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Pharmacogenetics research network and knowledge base second scientific meeting

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BACKGROUND

In April 2000, the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH) established a nationwide research network, the Pharmacogenetics Research Network (PGRN), joining several teams of pharmacogenetics and pharmacogenomics investigators.[1] In addition to NIGMS, other participating NIH sponsors include the National Cancer Institute, the National Heart, Lung and Blood Institute, the National Human Genome Research Institute, the National Institute of Environmental Health Sciences, and the National Library of Medicine. NIH created the PGRN after discussions with the scientific community identified a need to promote research in pharmacogenetics and pharmacogenomics. The scientific community also recognized the importance of full disclosure of data into the public domain. The PGRN is linked to a research database called the Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB: http://www.pharmgkb.org).

The second open scientific meeting of the NIH PGRN was held on 12 March 2002 at Stanford University in Stanford, CA. The program consisted of two keynote talks and research progress updates by PGRN team leaders. Rochelle Long (NIGMS), NIH program director for the PGRN project, invited the scientific community to provide feedback on the PGRN and on PharmGKB on a continual basis.

KEYNOTE: MICROARRAYS IN PHARMACOGENOMIC STUDIES

Pat Brown (Stanford University) delivered the morning keynote address on the applicability and use of microarray technology in pharmacogenomic investigations. Brown began by noting that Mendel's seminal experiments revealed rich phenotypic variation that can be seen in the expression of plant genes, which culminate in the formation of leaves, flowers, and stems. In a biomedical vein, the same result is obvious if one considers that every human cell contains an identical genome, yet markedly different properties. He described current efforts to systematically survey gene expression in a wide variety of cell types, under many different conditions.[2] Related to pharmacogenomics, microarrays may be useful in correlating genes and variable drug responses. While microarray applications to pharmacogenomic studies will undoubtedly be challenging, the methodology may also be useful in identifying tissue-specific drug toxicities, especially those occurring in tissues not routinely accessible to biopsy. Other potential microarray technologies that may find welcome use in pharmacogenomic research include the use of antibody microarrays, in which thousands of monospecific antibodies are printed on a chip to permit clinical monitoring of proteins in serum and other biological fluids for disease detection, drug response, and toxicity.[3]

PHARMACOGENETIC STUDIES OF ENZYMES AND TRANSPORTERS

Kathleen Giacomini (University of California, San Francisco) reported her group's progress on the analysis of membrane transporters as candidates for regulating drug responses. Giacomini's group is pursuing 25 transporters of two general classes: neurotransmitter transporters and xenobiotic transporters. A set of 247 ethnically diverse DNA samples derived from cell collections from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research (http://locus.umdnj.edu/) has been analyzed to assess genetic variation in transporter sequences across human populations. The nature and extent of variation differed widely; in general, neurotransmitter transporters exhibited less variation than xenobiotic transporter genetic sequences. Two clinical studies have recently been launched to evaluate how genetic variation in membrane transporter sequences may affect clinical response. The first study, SOPHIE (principal investigator: Esteban Burchard, UCSF), will contain a cohort of 500 healthy volunteers in the San Francisco Bay Area. Each volunteer's ethnicity will be defined by the geographical origin of his or her four grandparents, and subjects have consented to being called back in the future for further pharmacogenetic studies. Individuals participating in SOPHIE will be genotyped for genetic variation in the transporter gene OCT1 (organic cation transporter 1). A second study, GRAD (principal investigator: Cathy Schaefer, Kaiser Permanente), will investigate genetic components of the response to anti-SSRI antidepressants, a class of drugs that typically exhibits a wide range of drug

response: 30% of patients have no response, 70% have a variable response, and approximately 10% experience adverse effects. GRAD will enroll 1500 people diagnosed with depression. These people will be evaluated before and after therapy with an anti-SSRI drug to determine the drug's efficacy and adverse effects. All study participants will be genotyped.

Richard Weinshilboum (Mayo Foundation) presented recent results on the pharmacogenetics of phase II drug-metabolizing enzymes. Weinshilboum's group is continuing to resequence phase II metabolism genes, including approximately 10 sulfotransferases, approximately five methyltransferases, and one phosphoadenosine-5′-phosphosulfate synthetase.[4,5] Polymorphisms are common, and significant variability has been found both within and between ethnic groups. In general, the common nonsynonymous cSNPs identified lead to reduced quantities of enzyme production. Ongoing efforts include structural analysis of proteins to uncover potential explanations for variable drug response among different alleles. A case study was presented on the resequencing of the histamine *N*-methyl transferase (HNMT) gene.[6] An increase in allelic frequency of HNMT has been observed in asthma. Protein degradation is being actively investigated as a determinant for some of the phenotypic variability seen in phase II enzyme activity.

CARDIOVASCULAR AND PULMONARY PHARMACOGENETICS

Ronald Krauss (Lawrence Berkeley National Laboratory) presented his group's objectives and early data. Krauss' group is seeking to identify common genetic variants in candidate genes that contribute to interindividual variation in responsiveness to drugs used to reduce the risk of cardiovascular disease. Candidate genes involved in the response to treatment of the two most prevalent cardiovascular risk factors, hyperlipidemia and hypertension (with simvastatin and ramipril, respectively), are being sought. Two clinical studies will correlate SNP haplotypes with detailed phenotypes indicative of drug response. Primary phenotypic correlates, such as LDL, cholesterol, and blood pressure regulation, will be assessed, along with secondary phenotypic correlates, such as insulin levels and markers of inflammation. Candidate genes that have already emerged include those related to the angiotensin and renin pathways. For the planned clinical studies of relatively small numbers of patients, limited statistical power will necessitate follow-up confirmatory testing. In addition, haplotypes of potential importance may be examined in relation to clinical endpoints in larger clinical trials, such as the Heart Protection Study (http://www.ctsu.ox.ac.uk/~hps/).

Dan Roden (Vanderbilt University) presented research plans and early data pursuant to his group's study of the pharmacogenomics of arrhythmia therapy. Roden and his group are testing the hypothesis that allelic variants in candidate genes identified by an emerging understanding of molecular physiology and pharmacology contribute to the variable drug responses typical of anti-arrhythmic treatments. Candidate genes for ion channel proteins, drug-metabolizing enzymes, and components of intracellular signaling systems are being analyzed for allelic variation. To date, unique mutations have been found in several genes, including SCNSA (inward sodium flux), KCNQ1 (outward potassium flux), as well as in other potassium channel genes. *In silico* experiments are being conducted to reconstruct electrophysiological parameters with variant channel proteins, using Luo–Rudy action potential modeling methods. Early data from these experiments suggest that observed sequence changes do not affect the action potential, but do appear to exert an influence on repolarization reserve. Other studies in progress involve genotyping and monitoring the short- and long-term outcomes of 500–1000 patients with long QT syndrome to track responses to anti-arrhythmic drugs and warfarin.

Daniel T O'Connor (University of California, San Diego) described ongoing studies to investigate pharmacodynamic determinants of human drug responses, especially autonomic cardiovascular responses. O'Connor's group is investigating potential genetic determinants that may affect responses to autonomic control over systemic and pulmonary circulatory processes, such as blood pressure, pulmonary systems, dopaminergic responses, and presynaptic adrenergic mechanisms. The *in vitro* effects of identified SNPs will be measured, followed by an analysis of *in vivo* effects in transgenic animals. Cardiovascular drug targets such as alpha- and beta-adrenergic receptors are being used to identify potential genotype–phenotype relationships. To this end, variation has been observed in the ability of humans to metabolize the alpha-2-adrenergic agonist yohimbine. Studies are in progress to investigate adrenergic receptor and CYP2D6 genomic diversity.

Kelan Tantisira (Harvard University, representing PGRN member Scott Weiss) presented recent results on the pharmacogenetics of asthma treatment.[7] Ongoing studies seek to identify the genes responsible for the clinically apparent variable response to each of the three classes of medications used to treat asthma: beta-agonists, inhaled corticosteroids, and leukotriene modifiers. Asthma patients are being genotyped at loci associated with functional effects determined *in vitro*, then phenotyped according to individual response to treatment with the class of asthma medication of interest. Polymorphisms have been identified in the promoter region of the ALOX5 gene, which encodes a key enzyme in generating leukotrienes that trigger an asthmatic response.[8] Progress was reported on an ancillary genetics study component to the Childhood Asthma Management Program (CAMP), in which 1041 children with mild to moderate asthma are being evaluated with respect to the effects of methacholine administration on lung function, occurrence of respiratory symptoms, and duration of disease. DNA information has been obtained from most

participants in this longitudinal study; genotypic analysis will be performed to assess the nature of genetic contributions to asthma drug treatment. The differential transmission of alleles from parents to affected children is being investigated. Future studies will continue haplotypic association studies in the CAMP population, in an effort to define longitudinal response phenotypes. Genotyping of 14 candidate genes in 481 subjects participating in an inhaled steroid trial is also in progress.

KEYNOTE PHARMACOGENETICS AND INTELLECTUAL PROPERTY

Rebecca Eisenberg (University of Michigan) delivered the afternoon keynote address on pharmacogenetics and intellectual property. Eisenberg opened her talk stating that the 'big story' surrounding patent-related issues may not be about how to use patents, but rather about what is *not* getting patented. Eisenberg speculated on how patent-related issues may unfold in the fields of pharmacogenetics and pharmacogenomics, acknowledging that outcomes are yet to be determined. Pharmacogenetics as a whole may be value-enhancing for consumers of drugs, potentially providing pre-selected drugs for individuals, albeit likely at higher costs. To the benefit of pharmaceutical companies, so-called 'orphan drugs' may have a better chance of making it to market if target populations can be identified early on in the development process. The cost of drug trials may also be reduced if non-responders can be 'weeded out' early on in the clinical testing process. As a case in point, the industrial firms participating in the SNP Consortium have all agreed to place SNPs they identify into the public domain. Eisenberg suggested that this implied an example of an unusual application of the patent system, where companies intentionally put information into the public domain as a way of freeing potentially valuable information from third-party encumbrances. Such a strategy may speed access to resources that can be further developed by academia, thereby by fostering the development and maturation of scientific results in the pre-clinical realm. It may be that putting SNPs in the public domain prevents the fragmentation of resources, avoiding lengthy and costly licensing procedures. Another conjecture stems from recent media reports[9,10] suggesting that companies may use pharmacogenetics to patent tests that may never be used, hence blocking the markets of competitors; indeed, nothing in the US patent system prevents the use of patents to suppress an invention. Regardless of the motivations for the application of pharmacogenetics and pharmacogenomics to drug development, marketing, and distribution, the traditional allocation of payoffs will inevitably be altered. In contrast to a few 'blockbuster' drugs, there may be a larger number of products with smaller markets, and products previously seen as 'too risky' may find their way to market in carefully targeted clinical populations. Another change may relate to the types of firms that can develop drugs; an altered payoff structure may change the traditional attraction of investors to companies. Still other ramifications may include a shift in the balance between therapeutics and diagnostics, the latter conventionally a less remunerative market.

PHARMACOGENETIC STUDIES IN ETHNIC POPULATIONS

Julio Licinio (University of California, Los Angeles) presented an update on studies of genetic determinants of the response of Mexican-Americans to antidepressant medications. Only 60–65% of patients respond to antidepressant drugs. Licinio's group is in the process of collecting DNA from 500–600 Mexican-Americans, phenotyping individuals, treating them with fluoxetine or desipramine (following a one-week placebo trial), then assessing the drug responses on a weekly basis. DNA samples are being genotyped by collaborators at Los Alamos National Laboratory for later analysis with respect to drug response data. Other experimental approaches include applying genomic tools (in rat studies) to studying candidate systems known to be altered in depression, such as the neuroendocrine axis and the leptin system. To offset possible negative social effects of studying one particular ethnic group, community consultations have been conducted. Numerous small meetings have been held at various locations throughout Los Angeles.

PHARMACOGENETICS AND PHARMACOGENOMICS KNOWLEDGE BASE

Prakash Nadkarni (Yale University) described efforts to provide informatics support to enable end users to interface with the PharmGKB database. A primary goal of the Nadkarni group effort is to streamline the daily operations of the PGRN. While easy submission of data is an important aspect to optimizing the potential of PharmGKB, other day-to-day issues include utilizing PharmGKB as well as efficiently organizing laboratory data into smaller databases. Assistance is tailored to the expertise of the laboratory seeking help, and in general the strategy used is to 'teach people to fish *vs* fishing for them'. Nadkarni outlined the many advantages of storing experimental results in databases rather than spreadsheets, making data analysis and later data transfer a much more systematic process.

PharmGKB team leader Russ Altman (Stanford University) debuted the newly launched version of PharmGKB. The mission of PharmGKB is to become an imperative tool for the pharmacogenetics and pharmacogenomics research community, akin to databases such as GenBank® and PubMed. The goal is to make PharmGKB an *active*, not archival, resource of pharmacogenetic and pharmacogenomic data, and to disseminate results gathered by the PGRN to network members and the broader scientific community. The new launch of PharmGKB presents an organizational structure to house genotypic and phenotypic data according to four classifiers: functional assays, pharmacokinetics/pharmacodynamics, clinical drug response, and patient outcome. Discussions are under way to optimize this structure for data

input from PGRN members and the wider scientific community. A critical challenge is in determining how to get access to phenotype data. Ongoing improvements to PharmGKB include the possibility of posting a community-based submission query tool, in which any scientist working in the area of pharmacogenetics or pharmacogenomics could suggest a drug–gene relationship for further analysis. Also being investigated is the possibility of implementing a similar-minded data-mining tool that would automatically harness information and vocabulary terms from other existing databases such as PubMed and OMIM. PharmGKB is seeking feedback from end users; please visit http://www.pharmgkb.org to sign up to receive the PharmGKB newsletter, search for information, find resources, submit data, or provide feedback on the general utility and limitations of the database. Periodic data submissions to PharmGKB will be published in the journal *Pharmacological Reviews*.

CANCER PHARMACOGENETICS

Mark Ratain (University of Chicago) presented an update on the Pharmacogenetics of Anticancer Agents Research (PAAR) Group's investigation of the pharmacogenetics of anticancer agents. Ratain reported that a genotype (variant in the UGT1A1 promoter) correlates with the development of neutropenia in patients treated with irinotecan. Resequencing of DNA from human liver samples has identified several new polymorphisms; ongoing studies are investigating whether any of these displays a phenotype of interest. As part of an ongoing clinical trial of morphine glucuronidation, DNA from an ethnically diverse group of patients self-administering morphine is being screened for new polymorphisms in the UGT2B7 gene. Morphine metabolites have been measured, and one allele has been found to be more prevalent in low glucuronidators. Linkage disequilibrium was found with a known missense SNP that is thought to have minimal, if any, function. The PAAR Group is also using a novel molecular haplotyping method that utilizes long-range PCR and intramolecular ligation to perform successive rounds of allele-specific amplification.

David Flockhart (Indiana University) presented progress to date on examining the genetic influences of tamoxifen and other selective estrogen receptor modulators (SERMs) on various clinically important parameters. Tamoxifen was chosen as a SERM that exhibits many different pharmacologic effects of clinical importance, including therapeutic effects on breast cancer, osteoporosis, and atherogenesis. However, the drug also causes adverse effects such as hot flashes and blood clots. The Flockhart group's goals are to characterize tamoxifen metabolism *in vitro* and *in vivo*, to isolate, confirm, and synthesize active metabolites, and to test the association of genetic polymorphisms in cytochrome P450, eNOS, and transporter and estrogen receptors with clinically important SERM effects. Previous results of a pilot clinical study showed that the efficacy of tamoxifen was compromised in poor metabolizers; co-administration of paroxetine can mimic this effect. This study has now been expanded into a larger, prospective effort for which the initial response of bone, lipid and blood clotting parameters were presented. In order to identify useful genetic predictors of clinical response, and to determine their value relative to routinely used clinical predictors such as age and menopausal status, a two-step, iterative statistical procedure ('PRESS') was described. PRESS is being used to identify and authenticate genotypic associations with a variety of clinical parameters (eg bone density, cholesterol levels) in an effort to determine the pharmacogenetic profiles of women administered tamoxifen. The PRESS method can iteratively correct for multiple, independent variables and appears to be an innovative way to identify the most likely genetic predictors of clinical responses.

Howard McLeod (Washington University) presented the goals and emerging data of his research group, CREATE (Comprehensive Research on Expressed Alleles in Therapeutic Evaluation). In contrast to the traditional single gene-phenotype pharmacogenetic paradigm, CREATE aims to investigate the genetic variation existing in entire pathways influencing drug activity, using colorectal cancer as a model system. Colorectal cancer is a malignancy for which three drugs are in clinical use. In 113 genes, 840 SNPs have been discovered to date. Resequencing efforts are under way, and genotyping and gene expression studies are being conducted using microarrays and antibody arrays created using tissue from rigorously quality-controlled tumor banks. SNP frequencies are being analyzed in several populations to validate gene variants within drug pathways.

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DUALITY OF INTEREST

None declared.

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