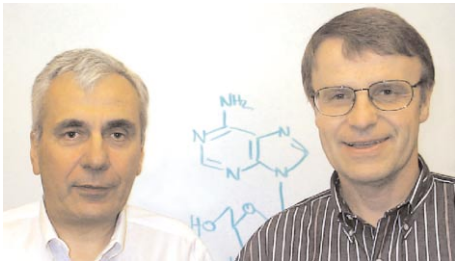
Pharmacogenomics: challenges and opportunities



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'Pharmacogenomics, by providing new potential targets, will lead to a novel bottleneck, namely, lead optimization.'

One of the most promising sciences for the pharmaceutical industry emerging in the post-genomic era is pharmacogenomics. With the discovery of gene diversity and the identification of single nucleotide polymorphisms (SNPs), pharmacogenomics is creating new challenges. Whereas pharmacogenomics refers to the field of new drug discoveries based on an increasing knowledge of the human genome, pharmacogenetics is the study of drug response resulting from heredity[1] (Box 1). These are broad and all-encompassing definitions for two related terms that are much-defined, often confused and interchangeably used. In this editorial we will:

- Define pharmacogenomics
- Present challenges posed by pharmacogenomics and pharmacogenetics; and
- Discuss opportunities related to these challenges.

Challenges always provide opportunities. Major opportunities are now emerging for the biotechnology and large pharmaceutical sectors of the pharmaceutical industry in the post-genomic era that will eventually benefit the patients.

Pharmacogenomics terms

Several definitions have emerged for the science of pharmacogenomics (Box 1), some of which are predictive of what this science will ultimately provide[2]. Pharmacogenomics, as broadly defined, is the study of the impact of genetic variation on the efficacy and toxicity of drugs, or the study of how a patient's genetic makeup determines the response to a therapeutic intervention. It is an emerging, authentic discipline that uses, and will more widely use, genetic and genomic information to predict the response of patient groups to drugs, and thus guide clinical trials and the drug development process. Pharmacogenomics has the potential to revolutionize the practice of medicine by using new genomics diagnostic tools (Box 2) to predict the specific drugs that will be most successful for treating individual patients. This new science should eliminate the trial-and-error technique commonly used by physicians to find the right drug for an individual patient, and should limit the exposure of patients to drugs that cannot work for them. Pharmacogenomics will be used for patient selection and exclusion, and provide differentiation criteria. In short, pharmacogenomics is a science that is gaining recognition and respect and could be the most promising of the several disciplines that have arisen from genomics to deliver three main genomics-based products: therapeutics, diagnostics and information.

Box 1. Definitions

Pharmacogenomics
The field of new drug development based on our rapidly increasing knowledge of all genes in the human genome.

Pharmacogenetics
The study of drug response as a result of heredity; this term is often used interchangeably with pharmacogenomics.

Functional genomics
The study of the relationships between particular genotypes and specific phenotypes.

Pharmacoproteomics
The study of subtyping patients on the basis of protein analysis.

Pharmacotherapy
The treatment of patients based on genetic analysis for the diagnosis and classification of diseases.

Pharmacodynamics
Variation of drug effects as a result of polymorphisms in receptors and other effectors, in contrast to polymorphisms in drug metabolism enzymes that lead to pharmacokinetic effects.

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Box 2. Pharmacogenomics tools

Subtractive cloning
Amplification by PCR of clones unique to diseased tissue, with respect to normal tissues, by using linkers that are ligated to the ends of the cDNA fragments (an inexpensive alternative to EST and cDNA microarray hybridization).

Differential display
Visualization of differences in gene expression on (polyacrylamide) gels where intensity of bands is quantitative (PCR-based).

Expressed sequence tag (EST) sequencing
The sequencing of clones from tissues of interest is performed to characterize changes in gene expression (using normalized or non-normalized cDNA libraries).

cDNA microarray hybridization
Expression levels and differential expression levels are measured by hybridization of RNA samples on cDNA microarrays and labeling cDNA pools, which are then produced from these hybridizations by incorporation of fluorescently labeled dCTP.

Serial analysis of gene expression (SAGE)
An accelerated version of EST sequencing in which unique sequence tags (13 or more bases) are generated for the cell or tissue of interest, to produce a library of clones where each clone has unique tags for 20 or more genes.

Specific examples are already emerging where the intervention of pharmacogenomics analysis is, or could be, useful in the clinical evaluation of drugs. A prime example relates to the six major families of cytochrome P450 enzymes that are important metabolizing enzymes in the liver. These enzymes can be affected by exogenous substances; for example, drugs can inhibit or induce the effectiveness of these enzymes. Just as importantly, drugs can be activated or inactivated by these enzymes. Thus, the variability of P450 isozymes can lead to drug underexposure or overexposure. Some people are deficient in or completely lacking these isoforms, for example, CYP2D6. In one specific instance, a patient in a clinical trial who lacked the 2D6 isoform was identified after hypotensive fainting-spells, which was caused by drug overexposure. Fortunately, the side effect in this instance was manageable. However, it is easy to imagine a scenario where a patient would be at great risk because of a lack of the 2D6 isoform. By contrast, no completely inactivating mutations have been found in the human CYP3A4 isoform that is responsible for the majority of drug metabolism, although a common polymorphism in the promoter has recently been described[3,4].

The term pharmacogenetics is often used interchangeably with pharmacogenomics, although pharmacogenetics is really a broader term that relates to the study of drug response resulting from heredity. More than 60 classifications of pharmacogenetic differences exist[5]. Most of the variations or polymorphisms occur in the drug metabolizing enzymes (DMEs) or cytochrome P450 enzymes. However, polymorphisms of drug transportation genes and genes that encode protein receptors and other effectors also lead to variations in drug response.

Significant data on both pharmacogenomics and pharmacogenetics is becoming available. Many examples of drug-induced changes in gene expression have been compiled[6]. For example, we know that gene expression levels of cyclooxygenase-2 (COX-II) and calcium-dependent phospholipase A_2 ($cPLA_2$) both change following ciprofibrate administration in rats[7]. In fact, an extensive table of cytochrome P450 drug interactions is available (http://www.dml.georgetown.edu/depts/pharmacology/davetab.html). This is an example of pharmacogenomics data. Likewise, we now have extensive compilations of clinically relevant genetic polymorphisms (SNPs) that influence drug metabolism[8]. For example, it is estimated that 2–10% of the population are homozygous for non-functional *CYP2D6* mutant alleles, which will not allow them to activate opioid analgesics[9]. This would explain why there is variability of pain relief among patients receiving the same dose of codeine, and is an example of pharmacogenetics. A comprehensive listing of genetic polymorphisms is also available (www.sciencemag.org/feature/data/104449.shl).

Functional genomics

Functional genomics, the study of the relationships between particular genotypes and specific phenotypes, is an important discipline that defines the causative connection between a particular genotype and a specific phenotype. Tracing these connections is a complex undertaking that has recently become more complicated. Drug response and disease susceptibility has centered on the identification of SNPs, which involve the changes of single bases within genes. However, recent studies suggest that the positioning and interaction of several SNPs in haplotypes might be more important to phenotype generation than single SNPs. Correlation of an individual's response to salbutamol was recently shown to be a result of multiple SNPs within a haplotype and correlation with individual SNPs could not be established[10]. In another study, it was shown that haplotypes for the μ-opioid receptor gene were associated with heroin and cocaine dependence. Although some are now convinced that the haplotype approach is crucial to recognize factors that predispose individuals

towards a pharmacogenetic phenotype and a particular disease trait, others still believe that single SNPs are just as important[11]. It is clear, however, that a diagnostic agent for single SNPs would be relatively straightforward and could involve a single assay, whereas an assay to define a combination of several SNPs would be costly. The combination of pharmacogenomics and pharmacoproteomics analyses will allow the assembly of responsive trial populations, which will allow us to monitor drug responses more efficently[12]. This subtyping will clearly lead us to therapy that is increasingly tailored to the genetic and molecular profiles of patient subgroups.

Pharmacoproteomics

Pharmacoproteomics is the study of patient subtyping on the basis of protein analysis. This mode of characterization is a more functional representation of patient-to-patient variation than is provided by genotyping, and includes the added effects of post-translational modification. Thus, pharmacoproteomics connects the genotype with the phenotype. This connection is not always predictable on the basis of genotyping alone. Consider the effects of 'silent' SNPs, which refer to base-pair changes in RNA that do not produce an altered amino-acid sequence in the proteins that are encoded[13]. One way in which a silent SNP can alter the phenotype is by a change induced in mRNA folding. This would lead to different interactions with proteins that regulate mRNA processing, stability and translation. In fact, understanding the mRNA conformational changes could lead to new drug targets, for example, an allele-specific target[14].

Pharmacotherapy

The anticipated and desired endpoint of pharmacogenomics is the ability to target a drug specifically to those patients who are genomically defined to respond well to the drug with no adverse effects. Thus, the new science of pharmacotherapy is the treatment of patients based on genetic analysis for the diagnosis and classification of diseases[15].

Polymorphism in drug metabolizing enzymes, such as the cytochrome P450 isozymes, was highlighted earlier and leads to pharmacokinetic effects following drug administration. In contrast to these effects is the variation of drug effects as a result of polymorphisms in receptors and other effector systems. This variation is termed pharmacodynamics.

Pharmacogenomics tools

At the heart of the science of pharmacogenomics is the evaluation of differential gene expression in biological tissues. Elucidation of these changes requires high throughput methods which are described in Box 2[16]

Challenges

The challenges to the industry created by pharmacogenetics and pharmacogenomics are significant and include the following:

- Genotyping will identify many new disease-related genes and provide an explosion of new targets to pursue; and
- Pharmacogenomics profiling will lead to patient stratification, and these new targets, as well as existing targets, will be divided into subsets.

It is estimated that genotyping will identify new disease-related genes that will lead to between 5,000 and 10,000 new potential targets. Because the current amount of targets is approximately 450 and is comprised of mainly four target classes, such as G-protein-coupled receptors (GPCRs), ion channels, nuclear hormone receptors and enzymes, these new targets will add genomic and medicinal diversity[17].

Pharmacogenomic profiling of patients will increase the amount of drugs that we will need to design to target a more segregated patient population. Thus, blockbuster drugs (or flagship products) will be replaced with subsets of compounds that, together, comprise a blockbuster drug class. Many benefits to patients will follow from this change. It has been estimated that, currently, as few as one-third of patients taking prescription medicines actually derive the intended benefit. Adverse drug effects among the remaining two-thirds could be as high as two million per year, with up to 100,000 of these being fatal[18].

The significant burst in new targets from genotyping and the subsetting of these targets as a result of patient stratification will lead to increased demands for lead optimization. Biotechnology companies such as ArQule (Medford, MA, USA), which specialize in the use of eADMET (early-ADMET) bioassays and predictive tools for designing drug-like molecules, can help to fill this gap by partnering with large pharmaceutical companies on lead optimization collaborations.

Opportunities

What conclusions can we draw regarding the challenges that lie ahead? We must conclude that as a scientific community we now have a multitude of opportunities to work together in delivering drugs to patients with the challenges of pursuing many new targets and the subsetting of these targets as a result of patient stratification. Past paradigms are good models for the present.

Medical advances in the global scientific community are hastened when industry, academia and government sectors

work together. Consider the strides that have been made by the international Human Genome Project in mapping the 3.1 billion base-pairs that comprise the human genome[19]. Add to this the progress made in genotyping by the collection of companies that comprise the SNP consortium in identifying the disease-related SNPs (or bundles of SNPs) in the hundreds of thousands of SNPs being identified. To handle the enormous amount of information that is being generated by these projects, the US Department of Energy (Washington, DC, USA), Celera Genomics (Rockville, MD, USA) and Compaq (Houston, TX, USA) is planning to team up to create a computing system that can process 100 trillion operations per second.

Multiorganizational genomics projects have also set an example for other major collaborations. For example, a group that has recently formed to tackle the huge challenge of mapping the complex mechanisms of cellular signaling is the Alliance for Cellular Signaling (http://www.cellularsignaling.org). This collection of 50 investigators at 20 universities will computationally create a virtual cell that could provide a drug discovery engine for virtual screening and thus lessen the need for expensive and time-consuming rodent, primate and human testing.

The industry now needs effective collaborations between motivated partners to pursue the many new potential targets that are emerging from genomics research and the many new subsets of targets that are being defined by pharmacogenomics and pharmacogenetics. Pharmacogenomics, by providing new potential targets, will lead to a novel bottleneck, namely, lead optimization. Pursuit of these newly defined targets with collaborative efforts, using rapid-cycle protocols, can efficiently produce the drugs of the future.

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