PHARMACOCOGENETIC APPLICATION IN DRUG DEVELOPMENT AND CLINICAL TRIALS

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ABSTRACT:
Pharmacogenetics examines the genetic characteristics of individuals to understand variations in response to therapeutics. This approach has the potential to significantly affect the development of new medicines. The application of pharmacogenetic principles could yield significant time and resource savings within the drug development process. In preclinical drug development, pharmacogenetics could be applied to compound screening and identifying potential side effects before entering full clinical testing. Subpopulations of patients with different drug responses and underlying genetic markers could be stratified in clinical trials by analyzing their genotype. These data can improve clinical trial design and offer the possibility of optimized drug prescription based on patient genotype. Pharmacogenetics can guide the development of therapeutic interventions by identifying nonresponder patient groups. Advances in high-throughput genotyping technologies have added potential by facilitating the technical hurdles and improving drug development strategies, clinical trial design, and postmarket pharmaco-vigilance. Pharmacogenetics, thus, impacts all phases of drug development and will fundamentally change the practice of medicine in the near future.

Pharmacogenetics is an emerging scientific discipline that examines the genetic basis of individual variations in response to therapeutics. Genetic polymorphisms modulate pharmacological and toxicological reactions in individuals upon exposure to drugs (Weber, 1997; Kleyen and Vesell, 1998; Evans and Relling, 1999). Kinetic variations in absorption, distribution, metabolism, and excretion of therapeutic agents are well known and have been studied extensively during the past two decades (Meyer, 1990; Kalow, 1992). More recently, pharmacodynamic variations, including receptor and transporter polymorphisms, have been shown to cause individual variations in drug responses (Evans and Relling, 1999). Understanding the role of genetic polymorphisms in drug responses will help to ensure drug efficacy and decrease the incidence of adverse effects by tailoring medications according to patients’ genetic profiles. Advances in this area have important implications in the design of dose regimens and the adequacy of drug prescriptions. During discovery and development of therapeutic agents, pharmacogenetics can expedite development of targeted therapeutic interventions when the pharmacophore is designed for specific responder patient groups. Genetic stratification of patients in clinical trials can enhance the statistical power and use a smaller number of subjects, providing substantial time and resource savings in drug development, not withstanding the time savings during the drug registry review. Therefore, advanced technologies that identify genetic polymorphisms rapidly, accurately, and economically are of significant value to pharmaceutical research and development.

Genetic Variations in Drug Response
Interindividual variations in therapeutic response often are genetically based and result in differences in metabolic pathways of drug action and elimination (Evans and Relling, 1999). Genetic differences in the absorption, distribution, metabolism, and excretion of therapeutics lead to different plasma concentrations or excretion profiles, resulting in a lack of efficacy or evoking toxic effects (Benet et al., 1996).

Pharmacokinetic variations impact drug responses, and when compounds are taken up, they typically undergo phase I and II metabolism (Benet et al., 1996). Recent research identified functionally important genetic variations in virtually all phase I and II enzymes, leading to variations in metabolic profile among individuals (Weber, 1997). Polymorphisms in drug-metabolizing enzyme genes were discussed in recent reviews (Weber, 1997; Evans and Relling, 1999).

Cytochrome P450 2C9 (CYP2C9) is an example of a well characterized phase I drug-metabolizing enzyme with multiple functionally important variants. This liver microsomal isozyme is responsible for the oxidative metabolism of the commonly used anticoagulant warfarin (Rettie et al., 1994). CYP2C9 has two clinically distinct phenotypes: normal (extensive) and slow (poor) metabolizers. In poor metabolizers, warfarin metabolism is significantly reduced, and the drug remains longer in the circulation, leading to prolonged drug effects. Therefore, a wide range of interindividual responses to a given dose of warfarin is recognized, necessitating dose titration for each patient from 1 to 60 mg/day (James et al., 1992; Hallak et al., 1993). A serious complication of prolonged oral anticoagulant includes severe hemorrhage with a high frequency in slow metabolizers of CYP2C9 receiving conventional doses of warfarin (Fihn et al., 1996; Aithal et al., 1999). Therefore, individualization of the dosing regimen is a routine clinical practice in order to avoid bleeding or other complications while achieving optimal therapeutic benefit. The two allelic variants CYP2C9*2 and CYP2C9*3 in the coding region of the gene are associated with impaired hydroxylation of warfarin. CYP2C9*2 has a single nucleotide polymorphism resulting in a cysteine substitution for arginine at codon 144 in exon 3. The CYP2C9*2 homozygous variant protein results in 12% of enzyme activity compared with the wild type (Rettie et al., 1994). CYP2C9*3
has an adenosine to cytosine polymorphism in exon 7, resulting in isoleucine to leucine substitution at codon 359 (Table 1). The homozygous CYP2C9*3 variant protein has approximately 5% of the normal enzyme activity (Haining et al., 1996).

In addition to altering drug-metabolizing enzymes, genetic variations in receptors and transporters can produce variations in drug response. A drug interacting with a polymorphic receptor may have reduced affinity. Therefore, this drug may have reduced efficacy in patients carrying this polymorphism. This situation exists for the β2-adrenergic receptor (β2AR). Agonists for this receptor are widely used in the treatment of asthma, with genetic polymorphisms of β2AR leading to specific responder and nonresponder phenotypes. Several β2AR receptor polymorphisms have been reported, one of which involves an amino acid substitution from arginine to glycine at codon 16 (Reihsaus et al., 1993; Turki et al., 1995). This substitution is associated with increased down-regulation of β2AR (Turki et al., 1995). Homozygous wild-type and heterozygous individuals respond more predictably to the β2AR agonist albuterol than do homozygous variant patients (Martinez et al., 1997).

Genetic polymorphisms of transporters also impact pharmacologic effects. The selective serotonin reuptake inhibitor fluvoxamine is a commonly prescribed drug for treatment of delusional depression (Catalano, 1999). The prime target of this drug is the 5-hydroxytryptamine transporter (5-HTT), which plays an important role in neurotransmission. A 44-base pair insertion polymorphism in the 5-HTT promoter region is associated with increased transcriptional activity (Lesch et al., 1996). Individuals with homozygous wild-type trait of the 5-HTT promoter respond better to fluvoxamine than do heterozygous or homozygous patients with the deletion polymorphism (Smeraldi et al., 1998).

Table 1 lists examples of drugs and the pharmacogenetic markers that cause or are recognized by variable drug responses. In general, drug responses can be linked to variations in three types of genes: 1) drug-metabolizing enzyme genes; 2) drug action pathway genes; and 3) disease-related or disease pathway genes. While these examples (CYP2C9, UDP-glucuronosyltransferase 1A1, β2AR, cholesterol ester transport protein, ApoE) represent effects mediated by a single gene, most pharmacogenetic and disease effects are polygenic. With the rapid progress of the Human Genome Project and high-throughput screening technologies, it is expected that more pharmacogenetic markers will be identified in the near future.

### Detection of Genetic Polymorphisms

Genetic polymorphisms are detected by phenotyping or genotyping. Phenotypes are collected, observable characteristics of a cell or organism, usually monitored by direct observations or through specific biochemical or functional analyses. In pharmacogenetics, phenotypes are monitored by low or exaggerated pharmacological effects, frequency of side effects, and different metabolic rate. Procedures for evaluating metabolic capacity involve administering a probe drug and measuring the ratio between the parent drug and its metabolite in urine, plasma, or other tissues (Fontana and Watkins, 1995). These procedures involve analytical techniques, which are usually time consuming, burdensome, and frequently require repeated sample collection. Metabolic phenotyping can be influenced by sample stability and external factors, such as age, nutritional state, general health, and concurrent medications (Linder et al., 1997). Once a genetic association is established, these limitations are circumvented by genotyping. Genotyping identifies individual DNA structure differences for particular traits independently of functional effects. This approach increasingly is being used in biomedical research and molecular diagnostics. Genotyping is relatively easy to perform and generally requires a small sample of peripheral blood or buccal swab from patients. Therefore, it is less invasive than phenotyping and is not influenced by drug-drug or drug-food interactions. Commonly used genotyping methods include polymerase chain reaction (PCR)-restriction fragment length polymorphism, allele-specific PCR, fluorescent dye-based high-throughput genotyping, mass spectrometry, and gene chip technology (for review, see Shi et al., 1999a).

The TaqMan Allelic Discrimination assay is a high-throughput genotyping method that uses the 5′-nuclease activity of Taq polymerase to detect a fluorescent reporter signal generated during or after PCR reactions (Livak et al., 1995). For genotyping single nucleotide polymorphisms, one pair of TaqMan probes and one pair of PCR primers are used. Each TaqMan probe consists of 20- to 40-base pair oligonucleotides complementary to the polymorphic region. The two TaqMan probes differ only at the polymorphic site, with one probe complementary to the wild type and the other to the variant. A 5′-reporter dye (6-carboxy-4,7,2′,7′-tetrachlorofluorescein; TET) and a 3′-quencher dye (6-carboxy-N,N,N′,N′-tetrachlorofluorescein; TAMRA) can be covalently linked to the wild type probe. Similarly, the variant probe can be labeled with a 5′-reporter dye (6-carboxy-fluorescein; FAM) and the same 3′-quencher dye TAMRA. When the TaqMan probe is intact, fluorescence is quenched due to the physical proximity of the two dyes (Clegg, 1992). During the PCR annealing step, the TaqMan probes hybridize to the targeted polymorphic site within the forward and reverse primer regions. During the extension phase of the PCR reaction, the 5′-reporter dye is cleaved by the 5′-nuclease activity of the Taq polymerase, leading to an increase in characteristic fluorescence from the reporter dye (Fig. 1). By measuring the fluorescent intensities of TET and FAM signals immediately after the PCR reaction, the specific genotype can be determined. Several high-throughput TaqMan genotyping methods for detecting single nucleotide polymorphisms and deletion polymorphisms have

### Table 1

<table>
<thead>
<tr>
<th>Polymorphic Genes</th>
<th>Drugs</th>
<th>Pharmacogenetic Effect</th>
<th>Reference</th>
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<tbody>
<tr>
<td>CYP2C9</td>
<td>Warfarin</td>
<td>Hemorrhage in poor metabolizers</td>
<td>Rettie et al., 1994</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>Irinotecan</td>
<td>Severe diarrhea in poor metabolizers</td>
<td>Iyer et al., 1999</td>
</tr>
<tr>
<td>β2-Adrenergic receptor</td>
<td>Albuterol</td>
<td>Responder with Arg-16 genotype</td>
<td>Martinez et al., 1997</td>
</tr>
<tr>
<td>CETP</td>
<td>Pravastatin</td>
<td>Responder with TaqB1 genotype</td>
<td>Kuivenhoven et al., 1998</td>
</tr>
<tr>
<td>5-Lipoxigenase</td>
<td>Zileuton</td>
<td>Responders with wild-type 5-LO promoter</td>
<td>Drazen et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nonresponders with variant 5-LO promoter</td>
<td></td>
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</tbody>
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CETP, cholesterol ester transport protein; UGT1A1, UDP-glucuronosyltransferase 1A1.
Significant response was elicited when patients were stratified according to ApoE subtype (Richard et al., 1997).

**Applying Pharmacogenetics in Clinical Research**

Pharmacogenetics holds great potential for facilitating the drug discovery process and subsequent clinical study. With the progress of the Human Genome Project and functional genomics, massive increases in the information available on individual genes and functionally important polymorphisms related to disease will emerge (Evans and Relling, 1999). In 1997, the U.S. Food and Drug Administration issued a guidance for industry and supported pharmacogenetic testing throughout the drug development process (United States Food and Drug Administration, 1997). Understanding how to adjust dose to minimize toxicity may allow marketing a drug that otherwise would have an unacceptable rate of adverse effects because its toxicity was unpredictable and unpreventable without pharmacogenetic tools. When genetic polymorphisms affect important metabolic routes of elimination, dosing adjustments may achieve the safe and effective use of a drug. Identifying metabolic differences in patient groups based on genetic polymorphisms would provide improved treatment recommendations and product labeling, thereby promoting the safe and effective use of a drug. An example of this is omeprazole (Prilosec), an inhibitor of the H\(^+\)/K\(^+\) ATPase enzyme system at the secretory surface of the gastric parietal cell, used for treatment of ulcer and gastroesophageal reflux disease (Bustamante and Stollman, 1999). In pharmacokinetic studies of omeprazole (single dose), an increase in area under the curve of approximately 4-fold was noted in Asian subjects compared with Caucasians (Johnson, 1997; AstraZeneca, 1999). The area under the curve difference was due to different metabolic rates of the drug, which is a substrate for CYP2C19 (Cupp and Tracy, 1998). Approximately 20% of Asians are homozygous for variants of the CYP2C19 gene resulting in poor metabolizer phenotype (De Morais et al., 1993). Therefore, the dose administered to Asian patients with poor metabolizer genotypes and patients with impaired hepatic function is reduced (Johnson, 1997). These examples of pharmacogenetic information will help to control or reduce adverse responses to drugs and reduce the costs associated with therapeutic failures. For drugs prescribed on a limited basis due to a high incidence of adverse effects, pharmacogenetics may provide the means to identify those most likely to benefit therapeutically without the development of adverse reactions.

Another potential application of pharmacogenetics is in the strategic design of clinical trials to increase the information obtained from each study. Identification of potential responder populations through genetic screening before clinical trial enrollment will allow demonstration of drug efficacy in a smaller set of subjects. This approach was relevant for trastuzumab (Herceptin), a monoclonal antibody for treatment of late-stage breast cancer, and only patients with tumor cells overexpressing HER2 gene would benefit from this drug (Shak, 1999). Therefore, patients were tested for this marker before receiving the drug. With the rapid progress in this area, the advent of validated pharmacogenetic markers could be included in clinical trials to increase the demonstration of therapeutic benefits without exposing “nonreceptive” subjects. Genotyping information also can be used to understand outliers in plasma concentration or therapeutic profiles related to genetic determinants.

With the emerging global development, new pharmaceutical agents are tested or developed in multiple countries. However, due to the differential distribution of genetic polymorphisms in ethnic groups (Weber, 1997), a well developed and extensively tested drug evaluated in one country might not be suitable for patients with pharma-
cogenetic traits from other countries or continents. Genotyping data from multiple ethnic groups will allow an early identification of drug efficacy and toxicity, and has the potential to reduce the need for conducting pivotal clinical trials in multiple countries, with significant resource savings, shortening development time and reducing the costs of new drugs.

Conclusions

The study of pharmacogenetic differences holds the potential to improve therapeutic effectiveness and limit toxicities of available drugs. Pharmacogenetics can provide substantial efficiency in clinical research by facilitating the conduct of smaller clinical trials by targeting groups of patients with similar genetic background. The approach of rigorous determination of genotype/phenotype relationships in individual drug responses will provide physicians and researchers with the key information that allows them to precisely prescribe or design the right drug, at the right dose, for the right patient. This singular individualized approach to therapeutics is enabled by high-throughput genotyping and will provide significant public health benefits to the population at large.

References


