Pharmacogenetics Research Network and Knowledge Base Third Scientific Meeting

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Introduction

The Pharmacogenetics Research Network and Knowledge Base (PGRN) held its third annual open scientific meeting on 3 March 2003 in Memphis, Tennessee. The PGRN effort is a nationwide pharmacogenetics research initiative sponsored by the National Institutes of Health. Network members submit data to a comprehensive knowledge base, PharmGKB (http://www.pharmGKB.org/). The meeting focused on the analytical and quantitative issues confronting modern genetics research. Morning sessions addressed population genetics, analytical challenges in molecular epidemiology and comparative genomics. Afternoon sessions featured selected research updates from PGRN investigators.

The PGRN was conceived as a network of multidisciplinary, collaborative research groups of investigators examining how genetic variation contributes to inter-individual differences in drug responses. The groups are charged with collecting comprehensive, integrative information about proteins and gene families known to be important in mediating the effects of therapeutics, from variations in drug metabolizing enzymes to target receptors. Once comprehensive biological information for particular proteins and genes and their corresponding variants is known, the network will catalogue this information in a manner that is accessible and interpretable to a wide range of scientists. PharmGKB has the capacity to collect genetic sequence information, display polymorphic forms, identify functional consequences of genetic variation and rigorously correlate this information with clinical drug responses [1].

Quantitative and analytical challenges in genetics research

While the history of pharmacogenetics research dates back nearly 50 years, changes in the scientific landscape have refocused attention on current challenges facing this field. Investigating multigenic human variation, especially as it relates to the molecular basis of complex diseases, is a critical element of modern genetics research. While Mendelian disease genes are typically of recent origin and alleles have large phenotypic effects, complex disease traits are often characterized by alleles exerting small phenotypic effects. In studying complex diseases, Aravinda Chakravarti of The Johns Hopkins University School of Medicine noted that functional tests of alleles and gene interactions must complement gene mapping efforts, and that an essential component of these studies is recapitulation of the disease trait in an experimental system. Good study design can tease apart the many factors that produce a complex disease, including episodic and age-dependent traits; gender, environment and lifestyle; unknown gene actions; positive or negative regulatory alleles; and epigenetic phenomena.

Chakravarti described recent data from his group on the complex genetics of Hirschsprung disease, in which pedigree studies of Mennonite populations combined with a genome-wide association screen have recently revealed two haplotypes in the receptor tyrosine kinase (*RET*) gene [2]. Although variant alleles of several genes have been associated with this condition, none is necessary or sufficient to cause Hirschsprung disease.

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Chakravarti reported that linkage disequilibrium studies have uncovered a genetic interaction between the endothelin receptor B (*EDNRB*) gene and multiple *RET* loci, and that preferential transmission of haplotypes may contribute to the phenotype of this disease.

Michael Province of Washington University in St Louis discussed means to controlling type I error in statistical data analysis. Province described the trade-offs inherent to selecting genotyping strategies, noting that researchers pay a statistical penalty for excessive genotyping. More data do not necessarily imply more information and, for any complex trait, most genetic variation is noise (including that present in candidate genes). To help explain the phenomenon, Province presented an analogy describing catching tuna with a fishing net, through which an occasional dolphin passes. In this case, the tuna represent the test hypothesis, and the dolphins are the null hypothesis. In the example, larger and more prevalent passage of dolphins constitutes 'noise'; however, increasing the fineness of the net (e.g. by applying a Bonferroni correction) severely restricts data collection such that hardly any tuna can swim through the net. This type of statistical correction can produce a large power loss, restricting the utility of such an approach.

Province suggested that pharmacogenetics research demands a separation between gene discovery and hypothesis testing. An approach pioneered in the 1940s by Hungarian mathematician Abraham Wald led to the development of a current technique called sequential multiple decision procedures, which can help remedy the problem of balancing false positives (type I error) and false negatives (type II error). Using this method lets the experimental data determine the point at which a hypothesis-generation phase should move to a true hypothesis-testing phase [3,4]. The approach helps to optimize sample size, while limiting both false positives and false negatives.

Joseph Terwilliger of Columbia University contrasted the goals and approaches of genetics and epidemiology. Whereas epidemiology is hypothesis-based and an ascertainment bias is undesirable, genome-scanning experiments are hypothesis-free and an ascertainment bias is necessary to maximize the efficiency of mapping studies. Genetic studies with small sample sizes tend to systematically overestimate linkage disequilibrium and underestimate variation [5]. Terwilliger expressed the view that genetic approaches need an ascertainment bias because penetrance is not equivalent to detection, and that genome-scanning methods should be limited to generating hypotheses, not to testing them.

Pharmacogenetics research may benefit from using comparative genomics strategies to glean information about variation in drug metabolizing enzymes, as well as to impact the design and interpretation of preclinical investigations. Anna Di Rienzo of the University of Chicago discussed comparative genomics approaches to resequencing studies, in which the same sequence block is analysed in multiple individuals. Di Rienzo described ongoing efforts of the Pharmacogenetics of Anticancer Agents Research Group to clarify the genetic basis for toxicity in response to irinotecan and flavopiridol therapy. Comparative genomics analyses in the human, dog, rat, mouse and primate versions of the *UGT1A* gene cluster have begun to identify conserved regions, using the MultiPipMaker computational tool (http://bio.cse.psu.edu/pipmaker) [6]. This Web-based tool draws percentage identity plots (pips) by comparing several genomic sequences, then creating alignments and identifying nucleotide identities. One of the technique's strengths is its unbiased analysis of any DNA sequence, regardless of whether the sequence is coding or non-coding. Di Rienzo predicted that future enhancements to comparative genomics strategies would include methods to assess statistical significance, large-scale studies to measure functional significance and new techniques to take into account phylogenetic distances between compared species.

PGRN research update

Selected members of the PGRN groups presented recent research findings on adrenergic pharmacology, membrane transporters and the pharmacogenomics of chemotherapy and asthma treatment.

Stephen Liggett of the University of Cincinnati presented recent findings on a synergistic interaction between β_1 and α_{2C}-adrenergic receptor polymorphisms that may contribute to the pathology of congestive heart failure. G protein-coupled receptors constitute the largest family of signalling molecules, many of which are involved in conveying drug action. Substantial interindividual variation exists in agonist and antagonist response directed to these receptors; the known variable response to β-blocker therapy represents a particularly relevant topic for pharmacogenetics investigation. Some of this variation may be attributed to polymorphisms in coding regions, which may act together to provide persistent adrenergic stimulation and promote heart failure and/or cardiac hypertrophy. Liggett described a small clinical study in which control or heart failure patients were genotyped at both the β_1 and α_{2C}-adrenergic receptor loci. Logistic regression analysis was used to determine the potential effect of each genotype and the interaction between them on the risk of heart failure. Data acquired from the African American cohort in this study were analysed separately, revealing a significantly increased odds ratio for risk of heart failure in African-American subjects who are homozygous for both polymorphisms [7].

Kathleen Giacomini, Ira Herskowitz, and colleagues at the University of California, San Francisco (UCSF) are in the process of cataloguing the extent of human sequence variation in membrane transporters, which are impacted by many classes of medications. The group is analysing human DNA for the existence of single nucleotide polymorphisms (SNPs) in the ATP-binding cassette transporter superfamily and in solute carrier transporters. Over 600 SNPs from both coding and non-coding regions of transporter genes have been identified, and the average heterozygosity (π), the likelihood that a nucleotide position will be heterozygous when compared across two chromosomes selected randomly from a population, appears to vary considerably among transporters. Giacomini reported segmental variation in amino acid diversity within transporters, with transmembrane domains having less variation than loop regions. Comparative genomics studies and functional analyses in cells have confirmed that sequence variation within evolutionarily conserved regions is more likely to alter transporter function [8].

In collaboration with Giacomini and Herskowitz, Esteban González Burchard of UCSF has created the SOPHIE cohort, a group of ethnically diverse research volunteers from San Francisco who have agreed to participate in future pharmacogenetics studies. To date, Burchard and colleagues have recruited 577 individuals for genotyping/phenotyping studies of medications that may be affected by genetic variation of membrane transporters. Individual members of the cohort, which contains approximately 125 individuals each of Mexican-American, African-American, Caucasian and Chinese descent, have consented to providing DNA samples to be used in future pharmacogenetics studies. Several UCSF investigators are currently making use of the SOPHIE cohort to examine racial and ethnic variation in genes involved in drug response. Burchard noted that, although racially admixed populations make up a significant proportion of the US population and it is important that the research community include these populations in genetics studies [9,10], population stratification as a result of racial admixture can be a serious obstacle to performing genetic association studies in racially admixed populations. Burchard urged the pharmacogenetics research community to consider and, when necessary, correct for the presence of population stratification in genetic association studies.

William Evans of St Jude Children's Research Hospital presented data on gene expression profiling to discriminate molecular subtypes and drug response in childhood acute lymphoblastic leukaemia (ALL). Despite a greater than 99.9% similarity between host and tumour genomes for most malignancies, several genetic characteristics distinguish host cells from cancer cells, the latter of which exhibit altered ploidy (i.e. chromosome number), as well as variable gene expression, gene fusions and gene translocations. Additional complexity arises from the fact that adult and childhood cancer differ at the molecular level. Evans reported the molecular classification of ALL based on gene expression profiling [11]. The primary microarray data have been deposited in the pharmacogenetics knowledge base, PharmGKB (http://www.pharmGKB.org/). Evans reported that, in some cases, differential gene expression can predict treatment outcome; however, such information appears to be treatment-specific. More recently, gene expression profiling has been used to determine how leukaemia cells respond to different antileukemic agents, revealing distinct genomic responses that are treatment-specific [12]. These approaches are now being used to identify genomic determinants of drug sensitivity in human leukaemia cells.

Pharmacogenetics approaches to asthma therapy have the potential to improve current treatment modalities by identifying genetic variation in drug response. These efforts may lead to diagnostic tests for asthma response to corticosteroids and other medications. Scott Weiss of Brigham and Women's Hospital presented data on two completed clinical trials investigating corticosteroid therapy in different populations. The goal of this research is to correlate treatment response with genotype. One of the trials was a 4-year, randomized, multicentre trial of glucocorticoid therapy in children. For this study, DNA samples were obtained from several hundred asthmatic children and their parents in the Childhood Asthma Management Program (CAMP). The trial has ended, but the population is still under investigation. The second study, an 8-week comparison of flunisolide and conventional steroid therapy, was conducted in adults with severe asthma. Weiss reported that no single SNP in the corticotropin-releasing hormone receptor type 1 (CRHR1) gene produced phenotypic effects in both populations, but that a common risk haplotype was present in both populations with identical effects on treatment response. The investigators modified the computer program 'haplo.score' (http://cran.r-project.org/doc/packages/haplo.score.pdf) [13] for use as a statistical tool in data analysis. The program performs score tests for association of traits to haplotypes in the presence of phase ambiguity.

In conclusion, the goal for PharmGKB, a component of the PGRN, is to provide an information resource of maximum utility to the entire research community, and to stimulate future hypothesis-driven research. PharmGKB has recently created a Web page where the pharmacogenetics community can enter gene–drug interactions where genetic variability has been observed and associated with phenotypic variability. The PharmGKB Community Submission Project aims to

catalogue important literature datasets in pharmacogenetics [14].

The fourth annual PGRN meeting will be held in Los Angeles, California on 8–9 March 2004.

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