

# Pharmacogenetics Research Network and Knowledge Base Fourth Scientific Meeting

AF Davis<sup>1</sup> and RM Long<sup>2</sup>

<sup>1</sup>Office of Communications and Public Liaison, National Institute of General Medical Sciences, National Institutes of Health, Bethesda, MD, USA; <sup>2</sup>Division of Pharmacology, Physiology, and Biological Chemistry, National Institute of General Medical Sciences, National Institutes of Health, Bethesda, MD, USA

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## INTRODUCTION

Now in its fifth year, the Pharmacogenetics Research Network and Knowledge Base (PGRN) is a multidisciplinary, collaborative research effort dedicated to probing relationships between genetic variation and interindividual differences in drug responses. The overarching goal of the program is to identify links between genotypic and phenotypic information, forging new knowledge about drug response and human physiology. The diversity of scientific interest spanning the group creates unique challenges but affords the opportunity for cross-fertilization of hypothesis generation, data analysis, and integrative exercises (eg creating biochemical/metabolic pathways to describe drug–gene–disease interactions). A guiding principle has been to create an open access resource of broad applicability to the scientific community.

## FOCUS ON STUDY DESIGN AND ANALYSIS

In recent years, research on the interindividual variation of drug response has undergone rapid change, in large

part due to the arrival of the genomic age. Distinguishing the terms pharmacogenetics and pharmacogenomics may be a subjective exercise that hinges on pragmatism. Furthering this notion, *Urs Meyer* (University of Basel) presented his view of the role pharmacogenomics currently plays in industrial drug discovery. While pharmacogenetics may be thought of as ‘one drug, many genomes’, pharmacogenomics could be better defined as ‘many drugs, one genome’. Pharmacogenomics encompasses the study of the effect of drugs on gene expression, and a key challenge remains analyzing the complex genomic and proteomic effects of drugs on human metabolism.

Meyer discussed recent studies on drug-induced transcriptional induction and repression.<sup>1</sup> Several xenobiotic response elements have been identified, many of which reside upstream of transcriptional start sites of genes encoding drug-metabolizing enzymes, drug transporters, and other metabolic proteins. Heterodimer combinations, ‘xenosensors’, bind to species-specific ligand-binding domains, although the general signaling pathways are highly conserved across evolutionary time. Meyer’s group is examining the pleiotropic effects of phenobarbital via microarray profiling of drug-inducible genes. Preliminary evidence suggests a molecular link between hepatic cholesterol and xeno-

biotic induction of drug-metabolizing enzymes. An algorithm, NUBIScan (<http://www.nubiscan.unibas.ch>), has been developed to predict nuclear response elements and inducible genes *in silico*.<sup>2</sup>

*Richard Spielman* (University of Pennsylvania) presented a genomic perspective of inherited variation in gene expression, positing that gene expression may be the critical link between genotype and phenotype. Spielman expressed the need for additional studies analyzing genome-wide variation in germline gene expression, taking into account both *cis*- and *trans*-acting control mechanisms. Asserting that most genome scans with a disease basis are still actually single-gene search efforts, Spielman called for a different approach that offers broader, unbiased genome searching for differential gene expression. Using CEPH pedigrees and lymphoblastoid cell lines, genome-wide linkage analysis and quantitative trait locus mapping approaches have identified networks of coregulated genes.<sup>3</sup> The expression level of each gene is considered a quantitative trait. An ‘agnostic’ search, not committed in advance to any expectation of genetic control, permits the gene expression data themselves to guide the way toward generating hypotheses of functional significance. The approach has yielded both *cis*- and *trans*-acting genetic determinants (that may have combined effects) that contribute to gene expression-induced genetic variation. Pharmacogenetic analyses may be well suited to this experimental design.

## PGRN RESEARCH UPDATE

Selected members of the PGRN teams presented recent research findings on methylation and sulfation enzymes, informatics, lipoprotein therapy, asthma treatment, antidepressant treatment, autonomic responses, and antiarrhythmic therapy.

*Richard Weinshilboum* (Mayo Clinic/Foundation) has employed a genotype-to-phenotype strategy to detect

and functionally characterize sequence variation in genes encoding phase II-conjugating enzymes. The main focus has been to define mechanisms by which nonsynonymous cSNPs influence phenotype. Functional genomic studies of nonsynonymous cSNPs in genes encoding methylation and sulfation enzymes have revealed that the most common mechanism whereby variation affects function results from decreased quantity of protein rather than altered enzyme function. In those cases that have been studied in detail, this is due to accelerated protein degradation (likely via a ubiquitin-proteasome pathway).<sup>4</sup> Protein misfolding may also contribute to this process. Preliminary results suggest that association with heat-shock proteins may also play a role in targeting variant gene products for proteasome degradation.

*Russ Altman* (Stanford University) showcased new developments in PharmGKB (<http://www.pharmgkb.org/>), the PGRN's centralized informatics resource.<sup>5</sup> The PGRN has made significant progress in the past year toward increasing the functionality of PharmGKB. Currently, several data types can be accepted, including genotype, phenotype, pathway information, MIAME-compliant microarray information, and literature annotations. PharmGKB has primary data from the PGRN on 192 genes (including either genotype or phenotype data), and it hosts 1262 annotations of the pharmacogenetics literature. Additional data types, such as image data, are not yet posted but new submissions are welcome. The knowledge base currently hosts drug pages, gene pages, and pathway pages. As of March 2004, six curated, clickable metabolic pathways had been posted on PharmGKB, and several more were in development. The PharmGKB staff has been continuously soliciting user advice on potential enhancements to the functionality of the knowledge base through initiating weekly 'focus group' conference calls. Plans are underway to accept haplotype submissions and to enable users to make haplotype calculations. In addition, Web-based templates for data submis-

sion are currently being developed to ease the data submission process for PGRN investigators and the broader scientific community.

*Ronald Krauss* (Children's Hospital Oakland Research Institute) discussed a comprehensive search for genetic polymorphisms predictive of lipoprotein response to diet and drugs. Ultimately, the goal is to unearth genotypic information that will be a predictive tool to offer protection against cardiovascular disease with specific drug treatments. Recent results have identified common SNP haplotypes in candidate genes involved in pathways that influence hepatic cholesterol metabolism and plasma lipoprotein transport. Of particular interest are data that associate a genetic variant with a diet-induced shift from larger to smaller LDL species.<sup>6</sup> Efforts to alter lipoprotein profile through diet and drug administration may reveal more polymorphisms of biological and/or clinical interest. Additional genetic influences on pathways regulating LDL subclass metabolism may also be revealed from analyses of tagged SNPs in candidate genes.

It is widely recognized that asthmatic patients react variably to treatment with the three major therapeutic modalities for this disease: steroids, leukotriene inhibitors, and beta-agonists. In particular, 10–15% of patients taking inhaled steroids (glucocorticoids) do not respond to this form of treatment, the most commonly prescribed anti-inflammatory medication for asthma. *Scott Weiss* (Brigham and Women's Hospital) reported the results of a strategy to find sequence variants in the glucocorticoid-asthma pathway. Sequence variants are genotyped in a clinical trial population, and the findings are replicated in second and third trial populations (both adult and child). Associations have been found with haplotype tag SNPs in the corticotropin-releasing factor receptor 1 (CRFR1, CRHR1) gene, which encodes a G-protein-coupled receptor with at least 14 exons and four known splice variants. Genetic variation may lead to a decrease in adrenocorticotrophic hormone, which may diminish endogen-

ous cortisol production and support an inflammatory response. Analyses of data with corticotropin-releasing factor (CRF, CRH) knockout mice revealed airway inflammation, decreased endogenous steroid levels, and a heightened inflammatory response as indicated by elevated inflammatory cytokines and chemokines. Combining mouse and human models may further elucidate the role of genetic variation in the heterogeneous response to steroid treatment of asthma.

Pharmacogenetic and pharmacogenomic approaches may be used to investigate treatment approaches for complex disorders of public health relevance such as depression. *Julio Licinio* (University of California, Los Angeles) discussed progress to date using a prospective, phenotype-to-genotype experimental strategy with a Los Angeles Mexican-American population. In the phenotype component, antidepressant response to fluoxetine or desipramine is assessed using a double-blind approach. Phenotype is characterized using the Hamilton depression scale as a primary outcome measure and several other validated scales for assessment of secondary outcomes. Evaluation of variable treatment response has led to the identification of several candidate genetic pathways. Consented DNA collection will permit genotype analyses to further investigate purported correlations. Initial results from a candidate genotyping approach focusing on corticotropin-releasing hormone receptor type 1 (CRHR1, CRFR1) strengthen pathophysiologic data that indicate corticotropin-releasing hormone (CRH, CRF) may act as a neuropeptidergic regulatory system in depression. Licinio pointed to the importance of involving communities in genetic research, describing ongoing, bilingual community consultation efforts with his Los Angeles study population.

*Daniel O'Connor* (University of California, San Diego) presented recent results on how allelic variation in autonomic pathway genes may determine cardiovascular response in humans. Variation exists in the response to drugs affecting the regulation of

catecholamine storage/release, heart rate, and vascular tone, all of which may lead to variable control of therapeutic targets (eg hypertension, heart failure, arrhythmias, renal failure, and edema). O'Connor's group is investigating the pharmacodynamic determinants of human autonomic cardiovascular drug response in systemic and pulmonary circulations. Initial phenotyping strategies measure local vascular response (via a regional circulatory bed assay, in the hand), to remove baroreflex and pharmacokinetic variables and attempt to isolate directly receptor, postreceptor, and effector drug response determinants. Using these parameters, SNP discovery efforts have produced several hypotheses that are currently being tested. Preliminary data suggest that genetic variation in the alpha2B adrenoceptor and alpha1B adrenoceptor genes have predictive value for mean arterial pressure response. Substantial mammalian interspecies variability exists within these genetic loci. Other data yielded major interindividual variation (1000-fold) in the metabolism of yohimbine, an alpha2 adrenoceptor antagonist.

Available antiarrhythmia drug therapies exhibit significant variability in response, leading to lack of efficacy or adverse effects. In particular, some

individuals are prone to proarrhythmic effects following administration of antiarrhythmia agents. Long QT syndrome (eg torsades de pointe) can be provoked by antiarrhythmia drugs or other classes of drugs<sup>7</sup> (eg terfenadine) and has become a model for investigating the pharmacogenetics of rare adverse drug reactions. Dan Roden (Vanderbilt University) presented recent data on variation within ion channel genes and other candidate loci that impact cardiac conductivity function. Nonsynonymous cSNPs have been found in the KCNA5 potassium channel-encoding gene, and functional analysis of one of these variants that produces a drug-insensitivity molecular phenotype suggests the absence of an alpha-helical protein motif. Roden's group has identified additional SNPs in other candidate genes, such as the gene that encodes the SCN5A cardiac sodium channel.<sup>8</sup> Phenotypic information is being collected from patients exhibiting postoperative atrial fibrillation, and future efforts will focus on forging genotype and drug response links to these data.

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#### DUALITY OF INTEREST

None declared.

#### Correspondence should be sent to:

Dr RM Long, Division of Pharmacology, Physiology, and Biological Chemistry, National Institute of General Medical Sciences, National Institutes of Health, Bethesda, MD, USA.

Tel: +1 301 594 1826

Fax: +1 301 480 2802

E-mail: longr@nigms.nih.gov

and

Dr AF Davis, Office of Communications and Public Liaison, National Institute of General Medical Sciences, National Institutes of Health, 45 Center Drive, Bethesda, MD, USA.

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