evidence for population structure, which may confound disease association studies, may only be observed when haplotype data are obtained, 3) deep sequencing and haplotype characterization is essential for disease association studies where allelic heterogeneity is a concern.

IMPROVED ASTHMA CONTROL WITH MONTELUKAST RELATIVE TO SALMETEROL IN ADRB2 ARG-16 HOMOZYGOUS CHILDREN WITH ASTHMA

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Background
Diminished efficacy of salmeterol for improving asthma control is increased in children with asthma homozygous for arginine-16(Arg16) allele of the *ADRB2* gene. Concerns have been raised regarding the efficacy and safety of long-term salmeterol use in patients with asthma. We investigated whether there is a genotype-specific difference in long-term asthma control with montelukast compared to salmeterol in individuals homozygous for Arg16 of *ADRB2*.
Methods
In this pragmatic randomised controlled trial, 62 children with asthma, carrying Arg/Arg16 genotype and exacerbation of asthma at least once within the previous year, were randomly assigned to receive Flixotide® (fluticasone propionate) via accuhaler (Diskus) dry powder inhaler device plus oral montelukast (Group I); or Seretide® (salmeterol plus equivalent dose of fluticasone) via accuhaler dry powder inhaler device plus placebo for montelukast (Group II). No effort was made to blind the prescribed inhaler. The primary end point was school absence, prospectively measured as individual events over the period of one year.
Results
No significant difference was observed in school absences (p=0.097) between the treatment groups. The use of reliever medication was significantly decreased in Group I compared to Group II (p= 0.004). Total exacerbations were reduced in Group1 compared to Group 2 (p=0.049). Self-reported symptoms were significantly improved in Group I compared to Group II (morning cough p=0.018; morning wheeze p=0.001; morning dyspnoea p=0.008; night wheeze: p=0.004; night dyspnoea: p=0.001). A significant improvement in quality of life as per the Juniper pediatric asthma quality of life questionnaire was observed in Group I compared to Group II (activity limitation score (p=0.004), symptom score (p=0.009), emotional function score (p=0.002)).
Conclusion
In individuals homozygous for Arg16 of the *ADRB2* locus, montelukast is an effective step up medication compared with salmeterol. Montelukast, as an asthma controller added on to inhaled steroid, improved asthma symptoms and quality of life, while reducing the use of reliever medication, in comparison to salmeterol. A larger randomised controlled trial is required, comparing asthma control with salmeterol versus montelukast in the genotypic sub-groups in *ADRB2*, and to explore the cost-effectiveness of genotype-specific controller therapies in children with asthma.

GENETIC PLOYMORPHISM IS RELATED TO DERMATOLOGIC ADVERSE DRUG REACTIONS OF ANTIEPILEPTIC AGENT.

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Antiepileptic drug-related adverse drug reactions (ADRs) are a major reason of discontinuation of treatment. Skin rash is one of these ADRs, which is showing mostly unpredictable and belongs to idiosyncratic type of ADRs. To find new genetic targets of dermatologic reactions to antiepileptic drugs, a whole genome-based SNP analysis was performed using Affymetrix 5.0 SNPchip containing 500,568 SNPs and samples from 132 patients treated with antiepileptic agents showing skin rash and those from another 132 patients showing no signs of dermatologic side effects. The SNP selection criteria were 1) minor allele frequency > 0.05, 2) genotyping success rate > 0.98, and 3) P > 0.001 in the Hardy-Weinberg Equilibrium test from QQ plot. The P values of association between these SNPs and antiepileptic agent-related skin rash were calculated in dominant, recessive, and co-dominant mode. A total of 21 SNPs showed with P values under 0.000001. Among these, 11 SNPs were in intragenic regions while the remaining 10 SNPs were in intergenic regions. The present study suggests that some genetic polymorphisms are highly related to the dermatologic side effects of antiepileptic agents. Our findings also encourage further studies, particularly confirmatory studies with larger samples, to validate and analyze the association between these SNPs and antiepileptic agent-related ADRs. (This study was supported by grant A030001 from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Korea)

METHYLATION DETECTION IN A MCF-7 CELL LINE USING ULTRA HIGH-THROUGHPUT BISULFITE-SEQUENCING WITH THE SOLID™ SYSTEM

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Epigenetic modification plays an important regulatory role in diseases such as cancer. While bisulfite sequencing is the most powerful method to study DNA cytosine methylation,approximately 99% of cytosine bases are converted to uracil thus creating essentially a 3 base bisulfite-converted genome that has less signature than a 4 base genome and is more difficult to map against with short reads. Traditional approaches to map the bisulfite-converted tag to both a bisulfite-converted reference and an unconverted reference can lead to loss of information when mapping reads with a moderate number of methylated cytosines. For example a read that has 6 methylated and 6 unmethylated bases would not map to either reference when mapped allowing up to 5 mismatches. In order to enable accurate mapping to the highly redundant bisulfite-converted genome,we have developed a mate-pair scheme in which only one mate-pair tag is bisulfite converted while the other tag remains unconverted. The adapters and non-converted tag are protected via incorporation of 5-methyl-cytosine,which is resistant to bisulfite conversion.We used this protocol to make a mate-pair library from a human MCF-7 cell line. The non-converted sequence provides an “anchor” in the genome and facilitates the identification and the methylation status of the bisulfite-converted tag. This approach reduces mis-mapping since the anchor sequence regulates the mapping of the bisulfite-converted tag and allows the non-anchored tag to tolerate more mismatches and thus map more of the moderately methylated tags and open a window to these previously difficult to obtain regions. This novel technique has the potential to provide a routine and reliable method for hypothesis free genome-wide methylation detection. Studying the role of methylation on gene expression will provide useful information on the role of epigenetic mutations in human breast cancer.
The SOLiD instrument is For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

OPIOID PATHWAY PHARMACOGENETIC ANALYSIS OF NEONATAL OPIOID TOXICITY FOLLOWING MATERNAL USE OF CODEINE DURING BREASTFEEDING

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Background: Codeine is globally one of the most widely used opioid analgesics for the treatment of mild to moderate pain. However, there are substantial interindividual differences in codeine response and toxicity. In North America codeine is routinely prescribed for maternal post-partum pain. It has been previously shown that maternal use of codeine during breastfeeding can be associated with central nervous system (CNS) depression in neonates. The identified genetic risk factors, CYP2D6-related ultra-rapid metabolism in combination with increased UGT2B7 activity, however, explained only 12% of the neonatal toxicity cases. We hypothesized that additional variations in genes involved in codeine and morphine metabolism and action pathway contribute to the risk of CNS depression in neonates whose mothers were using codeine and breastfeeding.
Methods: 26 mothers with infants exhibiting signs of CNS depression related to maternal codeine use during breastfeeding (cases) and 86 mothers of asymptomatic infants (controls) were identified. We genotyped 26 functional candidate polymorphisms in five genes involved in opioid metabolism and action pathway (*CYP2D6*, *UGT2B7*, *ABCB1*, *OPRM1*, *COMT*) by using SNaPshot®, TaqMan® Drug Metabolism Genotyping and Copy Number assays. Associations of single genetic markers, haplotypes, and non-genetic factors with the adverse outcome were investigated by regression and haplotype analyses.
Results: Extensive conversion of codeine into the pharmacologically active morphine and morphine-6-glucuronide metabolites (p=0.02) and decreased activity of the efflux transporter ABCB1 (p=0.0001) were significantly associated with neonatal CNS depression. Maternal codeine dose was also significantly higher in cases as compared to controls (p=0.004).
Conclusions: Genetic and non-genetic factors can be used to predict response to codeine and to prevent potentially life-threatening CNS depression.

ANALYSIS OF GENOTYPE-BASED WARFARIN DOSING APPROACHES AND THEIR OUTLIERS

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Various genotype-based dosing algorithms and tables are available to predict personalized doses for the anticoagulant drug warfarin. These dose prediction methods take into account whether a patient has variants in the CYP2C9 and VKORC1 genes that affect warfarin sensitivity, as well as other patient characteristics, such as age, sex, and body mass. Our goals were to: 1) compare the accuracy and properties of six such genotype-based algorithms and a genotype-based dosing table to each other and to 5 mg fixed-dose and empirical approaches to dosing, and 2) characterize the outliers resulting from inaccurate predictions. Residual blood specimens from a hospital clinical laboratory were obtained from male patients (n = 150) on stable maintenance doses of warfarin. DNA was extracted and genotyped for variants of the CYP2C9, VKORC1 and CYP4F2 genes. Clinical/demographic information and actual (i.e.empirical) warfarin initiation doses were obtained from the electronic medical record. Warfarin doses were predicted by the genotype-based approaches and compared retrospectively to the patients' actual stable maintenance doses. The accuracy of the predicted doses was assessed by a variety of measures. We found that the strength of correlation (R^2) between actual and predicted stable maintenance doses varied and fell into three tiers: 0.56-0.58 (for four algorithms), 0.41-0.47 (for two algorithms and the dosing table), and 0.10 (for empirical dosing). No genotype-based dose approach was better than empirical dosing in predicting starting doses within 0.5 mg of the stable maintenance dose. However, most genotype-based approaches increased the percentage of patients with starting doses within 1 mg and within 2 mg of the stable dose, and reduced the percentage whose doses differed by 2 mg or more from the stable dose, i.e. large outliers. All genotype-based approaches unexpectedly created new large outliers, but eliminated more than they created. Two algorithms yielded more large outliers than the others; one predominantly underestimated the doses (88%; 28/32), while the other predominantly overestimated (83%; 25/30). About 5% (8/146) of patients were large outliers with all algorithms. A CYP4F2 variant and less common CYP2C9 variants (*5, *6, and *11) did not account for most outliers. Some dosing approaches were more effective for low-dose CYP2C9-VKORC1 genotypes, others for intermediate dose genotypes, while all were equally effective for high-dose genotypes. Our results reveal that several genome-based dosing approaches outperform the others.

GENETIC VARIATION OF MEMBRANE-BOUND CATECHOL-*O*-METHYLTRANSFERASE AND SUSCEPTIBILITY TO SCHIZOPHRENIA AND ANTIPSYCHOTIC INDUCED ABNORMAL INVOLUNTARY MOVEMENTS IN AN AFRICAN POPULATION

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The catechol-*O*-methyltransferase (*COMT*) gene is an attractive candidate for schizophrenia-susceptibility as it encodes a catabolic dopamine enzyme and is located in an area of the genome that has been linked to schizophrenia on numerous occasions. The enzyme exists as two distinct isoforms, with the membrane bound protein (i.e. MB-COMT) being predominantly expressed in the brain. Since African populations remain underrepresented in psychiatric genetic and pharmacogenomic research, we performed a genetic association study on this gene in the local Xhosa population with regards to susceptibility to schizophrenia and antipsychotic induced abnormal involuntary movements.

The spectrum of common DNA sequence variation in the *MB-COMT* P2 promoter was determined by resequencing 15 Xhosa schizophrenia samples. Fourteen candidate single nucleotide polymorphisms (SNPs) were selected by means of a literature search and *in silico* analyses. Polymorphisms were subsequently genotyped in a cohort of 238 Xhosa schizophrenia patients and 240 healthy Xhosa controls. Additionally, samples were screened for deletions of the *COMT* gene locus using duplex real-time PCR. Statistical analyses were performed with regards to schizophrenia-susceptibility and the presence of abnormal involuntary movements, including tardive dyskinesia.

Two SNPs in the vicinity of the *MB-COMT* P2 promoter were significantly associated with schizophrenia-susceptibility reduction in the Xhosa cohort. These polymorphisms were in high linkage disequilibrium and have previously been associated with increased gene expression *in vitro*, which could possibly prevent excessive amounts of dopamine accumulating in the prefrontal cortex. No gene deletions were detected in the Xhosa population. The data generated by this study indicate that genetic variation of *MB-COMT* could be associated with schizophrenia-susceptibility in the local Xhosa population and may therefore be one of the genomic loci contributing towards schizophrenia-susceptibility in the South African community.

NOVEL VKORC1 MUTATIONS IDENTIFIED IN WARFARIN RESISTANT PATIENTS

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Warfarin is the most commonly used anticoagulant, yet it remains a very challenging and problematic drug to prescribe due to its narrow therapeutic index and large interindividual variability in dosing requirements. Genetic polymorphisms in two genes, *CYP2C9* and *VKORC1*, have been shown to explain over 35% of warfarin dose variability. Several studies have described VKORC1 missense mutations in patients resistant to warfarin who required higher warfarin doses. To identify rare mutations in the *VKORC1* gene in our cohort of 70 warfarin resistant patients, sequencing of the VKORC1 promoter and exonic regions was performed on the Applied Biosystems 3730 sequencer with big dye terminator cycle sequencing kit. Warfarin resistance was defined as the requirement of >10mg warfarin per day for at least three weeks in the absence of any comedication with enzyme inducers and evidence of adherence. Four novel mutations have been identified and one of these is located in the VKORC1 promoter region: -160G>C. To test whether this promoter variant could lead to changes in VKORC1 promoter activity, DNA from patients with the -160 GG or GC genotype was PCR-amplified, cloned into the pGL3 vector and transfected into HepG2 cells. Dual luciferase assay revealed that the VKORC1 promoter with the C allele had an 18% increase in activity when compared with the G allele. This increase in promoter activity may explain why the patient with the -160 GC genotype required higher warfarin doses. Further functional work is currently underway to elucidate the roles of the other novel mutations identified.

THE GENETIC CONTROL OF DRUG RESPONSES

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Although pharmacogenetics has, to date, made less of a contribution to the personalization of drug use than many had predicted, recent progress strongly suggests that we will soon witness a significant acceleration of clinically relevant pharmacogenetic discoveries. Here I first review our experience of studies of the genetic bases of variable response to treatment of hepatitis C. I next use this experience to outline appropriate discovery strategies for a variety of pharmacogenetic questions and attempt to identify important discovery opportunities.

A FOUR STEP GENOMIC APPROACH TO IDENTIFY PREDISPOSITION TO CARBAMAZEPINE-INDUCED HYPERSENSITIVITY SYNDROME IN CAUCASIAN PATIENTS

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Carbamazepine (CBZ) can lead to various forms of hypersensitivity ranging from maculopapular exanthema to severe blistering reactions. A strong association has been demonstrated between CBZ-induced Stevens Johnson Syndrome (SJS) and the HLA-B*1502 allele on Chromosome 6 in Han Chinese, but not in Caucasians. However, to date no association with HLA has been demonstrated for carbamazepine-induced hypersensitivity syndrome (also known as DRESS) in either Caucasians or Chinese patients. In the present study, we have investigated genetic susceptibility to carbamazepine-induced DRESS in 4 steps: 1) we performed genotyping for 1293 SNPs across the MHC region on chromosome 6 using the Illumina MHC SNP mapping panel and BeadArray technology; 2) we undertook a genome-wide association study using the Illumina 1.2M SNP chip to investigate whether other MHC and non-MHC gene variants are implicated in hypersensitivity reactions to CBZ; 3) conducted imputation analysis to identify any other SNP associations; and 4) performed sequence based-high resolution HLA-typing to identify the HLA marker.
In total 2913 patients and controls were included in the study. Twenty one patients developed DRESS. Two control groups comprised CBZ-tolerant patients (n=43) and WTCCC healthy individuals (n=2849). All patients and controls were Caucasians. SNP genotype frequencies in patients and controls were compared with frequencies in the HapMap, dbSNP and WTCCC databases. Data analysis was performed using the BeadStudio, Haploview and PLINK software packages.
Logistic regression analysis in the MHC showed an association between CBZ-induced DRESS and several SNPs in the coding region of HLA-A (p=4.7E-6). Genome-wide mapping showed a convincing association with several SNPs in the same region of the MHC, with genome wide significance (10-9). HLA-A sequence-based typing in patients and CBZ-tolerant controls showed a strong association with one HLA-A allele (OR 25.8 (95% CI 3.0 to 226.4, p=0.0003).
We have identified, in a multiple step approach, an association between one HLA-A allele and CBZ-induced DRESS in Caucasians, the first time this has been done in this ethnic group and with this phenotype. Functional studies are being performed to establish whether the HLA marker is the causal variant.

GENETIC RISK PROFILE PREDICTING ANTHRACYCLINE-INDUCED CARDIOTOXICITY IN CHILDREN

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Background Anthracycline-induced cardiotoxicity (ACT) is a serious adverse drug reaction of cancer treatment limiting its use as an effective chemotherapeutic agent and contributing to substantial morbidity and mortality in childhood cancer survivors due to subsequent cardiomyopathy. High cumulative doses are known to increase cardiotoxicity risk, though genetic factors are also likely to be important given the high inter-individual variability in tolerated doses.
Methods We carried out a comprehensive genetic association study using approximately 3,000 single nucleotide polymorphisms (SNPs) in 220 key drug biotransformation genes in an initial cohort of 156 children treated with anthracyclines in Vancouver, with replication in a second cohort of 188 children treated in pediatric oncology units across Canada as part of the Canadian Pharmacogenomics Network for Drug Safety (CPNDS).
Results We identified a highly significant association of ACT with a coding variant in a transporter gene ($P = 1.0\text{x}10^{-4}$). We found further evidence ($P < 0.01$) for association with risk and protective variants in other genes including several other transporters. Combining these multiple variants with important clinical risk factors into a single predictive model, we were able to classify patients into 3 risk groups. The positive predictive value in the high risk group was 75% with almost 40% of patients developing serious cardiotoxicity within the first year, 24% of whom went on to develop congestive heart failure (CHF). In contrast, very few patients in the low risk group experienced ACT, none of whom developed CHF, with a negative predictive value of 96%.
Conclusions We have identified multiple genetic variants associated with ACT. Combined with clinical risk factors, they can be used to identify high-risk patients who can then be provided with safer, alternative treatment options.

CNVS IN ASSOCIATION STUDIES

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Copy number variants (CNVs) account for a major proportion of human genetic polymorphism and it has been suggested that they may play an important role in genetic susceptibility to human disease. The WTCCC undertook a large, direct, genome-wide study of association between CNVs and 8 common diseases. Using a purpose-designed array we typed ~19,000 individuals into distinct copy number classes at over 3,000 CNVs, including an estimated 50% of all common CNVs larger than 500 base pairs. We will discuss some of the experimental and analytical challenges in CNV studies and sources of artefactual associations, along with the results of the study. Although we saw some instances of CNV associations with the diseases studied, common CNVs in general, and those showing association in our study, tend to be well-tagged by SNPs, and we conclude that common CNVs that can be typed on existing platforms are unlikely to be a major source of the missing heritability for common diseases. There would seem to be analogous, or even more formidable, challenges in performing reliable inferences for disease association at rare CNVs.

PROGNOSTIC IMPACT OF INHERITED GENETIC VARIATIONS IN SRD5A AND ANDROGEN INACTIVATING UGT2B GENES IN PROSTATE CANCER AFTER PROSTATECTOMY.

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Background. The relationship between genetic variations in androgen biosynthesis (SRD5A) and inactivating (UGT2B) genes and the risk of biochemical recurrence (BCR) after prostatectomy remains an unexplored area.

Methods. We studied a cohort of 526 men with organ-confined and locally advanced cancer with a median follow-up of 7.4 years. A total of 19 htSNPs distributed across the SRD5A 1 and SRD5A 2 genes were studied as well as copy number variations in UGT2B17 and UGT2B28 genes. htSNPs were selected with a strategy to maximize gene coverage and to reflect adequately the Caucasian haplotype genetic diversity. The htSNPs strategy allowed us to study 109 genetic variations in both SRD5A genes. Each genetic variation found to be associated with BCR was further analyzed by Kaplan-Meier analysis and Cox regression model.

Results. After adjusting for all clinico-pathologic risk factors, we found a strong association between the risk of BCR and 7 SNPs in SRD5A genes. The combination of 2 SNPs respectively in SRD5A 1 and SRD5A 2 were highly favourable, reducing drastically the risk of BCR for carriers of 3-4 alleles (HR=0.34; 95% CI=0.18-0.64; $P=9x10^{-4}$). Other variations specifically in the SRD5A 2 gene were associated with an increased rate of BCR, namely the coding SNPs rs523349 ($V^{89}L$) with a HR of 2.12 (95% CI, 1.21-3.75; P=0.009) and reaching a relative risk of 4.97 when combined with deleted copies of UGT2B genes (95% CI, 2.38–10.36; $P=2x10^{-5}$). BCR-free survival rate was reduced to 27% in patients with this combination of unfavourable genotypes compared to 75% for patients with favourable genotypes ($P=7x10^{-6}$).

Conclusion. Inherited polymorphisms in the SRD5A and UGT2B genes are independent predictors of biochemical recurrence after radical prostatectomy. With further validation, these findings may ultimately help refine our ability to identify individuals at low or high risk of cancer relapse after RP, beyond known prognostic variables, and for whom a more personalized approach might optimize outcome, especially in the era of 5α-reductase inhibitors therapy.

INHERITED GENETIC VARIATION IN *FZD3*, *EPHA5* AND *FGD4* AND PACLITAXEL (P) INDUCED PERIPHERAL NEUROPATHY (PN): RESULTS FROM A GENOME-WIDE ASSOCIATION STUDY (GWAS) IN CALGB 40101

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CALGB 40101 is an ongoing Phase III trial comparing the efficacy of standard therapy cyclophosphamide and doxorubicin (AC x 4 or 6 cycles) with single agent paclitaxel (P x 4 or 6 cycles) as adjuvant therapy for breast cancer in women with 0-3 positive axillary lymph nodes. AC and P are associated with significant toxicities, with peripheral neuropathy being the major toxicity of P. As part of this study, we have collected germline DNA for a pharmacogenetic GWAS to identify genetic predictors of toxicity. Germline DNA was isolated from 1040 patients on the P arm of CALGB 40101 and genotyped using the Illumina 610-Quad platform. Following QC analysis, genotypes were available for 520,679 SNPs. A principal components analysis identified 859 genetic Europeans that were the focus of this analysis. Since both the timing and degree of toxicity are important clinical considerations, sensory PN was modeled using two complementary approaches. A time to event approach defined "event" as first Grade 2 or higher sensory PN and "time" as the total prior drug exposure. The maximum grade of sensory PN occurring during treatment or follow-up was modeled using ordinal logistic regression and total prior drug exposure as a covariate. One SNP surpassed the level of genome-wide significance and three of the other top ten hits were in genes implicated in congenital or experimental PN. Patients carrying a variant *FZD3* allele had a decreased risk of P-induced PN ($p=3.1x10^{-9}$). *FZD3* is a *Wnt* signaling receptor affecting axonal growth and guidance. Variant ephrin receptor (*EPHA5*) and ephrin binding protein (*GRIP1*) alleles were also associated with increased risk of P-induced PN ($p=9.6x10^{-7}$, $4.3x10^{-6}$). Risk alleles were identified in two genes implicated in congenital PN, *FGD4* ($p=2.6x10^{-6}$) and *NDRG1* ($p=5.2x10^{-5}$). These findings are further supported by a significant enrichment for SNPs annotated in neuronal development pathways. Heritable variation in *FZD3*, *EPHA5*, *GRIP1*, *FGD4* and *NRDG1* may modify a patient's risk of developing P-induced sensory PN. If these findings are replicated, the ability to identify patients at risk of this common toxicity could influence the choice of adjuvant breast cancer therapy.

GENOME-WIDE SCAN IDENTIFIES GENETIC PREDICTORS OF SURVIVAL IN NON-SMALL CELL LUNG CANCER PATIENTS TREATED WITH PLATINUM-BASED CHEMOTHERAPY

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Background: Inter-individual variation in genetic background may influence response to chemotherapy and overall survival for advanced stage non-small cell lung cancer (NSCLC) patients.
Methods: To identify genetic variants associated with overall survival in these patients, we conducted a genome-wide scan to identify variants associated with survival for 327 advanced stage Caucasian NSCLC patients who received platinum-based chemotherapy with or without radiation at MD Anderson Cancer Center. A fast track replication was performed for 315 patients from the Mayo Clinic followed by a second validation at the University of Pittsburgh in 420 cases enrolled in the Spanish Lung Cancer Group PLATAX clinical trial.
Results: A SNP in the chemokine-like receptor 1 (*CMKLR1*), rs1878022, was identified as significantly associated with overall survival. It conferred a 1.59-fold increased risk of dying (95% CI: 1.32-1.92, $P = 1.42x10^{-6}$) in the MD Anderson discovery population. This highly significant increase in risk was replicated in the PLATAX clinical trial (HR: 1.23, 95% CI: 1.00-1.51) and in combined Mayo Clinic and PLATAX validation (HR: 1.19, 95% CI: 1.03-1.37). Meta-analysis of these three patient populations demonstrated a highly significant 1.32-fold increase in risk (95% CI: 1.18-1.48, $P = 1.42x10^{-6}$). Another candidate SNP, rs10937823, was significant in the MD Anderson and Mayo Clinic populations with a combined risk increase of 1.82-fold (95% CI: 1.42-2.33, $P = 1.73x10^{-6}$), but did not replicate in the PLATAX clinical trial (HR: 0.96, 95% CI: 0.69-1.35).
Conclusions: These results may have significant clinical potential in helping to achieve personalized chemotherapy for NSCLC patients.

PHARMACOGENOMICS OF TAMOXIFEN AND RALOXIFENE IN BREAST CANCER PREVENTION: GWAS AND FUNCTIONAL GENOMIC LINK OF A SNP SIGNAL TO *BRCA1* AND *BRCA2*

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Introduction: NSABP P-1 and P-2 are the two largest trials of tamoxifen and raloxifene for breast cancer prevention. We performed a genome-wide association study (GWAS) with DNA from subjects in P-1 and P-2 who developed breast cancer inspite of SERM therapy to identify SNPs and genes associated with the development of breast cancer in this setting.
Methods: A nested, matched case-control GWAS was performed that included 592 cases who developed invasive breast cancer or ductal carcinoma *in situ*, matched with 1171 controls who did not. GWAS genotyping with the Illumina 610-Quad chip and *CYP2D6* genotyping were performed. Functional genomic studies involved siRNA knockdown and overexpression in cultured cells, followed by qRT-PCR and Western blot analyses.
Results: 547,356 SNPs were used in the GWAS analysis, with correction for population stratification. The top 10 SNPs with p-values that ranged from 2.12E-06 to 7.28E-06 on chromosomes (Chr) 3, 4, 9, 13, and 16 were identified by conditional logistic regression—with rs8060157 on Chr 16 having the lowest p-value (OR=0.70, MAF in controls =0.47). Following imputation and fine mapping, functional genomic studies focused on five Chr 16 SNPs (p-values 2.12E-06 to 3.70E-06), all in *ZNF423*, a gene encoding a putative zinc finger protein. Incubation of U20S cells stably transfected with estrogen receptor (ER) α with 0.1 nM estradiol (E2) showed that expression of *ZNF423*, *BRCA1* and *BRCA2* was induced by estrogen exposure. However, in lymphoblastoid cell lines stably transfected with ERα, estrogen-dependent expression of all 3 genes occurred only with wild type (WT), but not variant ZNF423 SNP sequences, while blockade of ERα with ICI-182,780 or with 4-hydroxytamoxifen prevented induction with WT sequences, but resulted in allele dose-dependent increases in *BRCA1* and *BRCA2* expression after ER blockade in cells with variant SNP sequences—results compatible with the "protective" effect of the variant SNPs during clinical SERM therapy.
Conclusions: This GWAS of subjects enrolled in the NSABP P-1 and P-2 trials identified SNPs associated with risk for the development of breast cancer in high risk women treated with SERMs. The SNPs identified in *ZNF423* were associated with differential E2-dependent induction of *ZNF423*, *BRCA1* and *BRCA2*. These common *ZNF423* SNPs were associated with both reduced breast cancer risk during SERM therapy and with differential estrogen-dependent expression of *BRCA1* and *BRCA2*.

ASSOCIATION OF VARIANTS IN THE SELECTIN E PRECURSOR (*SELE*) WITH ADVERSE OUTCOMES IN THE INTERNATIONAL VERAPAMIL SR-TRANDOLAPRIL STUDY GENETIC SUBSTUDY (INVEST-GENES)

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BACKGROUND: We sought to identify novel genetic markers for treatment related outcomes in patients with hypertension and coronary artery disease (CAD).

METHODS: The Illumina Custom Cardiovascular Array, which contains about 50K SNPs in candidate genes and pathways for cardiovascular, inflammatory, and metabolic phenotypes, was genotyped in 1460 patients with hypertension and CAD from the INVEST-GENES which randomized patients to an atenolol-based β blocker (BB) strategy or verapamil SR-based calcium channel blocker (CCB) strategy with trandolapril and/or hydrochlorothiazide added if necessary for BP control. Primary outcome was defined as first occurrence of death, nonfatal myocardial infarction (MI) or nonfatal stroke. Initial association analysis was performed in each treatment strategy with PLINK, under an additive genetic model. Treatment interaction analyses were performed with SAS.

RESULTS: 45,319 SNPs met quality control procedures and were retained for analysis. Three SNPs in *SELE*, selectin E precursor, were among the top hits in the CCB strategy. SNPs rs3917410, rs3917452, and rs5361 were associated with the primary outcome ($P=3.32x10^{-5}$, $3.54x10^{-5}$, and $3.77x10^{-5}$, respectively, with Odds Ratio (OR) (95% Confidence Interval (CI)) = 2.46 (1.61-3.76), 2.44 (1.60-3.74), and 2.44 (1.60-3.73), respectively). All three SNPs are in high LD. rs5361 encodes for a missense mutation (Ser128Arg), and the Arg128 allele has been previously associated with higher risk for CAD in multiple populations. However, rs5361 showed no evidence of association with the primary outcome in the BB strategy (P=0.99, OR (95% CI) = 1.21 (0.66-2.20)). After examining SNP x treatment interaction, a significant risk was observed in patients with one or two copies of the Arg128 allele in the CCB strategy (interaction P=0.008).

CONCLUSIONS: While these findings require replication, these results suggest that in addition to the previous associations with CAD, rs5361 in *SELE* influences cardiovascular outcomes in a treatment specific manner in patients with hypertension and CAD. It is unclear whether CCBs increase risk in carriers of the Arg128 allele, or if the allele's risk is reduced by β-blocker treatment.

CAGI: THE CRITICAL ASSESSMENT OF GENOME INTERPRETATION, A COMMUNITY EXPERIMENT TO EVALUATE PHENOTYPE PREDICTION

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The Critical Assessment of Genome Interpretation (CAGI) is a community experiment to evaluate computational methods for predicting the phenotypic impacts of genomic variation. Participants will be given unpublished genetic variants and will make predictions of resulting phenotype. These predictions will be objectively assessed against experimental characterizations. The long-term goal for CAGI is to improve the accuracy of phenotype and disease predictions of rare variants in clinical settings.

CAGI, which is based on the framework of the long-running Critical Assessment of Structure Prediction (CASP), will entail four phases:
* Unpublished associations of genotypes with molecular, cellular, or organismal phenotypes will be collected by the organizers from experimental and clinical labs
* Participants will make computational predictions of phenotypes from provided genotypes
* Experimental and clinical scientists will assess predictions
* A community workshop will be held to disseminate results and evaluate our collective ability to make accurate and meaningful predictions.

From this experiment, we expect to understand the diversity of mechanisms of genome variation, identify bottlenecks in genome interpretation, inform critical areas of future research, and connect researchers from diverse disciplines whose expertise is essential to methods for genome interpretation. Preliminary data sets we have been offered include enzymatic activity of human metabolic enzymes, segregation of rare variants identified in from resequencing in cancer cases and controls, phenotypic impact of variants of a checkpoint protein, clinical phenotypes associated with complete genomes, and molecular mechanisms underlying GWAS disease associations. For more information, see http://genomecommons.org/cagi/.

UTILIZING A NOVEL ADME GENOTYPING PANEL TO IDENTIFY DRUG METABOLISM PHENOTYPE/GENOTYPE CORRELATIONS IN HUMAN LIVER SAMPLES

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In order to address the inter-individual variability observed in the pharmacokinetic response to many medications, we have created a broad-based custom genotyping panel that interrogates variation present in ~180 key genes involved in the Absorption, Distribution, Metabolism and Excretion (ADME) of many therapeutic agents. The assay consists of ~3,000 SNPs and is composed of two types of variation, published markers that alter gene function (~360 SNPs) and haplotype tagging markers that define blocks of linkage disequilibrium across ADME genes. After several phases of development and optimization, the panel has a conversion rate of close to 100% with the flexibility to add additional marker content. Moreover, the accuracy of the panel has been validated using ~50 HapMap samples of known genotype and genotyping results in 24 samples have been compared across two commercial ADME products; DMET-Plus (Affymetrix) and Veracode ADME Core Panel (Illumina). To test the utility of the ADME genotyping assay in a research project, we screened 150 extensively characterized liver samples from the Institut für klinische Pharmakologie (IKP) in Stuttgart. The livers have been previously tested using several genomic approaches such as whole-genome genotyping, gene and protein expression and selected ADME enzymatic activity. Additionally, there is also extensive clinical and demographic information for each sample including disease status and concomitant medications. To date we have identified a considerable number of statistically significant genotype/phenotype associations between our ADME genotyping results and gene expression (eQTLs). Furthermore, we have reproduced the identification of several previously published eQTLs for our ADME genes. We are currently testing some prioritized targets for further functional characterization. In addition, we were able to make correlations between ADME genes variation, ADME protein expression and ADME enzymatic activity. With the demonstration of the usefulness of our panel to identify new and previously identified phenotype/genotype correlations in the IKP liver samples, we believe that our ADME panel would have broad applicability to any study or clinical trial that would benefit from the evaluation of an extensive list of ADME genes.

HERITABILITY OF CHEMOTHERAPEUTIC-INDUCED APOPTOSIS

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Chemotherapeutic-induced cell cytotoxicity as measured by cell growth inhibition in HapMap lymphoblastoid cell lines (LCLs) has been shown to be heritable and amenable to genetic dissection. As a result, LCLs have served as a model system to identify genetic variants associated with chemotherapeutic toxicity in patients. However, cytotoxicity is a broad phenotype encompassing both apoptotic and non-apoptotic avenues of cell death. Apoptosis is considered a more refined phenotype that may be a better representation of cell death for some tissues (and some tumors). To evaluate whether chemotherapeutic induced apoptosis is heritable and how this phenotype compares to cytotoxicity, we measured caspase 3/7 activity along with cell growth inhibition in LCLs derived from Caucasian monozygotic twins, siblings and unrelated individuals. Drugs included platinum agents, cisplatin and carboplatin; antimetablites, Ara-C, capecitabine, pemetrexed; and a mitotic inhibitor, paclitaxel. Apoptosis was evaluated 24 hours following exposure to increasing concentrations of each drug. Among these six drugs, paclitaxed induced the greatest degree of apoptotic response followed by Ara-C with capecitabine and pemetrexed producing the lowest apoptotic response among the six drugs. Interestingly, chemotherapeutic induced apoptosis was significantly correlated with cell growth inhibition at low concentrations of Ara-C, capecitabine and paclitaxel but not higher concentrations of these three drugs implying that the route of cell death to these three drugs treatment may vary depending on the drug concentration with higher concentrations going through non-apoptotic pathways. This is consistent with our data demonstrating genetic variants differ for high versus low concentrations of drug. The variance between each twin set (intra-twin variation) was significantly lower than the variance among the unrelateds across all six drug treatments. For example, the variance among the twins after 20μM capecitabine treatment is only 28% of the variance among the unrelated. This suggests that anticancer agents induced apoptosis is a heritable trait. Our results demonstrate the value of evaluating apoptosis as a separate phenotype in the identification of genetic variants associated with chemotherapy.
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EUKARYOTIC TRANSCRIPTOMES: COMPLEX, MULTIFUNCTIONAL, COMPARTMENTALIZED AND ELEGANT

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Deep sequencing and tiling array analyses of transcriptomes obtained from various cells and tissues of multiple organisms reveal that information stored in DNA sequences is complex, layered and compartmentalized. It is complex based the multiple types of novel RNAs continuing to be identified and that serve specified functions. Genetic information is also layered because within a given length of genomic sequence multiple RNAs can be produced serving different functional roles. Finally, it is compartmentalized because cells shuttle specific RNAs to designated compartments within cells after they are made. This compartmentalization provides an added level of control of expression and function. Multiple examples of RNAs that illustrate these characteristics of complexity and multifunctional layering will be described. One example is the transcripts enriched within sub-compartments within the nucleus of cells. These RNAs have specific properties and their isolation speak to a poorly understood mechanism of post transcriptional regulation of expression. Their functional roles will be discussed.

POLYMORPHISMS OF THE ASPARAGINASE PATHWAY AND CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA OUTCOME

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Due to the introduction of effective combination risk-adapted therapies the treatment of pediatric acute lymphoblastic leukemia (ALL) has greatly improved in the past four decades. Nevertheless, therapy resistance in a significant number of children is a major obstacle to successful treatment whereas intensive treatment has also important drug side effects. Pharmacogenetic studies that have been conducted in ALL identified genetic variations in several drug pathways that may contribute to variability in treatment responses. Asparaginase (ASP) which depletes cells of essential amino acid asparagine is one of the important components of ALL treatment. Clinical studies have shown a direct relationship between outcome and dose intensity. Nevertheless, a number of patients fail to respond well to treatment with ASP. Several studies conducted in vitro have shown that asparagine synthetase (ASNS), an enzyme that is critical to the biosynthesis of asparagine, plays a role in mediating drug resistance. The data obtained by microarray profiling using ASP-sensitive and -resistant ALL cells pointed to several other proteins that may, in addition to ASNS, be of relevance to resistance to ASP treatment. ATF5 stimulated ASNS expression in response to asparagine starvation and the increase in expression of arginosuccinate synthase 1 (ASS1) was also found. No data are yet available whether polymorphisms in these genes may affect the outcome of childhood ALL. Here we report the analysis of the polymorphisms in ASNS, ATF5 and ASS1 genes and their association with ALL outcome. Eleven polymorphisms located in the regulatory and coding region of these genes were analyzed in 270 children diagnosed with ALL and CHU Sainte Justine, Montreal and treated with the Dana-Farber Cancer Institute ALL Consortium protocols DFCI 87-01, 91-01, 95-01 or 2000-01. The association between obtained genotypes and event free survival (EFS) and overall survival (OS) was performed. Additionally, the same analyses were done following stratification by the treatment protocol given the differences in the type of ASP and administered dose. After the correction for multiple testing, C231T in ATF5 showed highly significant association ($p<0.001$) with reduced EFS in ALL patients treated with DFCI 91-01 protocol. The C231T substitution is located in 5'UTR of ATF5 gene, potentially affecting level of expression. We are currently testing promoter activity in relation to the alleles of this polymorphism.

THE DIHYDROPYRIMIDINE DEHYDROGENASE GENE AS A MAJOR PREDICTOR OF SEVERE 5-FLUOROURACIL TOXICITY: A CLASSIC REBORN?

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Dihydropyrimidine dehydrogenase (DPD) is the key enzyme in the catabolism of 5-fluorouracil (5-FU), one of the most commonly prescribed chemotherapeutic agents worldwide. However, the importance of polymorphisms in the DPD gene (DPYD) for the prediction of severe toxicity in 5-FU based chemotherapy has been under debate. Early studies estimated that around 25% of 5-FU related toxicities are caused by genetic variants in DPYD, whereas subsequent studies suggested that the deleterious mutations known so far only accounted for a small proportion of severe 5-FU related adverse effects, and thus were unsuitable as makers for general pre-treatment testing. Recently, however, we identified two novel DPYD-haplotypes (HapB3 and HapB6) containing only synonymous and non-coding mutations being significantly associated with severe 5-FU toxicity in 111 cancer patients. One of these haplotype (HapB3), has recently been found to contain a deep intronic mutation (c.1129–5923C>G), which affects pre-mRNA splicing and leads to reduced DPD activity. Here, we report the results of a genetic analysis of DPYD in an extended population of 243 cancer patients receiving 5-FU-based chemotherapy. The significant association of HapB3 with severe toxicity was confirmed in the extended data set ($p = 0.0018$) with a HapB3 frequency of 9.3% in 43 patients with grade ≥3 toxicities, and 1.8 % in the remaining patients ($n = 200$). This is in agreement with a recently reported retrospective analysis of 203 cancer patients showing that the HapB3 associated c.1129–5923C>G splice mutation was significantly enriched in patients with severe 5-FU toxicity (9.1%; 66 patients) compared to patients without toxicity (2.2%; 137 patients). Overall, we found toxicity-associated DPYD variants (including IVS14 +1 G>A, HapB6 and c.1679T>G) in 10 (23 %) out of 43 patients with grade ≥3 toxicities, while the prevalence of these variants was only 4.5% in patients without toxicities. Remarkably, both cases with lethal toxicity were either homozygous for HapB3 or carrier of the rare HapB6. These results suggest that genetic variants in DPYD represent important pharmacogenetic markers for severe 5-FU toxicity and that their application in pre-treatment screening needs to be re-considered in the light of the discovery of additional functionally relevant DPYD variants located outside the coding regions.

CANCER PHARMACOEPIGENETICS: GENES AND DRUGS

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The recent unmasking of genetics lesions in tumors has optimized cancer treatment regimens, specifically with the identification of the presence of c-ERBB2/neu oncogene amplifications, the BCR-ABL translocations and EGFR mutations. It is predicted that in coming years, the hypermethylation patterns of particular genes will also predict response to specific treatments. The most promising epigenetic candidates to predict pharmacoepigenetic response are the DNA repair genes undergoing epigenetic inactivation in tumors, such as the 06-methylguanine DNA methyltransferase (MGMT), the mismatch repair gene hMLH1, the Werner gene (WRN) or the breast cancer susceptibility gene BRCA1. In healthy tissues these enzymes are responsible for repairing the DNA damage that occurs during our lifetime and prevent the formation of mutations and other type of genomic damage. However, in cancer cells they become our terrible foes because they repair the DNA damage induced by many used chemotherapy agents, thus, generating chemoresistance to drugs. However, there is another side to this story: these DNA repair genes undergo hypermethylation-associated silencing in a fraction of human tumors that progress with a mutator pathway phenotype, but this is also an Achilles' heel because these hypermethylated-malignancies will not be able to repair the DNA damage caused by the chemotherapy agent. With the emerging epigenomic technologies we now have the techniques that will help address the DNA methylation chemoprofiles in an unbiased manner, and complete the promising pharmacoepigenetics landscape.

AROMATASE INHIBITOR PHARMACOGENOMICS: FUNCTIONAL GENOMICS OF TCL1A GWAS SIGNAL FOR MUSCULOSKELETAL ADVERSE EVENTS

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Background: Aromatase inhibitor (AI) therapy of estrogen receptor (ER) positive breast cancer greatly reduces disease recurrence. However, up to 50% of post-menopausal women treated with AIs experience new or worsening muscle and joint pain (musculoskeletal adverse events, MS-AEs), and approximately 8% discontinue therapy because of this drug reaction. In a previous case-control genome-wide association study (GWAS) of DNA samples from the NCIC-CTG-NCI MA.27 adjuvant therapy trial of AIs in early breast cancer, we identified four SNPs on chromosome 14 that were associated with MS-AEs (Ingle et al., J Clin Oncol, in press). Those SNPs had p-values that ranged from 2.23E-06 to 6.67E-07 and mapped near the 3'-end of the T-Cell Leukemia 1A (*TCL1A*) gene. Therefore, we set out to study the possible role of TCL1A in mediating musculoskeletal pain during AI therapy.
Methods: Functional genomic studies in pursuit of this GWAS SNP signal included qRT-PCR using RNA from U2OS cells stably transfected with ER(α) or ER(β), siRNA knockdown of TCL1A and other genes, qRT-PCR and Western blot analyses, and dual luciferase reporter assays to study NF-kB transcriptional activity.
Results: We showed that TCL1A expression was increased by estrogens and regulated expression of the interleukin 17 receptor (IL-17RA), IL-17 and NF-kB transcriptional activity—all markers for joint inflammation. Increased TCL1A expression was also associated with the variant SNP genotypes in estradiol-treated lymphoblastoid cells stably transfected with ER(α). Finally, exposure to estradiol, followed by ER blockade with ICI-182,780, resulted in greatly increased NF-kB transcriptional activity. These studies have linked the variant SNP sequences near *TCL1A* to drug effect and clinical phenotype, in both cases through TCL1A.
Conclusions: Our case-control GWAS of DNA samples from a large AI clinical trial identified 4 SNPs on chromosome 14 located near the 3'-end of *TCL1A* that were associated with AI-dependent MS-AEs. In the present functional experiments, we found that estrogen regulates TCL1A and that TCL1A regulates IL-17RA and IL-17 expression and suppresses NF-kB transcriptional activity. These results provide a mechanistic pharmacogenomic explanation of risk for the occurrence of a clinically important adverse drug reaction. They may also provide insight into estrogen-dependant mechanisms for musculoskeletal pain.

CORRELATION OF ACTIVE TAMOXIFEN METABOLITE LEVELS WITH GENOTYPES OF DRUG METABOLIZING ENZYMES IN PATIENTS WITH EARLY BREAST CANCER

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Background: The formation of active metabolites during tamoxifen (Tam) treatment of early breast cancer is anticipated to be critical for drug efficacy. Plasma levels of (Z)-4-hydroxytamoxifen (4-OH-Tam) and (Z)-endoxifen (endoxifen) are highly variable and this is attributed to polymorphisms of drug metabolizing enzymes. Clinical studies showed a strong relationship between cytochrome P450 2D6 (CYP2D6) genotypes and tamoxifen outcome. Translation of these findings into clinical practice however requires guidelines to personalize endocrine treatment of early breast cancer. Therefore, it is important to establish the correlation of steady-state tamoxifen metabolite plasma levels and genotypes and moreover, identify other factors potentially contributing to this variability.
Methods: Postmenopausal patients with ER positive breast cancer were recruited within an ongoing observational clinical trial (German Study ID 456) for the identification of endocrine treatment predictors. Patients were treated with 20 mg/d Tam. We quantified Tam, desmethyl-Tam, endoxifen, 4-OH-Tam, and 19 other phase I and phase II metabolites by rapid resolution LC-MS/MS in plasma of 236 patients. Patients were genotyped for CYP2D6 *3, *4, *5, *6, *7, *8, *9, *10, *41; CYP2C19 *2, *3, *17; CYP2C9 *2, *3; CYP 3A5 *3; CYP 2B6 *6; UGT1A4 *2; UGT 2B7 *2; and UGT 2B15 *2 by Taqman allelic discrimination and copy number assays.
Results: There was an allele-dose dependent relationship between reduced-function/deficiency CYP2D6 alleles and endoxifen levels ($p<10^{-16}$). Median steady-state endoxifen concentrations were 77.0 nM, 30.8 nM, 13.4 nM and 9.0 nM for UM, EM, IM, and PM, respectively. 5 of 9 EM patients had endoxifen levels similar to those of PM patients (2 of these reported co-medication with CYP2D6 inhibitors). With respect to CYP2C9, carriers of reduced function alleles showed decreased plasma levels of endoxifen and 4-OH-Tam. No correlations were found for CYP2B6, CYP2C19, and CYP3A5 polymorphisms.
Conclusions: Endoxifen plasma concentrations correlate with genotypes and therefore, the CYP2D6 genotype can be used to predict expected plasma concentrations. The phenotype prediction benefits from comprehensive allele coverage and this should be useful in treatment outcome prediction. CYP2C9 genotype and co-medication with CYP2D6 inhibitors together with other yet unknown factors may also play a role.
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EXPLORING THE RELATIONSHIPS BETWEEN GENETIC VARIANTS WITHIN THE UGT1A LOCUS, CELLULAR DETOXIFICATION AND RISK OF BLADDER CANCER

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A recent genome-wide association study (GWAS) for urinary bladder cancer (UBC) has identified multiple novel genetic risk factors (Rothman et. al, in press). One of these factors was an intronic single nucleotide polymorphism (SNP) rs11892031 located within the UGT1A locus on chr 2q37. The human uridine 5' diphosphate (UDP)-glucuronosyltransferases (UGTs) belong to a superfamily of proteins that represent the major biochemical mechanism of cellular defense and detoxification of diverse endo and exotoxins that can function as carcinogens. UGTs transform these compounds into water-soluble glucuronides that are eliminated from the body via bile and urine. Genetic variants within UGT1A locus associated with decreased activity of UGT enzymes have been previously associated with increased risk of different cancers and toxicity of cancer drug irinotecan. UGT1A locus represents a cluster of 9 coding and 2 non-coding genes that share exons 2-5 and have unique but highly similar exons 1 that define substrate specificity. Due to the complexity of the region and ~90-95% similarity between exon 1 sequences of UGT1A genes, this region is poorly represented in public databases (HapMap, 1000 Genomes, dbSNP). To ensure specificity of detection, we generated long-range amplicons and sequenced all UGT1A exons in HapMap individuals (CEU) and in 44 bladder cancer patients and identified 25 non-synonymous coding variations. All these variations are being genotyped in 1000 bladder cancer cases and 1000 controls. Our preliminary analysis identified a risk haplotype of several variations previously associated with altered detoxification and risk of other cancers, and a novel protective haplotype. Both haplotypes are specific for bladder cancer patients with previous/current history of smoking, suggesting that UGTs play a significant role in detoxification of tobacco-borne carcinogens and risk in bladder cancer.

FUNCTIONAL GENOME-WIDE ASSOCIATION STUDIES OF CHEMOTHERAPEUTIC RESPONSE IN AFRICAN POPULATIONS

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Chemotherapeutic agents are used in the treatment of many cancers, yet variable resistance and toxicities among individuals limit successful outcomes. Understanding the contribution of genetics to chemotherapeutic response is key to maximizing drug efficacy and minimizing adverse side effects. We used HapMap lymphoblastoid cell lines to measure chemotherapeutic-induced cytotoxicity in populations of African descent for which there is a disparity in the number of pharmacogenomic research studies conducted. We performed genome-wide association studies (GWAS) in Yoruba (YRI) and African American (ASW) populations and identified SNP predictors of cisplatin, carboplatin, capecitabine, ara-C, and pemetrexed-induced cytotoxicity. Traditional GWAS test single markers and are powered to detect SNP associations with relatively strong effects, but are unable to detect multiple smaller effects working together. We found that the chemotherapeutic susceptibility SNPs in the YRI population are enriched for expression quantitative trait loci (eQTLs, $p<10^{-3}$). Most of these are *trans* eQTLs and thus simply examining the region directly surrounding a SNP that associates with a phenotype of interest for possible functional genes is inadequate. The SNP may be affecting many genes across the genome and one gene may be affected by many SNPs across the genome. We used a novel approach called functional GWAS (fGWAS) that combines multiple SNPs that affect the activity of each gene into one gene-level test for association with chemotherapeutic response. The functional SNP set for a particular gene included any nonsynonymous SNPs within the gene and eQTLs associated with the gene in *cis* or *trans*. fGWAS in the YRI resulted in the genome-wide significant association of 234 genes with cisplatin IC_{50}, 81 genes with capecitabine AUC, 64 genes with carboplatin IC_{50}, 47 genes with pemetrexed AUC, and 13 genes with ara-C AUC ($p<10^{-6}$). Similar fGWAS are ongoing in the ASW. Importantly, by the nature of fGWAS, functional information for how these associated genes may be acting to affect phenotype is already known and will drive future studies of their mechanisms in chemotherapeutic response.

RADIATION PHARMACOGENOMICS: NOVEL BIOMARKER IDENTIFICATION AND FUNCTIONAL GENOMICS

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Background: Radiation therapy is used to treat half of all cancer patients. Response to radiation therapy varies widely among patients. Several clinical factors are known to influence radiation response, including radiation dose, volume and fraction, but it is also known that genetic inheritance can play an important role in variation in radiation response. Therefore, we performed a genome-wide association study (GWAS) to identify biomarkers to help predict radiation response using 277 ethnically defined human lymphoblastoid cell lines (LCLs).
Methods: Basal gene expression levels and 1.3 million genome-wide SNP markers from both Affymetrix and Illumina platforms were assayed for all 277 human LCLs. MTS assays for radiation cytotoxicity were also performed to obtain area under the curve (AUC) as a radiation response phenotype for use in the association studies. Functional validation of candidate genes, selected from an integrated analysis that used SNP, expression and AUC data, was performed with multiple cancer cell lines using specific siRNA knockdown, followed by MTS and colony-forming assays. Further mechanistic studies were also performed for two candidate genes.
Results: 27 loci, each containing at least 2 SNPs within 50kb with p-values $<10^{-4}$, were associated with radiation AUC. 270 expression probe sets were associated with radiation AUC with $p<10^{-3}$. The integrated analysis identified 50 SNPs in 14 of the 27 loci that were associated with both AUC and the expression of 39 genes that were also associated with radiation AUC ($p<10^{-3}$). Functional validation using siRNA knockdown in multiple tumor cell lines showed that *C13orf34* (*BORA*), *MAD2L1*, *PLK4*, *TPD52* and *DEPDC1B* each significantly altered radiation sensitivity in at least 2 cancer cell lines. Preliminary mechanistic studies for two candidate genes, *TPD52* and *Bora*, demonstrated novel functions of these two genes. TPD52 is involved in regulation of the NFκB pathway through the regulation of IKKγ ubiquitination, while Bora is involved in regulation of DNA damage and repair pathways.
Conclusions: In this study, we identified and functionally validated five novel candidate radiation responsive genes. Studies performed with LCLs can help to identify novel biomarkers that might contribute to variation in response to radiation therapy and enhance our understanding of mechanisms underlying that variation.

COST-EFFECTIVENESS OF PHARMACOGENOMICS: CHALLENGES AND OPPORTUNITIES IN AN ERA OF DECREASING TEST COST

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Payer reimbursement policies for pharmacogenomic (PGx) technologies are variable, but often do not include coverage because of stated lack of evidence of clinical utility. However, evidence thresholds may change as the average cost per actionable test result decreases and the possibility of 'preemptive' PGx information becomes a possibility. The strengths and weaknesses of current evidence and cost-effectiveness frameworks will be presented, and novel approaches using value of information analyses, with consideration of PGx vs. non-PGx drug information, will be discussed. Specifically, development of recommendations for inexpensive tests that fall in the traditional 'insufficient evidence' category will be evaluated.

RETURN OF INDIVIDUAL GENOMIC RESEARCH RESULTS:PERSPECTIVES OF IRBS

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Background: It is no longer hypothetical that genomic technologies will increase the likelihood that clinically relevant results will be obtained in the course of research. This coupled with the NIH GWAS policy suggesting that researchers and IRBs consider developing a plan to manage this occurrence has moved the controversial issue of return of individual research results (ROR) to the forefront of pharmacogenomic research.
Methods: In-depth interviews were conducted with 31 IRB members and staff at 6 US sites. Interviews were conducted in person or by phone, audio-recorded, and transcribed with individual identifiers removed to protect privacy. Coding and content analysis of interview text was performed independently by two analysts and compared for consensus. In depth analysis was conducted by team review.
Results: IRB members and staff see returning individual genomic research results as a complex issue, especially due to the fast paced, rapidly changing nature of the research and their lack of familiarity with genomic research. Positions on the issue of ROR were often expressed as being context dependent, where variables such as disease type and validity and utility of the result both now and in the future must be considered. Conditions for returning a genomic result favored a clinical utility perspective, where results should have clinical significance, be obtained in a CLIA approved lab, be life threatening, and be medically actionable. A personal utility perspective, based on respect for the research subject and opportunity for non-medical interventions was also considered by some as a condition for return. All participants expressed the need to honor a subject's right to know and right not to know results. Participants expressed different views on the IRB's role in ROR: active involvement or oversight of the process of decision-making.
Conclusions: In the context of genomic research, a complex web of tensions and thresholds for uncertainty exist among different IRB members and staff. ROR blurs the line between genomic research and clinical care and thus IRB responsibility related to this issue. This is complicated by the varying levels of knowledge/comfort with reviewing such protocols. Consideration of these findings can inform the development of policy to guide IRBs and researchers in decision-making related to ROR.

PHARMGKB: FROM KNOWLEDGE ACQUISITION TO CLINICAL APPLICATIONS

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The Pharmacogenomics Knowledge Base (PharmGKB: http://www.pharmgkb.org) began in 2000 as a repository of pharmacogenetic and pharmacogenomic primary data and simple associations between drugs and genes found in the literature. PharmGKB was one of the first resources of its kind, and it remains a pre-eminent resource in the field. PharmGKB's mission has evolved over the last ten years to reflect the changing field of pharmacogenomics. We now focus on pharmacogenomic knowledge acquisition and the application of this knowledge to the clinical arena.

Knowledge acquisition entails aggregating and curating pharmacogenomics information, primarily in the form of drug-centered pathways, important pharmacogene summaries and detailed annotations of the relationships among genetic variation, drugs and diseases. Pathways and gene summaries are peer-reviewed and published routinely in the journal *Pharmacogenetics and Genomics*. Our group has developed custom annotation tools that capture complex pharmacogenomic relationships, including statistical measures of study significance and study population parameters, in a structured manner. We also continue to develop automated and semi-automated methods for knowledge curation.

PharmGKB now pursues clinical applications for the pharmacogenomics knowledge we curate. Such applications include personal genome annotations and practical guidelines for pharmacogenomics in the clinic. We also organize research consortia and facilitate the sharing of data across research groups. The International Warfarin Pharmacogenetics Consortium (IWPC), the International Warfarin Pharmacogenetics Consortium – Genome Wide Association Studies (IWPC-GWA) and the International Tamoxifen Pharmacogenomics Consortium (ITPC) are examples. PharmGKB is currently developing several other consortia, including a knowledge-centric consortium. The Clinical Pharmacogenetics Implementation Consortium (CPIC) uses well-defined criteria to evaluate the evidence for clinical applications of pharmacogenetics and intends to publish corresponding clinical guidelines.

PharmGKB has already used its manually curated database of drug-genetic variant associations to annotate the human genome. Over 1600 variant annotations from PharmGKB were screened for quality of evidence and an MD determined the clinical relevance of the annotations. PharmGKB is now working to build an automated pipeline of human genome annotation.

INFLUENCE OF GENETIC VARIANTS OF OATP1B1 ON STATIN DRUG EFFICACY- RESULTS FROM A POPULATION BASED SURVEY

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OATP1B1, a liver specific transporter facilitates uptake of several drugs in clinical use including statins. The identification of frequent naturally occurring single nucleotide polymorphisms (SNPs) with effect on transport activity was basis of in vivo studies. Various authors could show that reduced hepatic uptake translates into significantly higher exposure with substrate drugs. In accordance OATP1B1 polymorphisms were identified as predictor of statin induced myopathy.
In order to evaluate the impact of OATP1B1 polymorphisms on pharmacodynamics of statins we employed a population-based survey. The study of health in Pomerania (SHIP) gathered clinical parameters of 3576 individuals, a total of 191 individuals with dyslipidemia during the first recruitment (SHIP-0) were treated with statins in the 5-year follow-up (SHIP-1). In this treatment group individuals harbouring the 521C variant exhibited an 11.6% and 12.6% lower reduction of LDL- and total cholesterol plasma levels, respectively. Stratification for compounds revealed a genotype effect on simvastatin and pravastatin efficacy, while atorvastatin appeared to be independent of OATP1B1. A second measure for statin efficacy was the likelihood to achieve ASC-target cholesterol levels. 377 individuals treated with statins in SHIP-1 were included in this analysis. In the case of simvastatin and pravastatin treatment those individuals harbouring the 521C allele were less likely to achieve the treatment goals. Again, no such genotype effect was detectible in patients treated with atorvastatin.
In conclusion, genetic variants of OATP1B1 are associated with changes in statin efficacy in a general population. Importantly the genotype effect profoundly varies for individual statins.

PHARMACOGENETICS OF DIABETES DRUGS: CANDIDATE GENES ENCODING DRUG TRANSPORTERS AND METABOLISM ENZYMES

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Background:
Considerable variation exists in type 2 diabetes patients' response to common Oral Hyperglycaemic Agents (OHA), which include metformin, sulphonylureas and thiazolidinediones (TZDs). Pharmacokenetic studies in healthy volunteers have established that genetic variants in OHA transporter genes (eg. SLC22A1 for metformin and SLCO1B1 for TZDs) and metabolism enzyme genes (eg. CYP2C8 for TZD and CYP2C9 for sulphonylureas) can affect the disposition of these drugs, hence change the exposure levels. Whilst effort is being channelled into hypothesis free genome wide association studies of OHA response, candidate gene studies of these drug transporters and key metabolism enzymes still have considerable merit. We aim to explore whether these genetic variants have an impact on OHA response in clinical practice.

Methods
Data was obtained from 8000 type 2 diabetes patients in the Go-DARTS cohort. As a measure of response we determined failure to achieve a treatment target of HbA1C<7%, in around 3000, 2000 and 850 patients for metformin, sulphonylureas and TZDs treated patients respectively. We assessed the associations between response and genetic variants in CYP2C8, CYP2C9, SLC22A1 and SLCO1B1 by logistic regression analysis with adjustment for appropriate covariates such as dose, duration, and adherence.

Results
Among sulphonylureas treated patients, those homozygote for CYP2C9 *2 or *3 alleles were 3.4 times (p=0.0009) more likely to achieve the treatment target than wild type carriers. No association was observed between SLC22A1 variant 420del/R61C and metformin response. Among TZD treated patients, those with two copies of the gain of function CYP2C8 *3 or *4 allele were 1.4 times (p=0.04) more likely to fail the treatment target whilst those with one or more copies of the SLCO1B1 *5 and *15 haplotypes were more likely to achieve the treatment target than wild type carriers (p=0.03). This result is of importance due to conflicting *in vitro* and *in vivo* evidence regarding the role of SLCO1B1 in the disposition of rosiglitazone and pioglitazone.

Conclusions
Using data from this large type 2 diabetes cohort, we have shown that genetic variants in OHA transporters and metabolism enzyme genes influence the treatment efficacy in clinical practice. The effect sizes observed in this study also suggest that even larger samples are required for genome wide association studies to overcome the multiple test penalty and unequivocally demonstrate the genetic effects in these genes with such strong functional candidacy.

ACCURACY OF PHARMACOMETRIC *A PRIORI* AND *A POSTERIORI* DOSE PREDICTIONS OF WARFARIN

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Objective: To evaluate the accuracy of a pharmacometric *a priori* and *a posteriori* warfarin dose prediction model [1].
Background: Warfarin therapy is challenging due to its narrow therapeutic range and pronounced variability in individual dose response. We have developed a NONMEM model for the relationship between warfarin dose and INR response, which includes CYP2C9 and VKORC1 genotype, and age [1]. Six published dosing algorithms for *a priori* dose predictions were recently compared [2], and the IWPC [3] and Gage [4] algorithms were found most accurate.
Method: Warfarin patients enrolled in a prospective UK study [5,6] and who met all of the following criteria were included in the evaluation dataset: a target INR of 2.0-3.0, achieved a stable, therapeutic warfarin dose (three consecutive INRs within target at same daily dose), information on CYP2C9 and VKORC1 genotype, sex, age, weight, height and concomitant treatment with amiodarone or enzyme inducers. The IWPC and Gage algorithms [3,4] and a 5 mg fixed dose were included in our evaluation of *a priori dose* predictions. The dose revision algorithm by Lenzini [7] was included in the evaluation of *a posteriori* dose predictions. Predictive accuracy was based on the difference between predicted and actual dose, including calculation of mean absolute error, extent of predicted doses within an acceptable range, and extent of severe over-prediction [2].
Results: Our model can be used to predict warfarin dose during all phases of treatment, irrespective of target INR. Preliminary results suggest that it gave slightly less accurate *a priori* dose predictions than the two published algorithms [3,4]. The evaluation of *a posteriori* dose predictions is ongoing.
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CLINICAL UTILITY OF *TOMM40* RS10524523 FOR THE PREDICTION OF ALZHEIMER'S DISEASE

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A phylogenetic analysis of sequence data from the linkage region containing *TOMM40* and *APOE* identified an association between genotype at a polymorphic poly-T tract, rs10524523 (523), in *TOMM40* with age at onset distributions of Alzheimer's disease (AD). This study also revealed the unique linkages between 523 and *APOE* alleles: *APOE4* is linked to a long (L) 523 allele whereas *APOE3* is linked to a short (S) or a very long (VL) poly-T in two, evolutionarily distinct haplotypes. Longer poly-T tracts (L and VL) are associated with earlier age of AD onset. This association is now confirmed in a prospective study of aging (Mayo Clinic, Scottsdale).

Poly-T length is also associated with memory, learning and brain grey matter volume in cognitively normal, adult children of AD patients (Wisconsin Alzheimer's Institute). *APOE3/3*, 523 VL homozygotes perform significantly worse on the Rey Auditory Verbal Learning Test of memory and learning than S/S homozygotes. *APOE3/3* VL/VL subjects also have reduced grey matter volume in the ventral posterior cingulate and precuneus, regions known to be affected early in AD, relative to *APOE3/3* subjects who carry two short 523 alleles. These results indicate that genotype at the poly-T locus identifies individuals who, although cognitively normal, express important biomarkers of incipient disease. The associations between 523 genotype and other dynamic biomarkers are being studied in this normal cohort to frame recently published hypothetical data regarding the timing of appearance of these biomarkers.

In contrast to 523, SNPs that have been identified by GWAS of AD, with the exception of SNPs in the *APOE-TOMM40* linkage region, have low predictive utility for disease risk or age of onset, have a more tenuous connection to known disease biology, and are unrelated to expression of known disease biomarkers. Without a biological context or a compelling statistical case for disease prediction, these GWAS findings are distant from translation into drug discovery and development or clinical practice.

The performance of 523-genotype as a diagnostic will be tested as one objective of a pharmacogenetically assisted delay of AD onset clinical trial, OPAL. For OPAL, cognitively normal individuals, judged to be at high disease risk based on 523 and *APOE* genotypes and age, will be randomized to receive drug or placebo. Low risk subjects will receive placebo, but the inclusion of this group provides data for assessment of the clinical performance characteristics of the genetic test of disease risk.

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Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar
Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room

Messages, Mail, Faxes

Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking

June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m. Two tennis courts open daily.

Russell Fitness Center

Dolan Hall, east wing, lower level
PIN#: **Press 64565 (then enter #)**

Concierge

On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones

Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

Take Syosset Taxi to Syosset Train Station ($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
US Airways	800-428-4322