DDT Vol. 6, No. 4 February 2001

Clinical pharmacogenomics: applications in pharmaceutical R&D

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Within the pharmaceutical industry, the application of clinical pharmacogenomics promises to enhance the discovery of drug response markers, reduce the size and expense of clinical drug trials and provide a new tool for addressing regulatory approval issues. Today, pharmacogenomics is primarily applied early in clinical drug development by prospective genotyping in Phase I trials, to ensure that a subject population is representative with respect to drug metabolism phenotypes. The banking of genetic material from later stage trials for retrospective studies on drug response is becoming more frequent, but is not yet standard in the industry. This article provides an overview of the driving forces that are encouraging pharmacogenomic strategy development in the pharmaceutical industry, and the significance of polymorphisms in drug metabolizing enzymes (DMEs) and target proteins.

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▼ In his 1997 book, *Pharmacogenetics*[1], Wendell W. Weber quotes from Somerset Maugham's account of his experiences as a young medical student[2]. This quote aptly introduces the theme of Weber's excellent work, and because pharmacogenomics is an extension of pharmacogenetics, it is also a fitting introduction for this article:

> I have always worked from the living model. I remember that once in the dissecting room when I was going over my 'part' with the demonstrator, he asked me what some nerve was and I did not know. He told me; whereupon I remonstrated, for it was in the wrong place. Nevertheless he insisted that it was the nerve I had been in vain looking for. I complained of the abnormality and he, smiling, said that in anatomy it was the normal that was uncommon. I was annoyed at the time, but the remark sank into my mind and since then it has been forced upon me that it was true of man as well as of anatomy. The normal is what you find but rarely. The normal is the ideal. It is a picture that one fabricates of the average characteristics of men, and to find them all in a single man is hardly to be expected.

Maugham's observation – that the normal is rare – is at the heart of the challenge and the promise of pharmacogenomics.

Historical perspective

Friedrich Vogel originally proposed the word 'pharmacogenetics' to describe studies of the genetic basis of therapeutics[3]. The first book on the subject, Kalow's *Pharmacogenetics – Heredity and the Response to Drugs*, was published in 1962[4]. Pharmacogenetics is the combination of genetics, pharmacology and biochemistry, whereas pharmacogenomics is a broader and more recent term that adds the newer sciences of molecular biology, genomics and bioinformatics and their associated technologies. Although the field has grown and its scope has enlarged dramatically over the past decade, its purpose remains the same: to understand the underlying reasons for differential human response to xenobiotics.

The notion that people differ in their response to medicines is not new. Although a broad generalization, it has been estimated that as few as one-third of individuals can derive the intended therapeutic benefit from a prescribed medicine. In the remaining two-thirds, the medication either does not work as intended or is poorly tolerated. Certainly, the observation of extraordinary responses is relatively common. Adverse drug effects among hospitalized patients might be as high as two million per year; up to 100,000 of these cases prove fatal[5]. What is relatively new is our knowledge of the influence of heredity on these individual responses, and our opportunity to apply that knowledge.

1359-6446/01/$ – see front matter PII: S1359-6446(00)01648-2

At the turn of the 20th century, Archibald Garrod suggested that genetic material could play an important role in the biotransformation of chemicals[6]. However, it was not until the 1950s that the relationship of drug response to individual genetics was described for a few compounds, including primaquine and isoniazid[7].

With the completion last year of the initial draft of the human genome map, we find ourselves in what has been described as the post-genomic era. Numerous articles have predicted the effects that the genomics revolution will have on the drug development industry and on patient healthcare in this new age. Most of the excitement in the field is centered on understanding the genetic basis of susceptibility to common, complex disease, and on elucidating differences in patient response to drugs. The successful completion of the mapping and sequencing of the human genome will certainly lead to the discovery of more disease-causing genes. However, the first clinically useful application of this effort will probably be the ability to predict, from genetic tests, who is likely to benefit from a medicine and who is likely to suffer a toxic side effect. This ability to use medicines more effectively is the ultimate application of clinical pharmacogenomics.

The value of pharmacogenomics to the pharmaceutical industry

In the context of drug discovery and development, clinical pharmacogenomics is the use of genetic information, from a population or from an individual, to predict the safety, toxicity and efficacy of drugs, either as part of a drug development program or as part of an individual's diagnosis and treatment regimen. It encompasses genetically determined variability in drug response in and across populations. Much of this variability is because of both polymorphisms in drug metabolism (i.e. pharmacokinetic effects), and polymorphisms in drug receptors and other effectors (i.e. pharmacodynamic effects). The scope of clinical pharmacogenomics includes:

- the identification and characterization of candidate genes and polymorphisms;
- the correlation of polymorphisms with therapy, clinical outcomes and drug effects; and
- the development of molecular genetic tests for prediction of drug response, or drug selection and dosing based on genotype or gene expression.

Innovation deficit

Pharmaceutical companies are facing an 'innovation deficit' in drugs entering the development pipeline and they are looking for new ways that significantly improve their productivity and increase the number and quality of new drugs in their development pipelines. Traditional approaches to small-molecule drug discovery and development are reaching the limit of their ability to yield innovative new drugs. In 1995, 75 of the top 100 drugs on the market targeted only four families of molecular targets. The number of targets addressed today is less than 450 of the 10,000 targets estimated in the human genome. Target diversity is limited to G-protein-coupled receptors (60% of drugs on the market), ion channels, nuclear hormone receptors (e.g. steroid receptors), and enzymes (e.g. serine proteases). It is also significant that targets are not medically diverse: one-third of the drugs on the market (excluding antibiotics) address CNS disorders and another third address cancer and blood diseases[8].

Rising R&D costs

The financial community expects the pharmaceutical industry to grow by 13% Earnings per Share (EPS) annually. However, the projected growth under traditional models of drug development is less than 8% EPS. Financial projections suggest that pharmaceutical companies will require 3–5 new chemical entities (NCEs) approved each year to reach even a 10% EPS growth rate. To increase its annual-growth rate, the pharmaceutical industry must reduce the time and cost for drug discovery and development, which currently requires an average of 10–12 years and US$500 million or more per compound[9].

Regulatory approval

Failure to address specific issues during the regulatory review process is often cited as the cause of delay or failure to approve new drug applications (NDAs). In particular, three areas have been repeatedly identified by FDA advisory committees as problematic[10]:

- problems characterizing dose–effect relationships, including inadequate delineation of dose tolerance and therapeutic ratios;
- issues in experimental design and clinical development plans, including failure to adequately address the proposed indication; and
- difficulty in measuring risk:benefit endpoints: during the drug development and regulatory review process, both sponsoring pharmaceutical companies and regulators must assess the toxicological price associated with the approval of a new drug. Factors include the overall safety of the compound relative to other available therapeutic agents, the proposed indication and severity of disease, and the likelihood of use in high-risk populations.

Clinical pharmacogenomics has the potential to improve productivity and increase the number and quality of new drugs in drug development pipelines by:

- validating more genomically diverse and higher-quality drug targets;
- eliminating unsuitable drug candidates and targets at the earliest and lowest cost stages of development;
- speeding up clinical development by designing better trials that clearly show improved safety, efficacy and compliance; and
- developing drugs with optimized risk:benefit profiles that clearly demonstrate improved medical outcomes in targeted patient populations.

Industry analysts predict that, by improving medical outcomes by the use of pharmacogenomics-enhanced drugs and diagnostics, pharmaceutical companies could benefit to the order of US$200 million to US$500 million in extra revenue for each drug. Patients, physicians and managed-care organizations will also benefit from more effective treatments and lower overall healthcare costs. For these reasons, pharmaceutical companies have begun to integrate pharmacogenomics into drug development programs. During the next three to five years, it will become an essential tool in the industry.

Clinical discovery using DNA banking

Although individual human genomes are 99.9% identical, the 0.1% difference predicts as many as three million polymorphisms, the most common being the single nucleotide polymorphism (SNP). Many polymorphisms in the 100,000 or so genes in the human genome will have no effect. Some, however, will affect protein expression and function, resulting in phenotypes affected for disease and drug response. Industry strategies for the discovery and validation of relevant polymorphisms include banking DNA from late-stage drug trials where patients are well defined with respect to ethnicity, disease characteristics and drug response. Retrospective studies on these genome banks attempt to identify and validate relevant polymorphisms by employing high-throughput sequencing and genotyping methods, in conjunction with commercial, public or proprietary DNA databases and bioinformatics tools. Once identified and characterized in terms of expression, functionality and frequency, an association of the polymorphism with disease, disease progression or drug effect must be established to confirm commercial potential. These validated genetic markers will have potential applications in accelerating the identification of lead compounds, in choosing patients for clinical trials and in developing molecular diagnostics.

The current barriers to this approach arise from the likelihood that the number of SNPs targeted and the number of subjects required to investigate them is quite large[11]. In addition, the current costs for high-throughput methods (around US$1 per SNP) will probably hinder whole genome-based studies for the immediate future and favor a less inclusive candidate gene approach. For the moment, success in this area depends on access to sophisticated bioinformatics tools and data, effective candidate gene selection and access to appropriate populations.

Pharmacogenomic profiling in clinical drug trials

Today, the clinical application of pharmacogenomics occurs relatively early in clinical drug development, mainly in Phase I studies, and is centered mostly on the underlying genetic differences that result in variation in an individual's ability to metabolize drugs. Initial Phase I applications consist of prospective approaches that attempt to control pharmacokinetic variability or adverse events. This can be achieved by enrolling subjects based on genotypes that predict a subject's metabolic capacity via specific pathways. Genetic polymorphisms in these pathways are prevalent in individuals and within ethnic groups. Because there is a limited number of subjects enrolled in a Phase I trial, it is beneficial to genotype prior to enrollment to ensure a representative population, including appropriate percentages of each metabolic type.

Fig 1. depicts the observed CYP2D6 metabolic type variability across 15 clinical sites. The sites were all within the USA and the population primarily Caucasian. The percentage of the poor metabolizer type varied from 0% to 15%[12]. This site-to-site variability can have a significant impact on Phase I safety studies. If the sites selected include only sites 1, 4 and 15 (100% extensive metabolizers), systemic drug levels and kinetics would probably be more consistent than if sites 2, 3, 11 or 12 (11%–15% poor metabolizers) were included. Because systemic drug levels and kinetics are causative factors influencing adverse events, decisions on dosing levels in Phase II trials or drug progression are impacted. By prospective genotyping to ensure a representative population across all sites, this source of variability can be controlled, and a higher level of confidence achieved to support decisions on continuing drug development.

As an alternative or supplement to prospective genotyping, archiving DNA from subjects enrolled in Phase I studies yields a DNA library that enables retrospective genotyping. Retrospective genotyping can help explain pharmacokinetic outliers and identify subjects that could be at risk of adverse events in later trials.

It is generally recognized that clinical drug development strategies need to consider the potential of pharmacogenomics to impact both pharmacokinetics and pharmacodynamics. There is a trend, therefore, to apply pharmacogenomics in later-stage trials where markers of drug efficacy might be employed or identified and fed back into

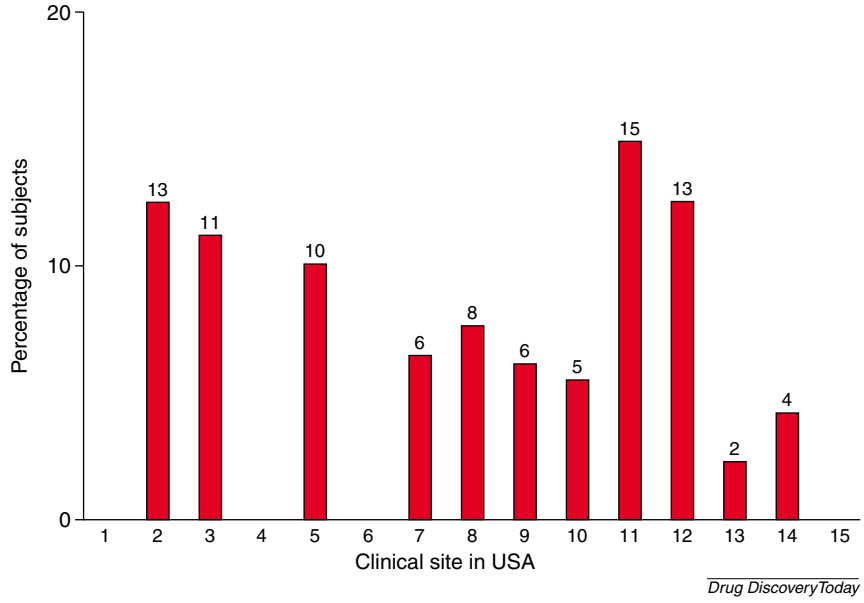

Figure 1. The incidence of CYP2D6 poor metabolizers (PMs) across 15 clinical trial sites in the USA. The population is primarily Caucasian. The average percentage of PMs across all sites was 6%; the range was 0%–15%.

the discovery process. Pharmacogenomic profiling can be used to stratify trials based on patients who are most likely to benefit from therapy. Moreover, profiling can be used to exclude subjects that are likely to have adverse events related to DMEs, thus enhancing tolerance and compliance during the trial and during post-marketing use. Profiling can also help to differentiate novel drugs from compounds already on the market. In these late-Phase trials, the archived DNA is more valuable because it adds the opportunity to identify pharmacodynamic markers that distinguish responders from non-responders. As archived DNA accumulates, it provides a highly specific, well-attributed DNA library for future research.

The main opportunity for clinical pharmacogenomics is between Phase II and Phase III research, when crucial economic decisions are made. A Phase II trial that is failing because of toxicity or lack of efficacy could be redesigned using pharmacogenomic markers. Efficacy can generally be demonstrated only in a proportion of trial patients. For instance, the non-response rate to tricyclic antidepressants can range from 20% to 50%, beta-blockers from 15% to 35% and anti-neoplastics from 20% to 70%. Limited efficacy affects the size and cost of Phase III studies. Advancing a drug from a large Phase II drug trial using traditional approaches means that any resulting Phase III trial needs to be large, because the number of patients who did not respond dilutes the measurement of efficacy. Generating a pharmacogenomic profile to identify the Phase II trial patients who are likely to benefit from a drug means that a resulting Phase III trial can be more efficient in size, time and costs. Several of the many existing compounds that failed to show efficacy in large trials might then prove safe and effective in a smaller but identifiable patient population.

The concern that such pharmacogenomic stratification of patients restricts the patient population for a given drug is valid; but that population represents a market where the drug has most benefit. The high efficacy and low adverse events expected in those populations translate into high clinical utility and shorter, easier development. In addition, any drug developed using this strategy would tend to exclude 'me-too' competition and be highly valuable to patients. The application of pharmacogenomics does not limit the population in which a drug will ultimately prove to be effective; it will simply allow the recognition of that population early in the drug's life cycle and mitigate the lengthy, expensive and sometimes tragic validation process of prescribing by trial and error.

Pharmacokinetics and polymorphisms in DMEs

Pharmacokinetics was the initial area of clinical research to apply pharmacogenomics, and it remains the most active. One reason is that there are few DMEs responsible for metabolizing the majority of today's marketed drugs, and there are also few relevant polymorphisms within those enzymes[13]. Table 1 shows the primary routes of Phase I metabolism for most drugs. It should be qualified by

Table 1. Drug metabolism[a] by the major families of CYP450 enzymes

CYP450 isoform	Percentage of drugs metabolized
CYP3A4	55
CYP2D6	20
CYP2C19	15
CYP1A2	5
CYP2E1	1
Others	4

[a]It should be noted that there are a minority of drugs that do not undergo biotransformation by CYP450 enzymes prior to elimination.

noting that there are a minority of drugs, that do not undergo cytochrome P450 metabolism but are eliminated directly or through other biotransformation processes.

Genetic polymorphism in DMEs gives rise to three distinct subgroups that have measurable differences in their ability to metabolize drugs to either inactive or active metabolites. Individuals capable of efficient drug metabolism are called extensive metabolizers (EMs) and individuals with deficiencies in metabolism, which typically requires mutation or deletion of both alleles of a gene, are termed poor metabolizers (PMs). Conversely, gene amplification and subsequent over-expression results in ultra rapid metabolizers (UMs). Standard doses of drugs with a steep response curve or a narrow therapeutic range could produce adverse drug reactions, toxicity or decreased efficacy in PMs. However, when taken by UMs, the standard dose might be inadequate to produce the desired effect, or, if the active agent is a metabolic product, could result in an effective overdose.

CYP2D6

Mutations leading to CYP2D6 enzyme deficiency are found in 7%–10% of Caucasians and 1%–2% of Asians. In the context of treatment or clinical trial research, these variants can affect initial dose setting and result in either overdose or in the inability to maintain a therapeutic efficacy. For instance, an effective, tolerable nortriptyline dose in PMs is 10–20 mg, compared with the usual 75–150 mg dose given to most patients. Conversely, UMs can require a dose increase to at least 500 mg[14]. Medicines that are metabolized by CYP2D6 include many commonly prescribed analgesics, antidepressants, antipsychotics and cardiovascular drugs. The interpretation of genotype–efficacy/toxicity relationships, particularly for CYP2D6, is complicated further by the metabolic production of pharmacologically active metabolites in several drug classes, including antidepressants.

CYP2C19

Mutations in the *CYP2C19* gene that result in compromised drug metabolism are found in 2%–5% of Caucasians and 18%–23% of Asians. Three-quarters of all PMs are accounted for by one allele; there is a unique allele in Asians that accounts for 25% of PMs in that population[15]. There is a correlation between those polymorphisms and both the pharmacokinetics and pharmacodynamics of drugs, such as citalopram, clomipramine, diazepam, propranolol, omeprazole and the tricyclic antidepressants. As an example, individuals with CYP2C19 polymorphisms resulting in inactive enzyme show higher omeprazole levels and increased drug response, as measured by the surrogate marker, plasma gastrin[16].

CYP3A4

CYP3A4 is responsible for metabolizing an estimated 55% of all clinically used drugs. Substrates of CYP3A4 include acetaminophen, alprazolam, carbamazepine, cyclosporin, erythromycin, lidocaine, lovastatin, nifedipine, tamoxifen, verapamil and vinblastine. However, CYP3A4 does not exhibit extensive genetic variation, and non-genetic factors are likely to play the dominant role in explaining the differential human response to drugs metabolized by this pathway. Despite speculation on the presence of genetic variants in CYP3A4 that might account for the observed high interindividual variability in expression, only recently has a variant been discovered in the 5′ region of the gene. In 1998, Rebbeck and colleagues[17] reported a polymorphism in the nifedipine-specific response element of the promoter region of CYP3A4. This polymorphism (CYP3A4-V) occurs in 9% of Caucasians and 53% of African-Americans but not in Taiwanese-Asians[18]. In one study, CYP3A4-V appears to be associated with a lower occurrence of treatment-related leukemia following administration of chemotherapeutic agents metabolized by CYP3A4[19]. A study using erythromycin and nifedipine probes found no difference in the rate of CYP3A4-dependent metabolism associated with this mutation[20]. However, Wandel and coworkers have associated this mutation with significantly lower systemic clearance of midazolam and reduced hepatic CYP3A activity[21].

Pharmacodynamics and drug targets

Many drugs work by interacting with specific protein targets, such as receptors, enzymes and transporters. Many of these targets have been shown to have polymorphisms that can influence an individual's response to specific medicines. Furthermore, polymorphisms in known disease pathways can predict the potential efficacy of a specific drug. There are several examples in which retrospective genotyping studies have elucidated clinically relevant associations between genetic polymorphisms in drug targets and disease pathways, and with certain drugs.

Cholesteryl ester transfer protein (CETP)

A polymorphism, termed B1, in CETP determines the efficacy of pravastatin in patients diagnosed with coronary atherosclerosis. The absence of the polymorphism is referred to as B2 and is associated with reduced pravastatin efficacy. Patients homozygous for the polymorphism (B1B1 individuals) tend to exhibit higher baseline plasma CETP levels and lower high-density lipoprotein (HDL) levels than those that are either heterozygous (B1B2) or homozygous (B2B2). The cholesterol-lowering drug pravastatin is more effective in B1B1 individuals, less

so in B1B2 individuals, and poorly effective in B2B2 individuals[22].

β Adrenoceptors

Recently described polymorphisms in β adrenoceptors affect their sensitivity to agonists such as albuterol. Asthma patients carrying the ^{16}Gly polymorphism show increased desensitization to albuterol-mediated downregulation of the receptor, compared with those carrying ^{16}Arg. When compared with homozygotes for ^{16}Gly, homozygotes for ^{16}Arg and heterozygotes were fivefold and twofold more likely to respond to albuterol, respectively[23].

Serotonin neurotransmitter receptors (5-HT$_{2A}$)

Meta-analysis performed on several studies of patients taking the antipsychotic drug clozapine have associated polymorphisms in the 5-HT$_{2A}$ receptor with improved drug efficacy. Patients carrying a thymidine cytosine conversion at position 102 are particularly likely to respond to clozapine therapy[24].

Other reported associations with putative drug targets include angiotensin-converting enzyme (ACE) and sensitivity to ACE inhibitors, and apolipoprotein E (APOE) in the responsiveness of patients with Alzheimer's disease to tacrine therapy. The discovery and validation of new polymorphism associations will arise from the collection of DNA libraries as a part of current clinical trials and is a mission for future retrospective studies.

Conclusions

Pharmacogenomics is an important tool in the design and interpretation of clinical trials because it contributes to a more precise definition of disease, correlates drug response to genetic markers, predicts dose response and adverse events, allows for representative subject populations and allows patient stratification.

The potential benefits of pharmacogenomic profiling in clinical trials include:

- reduction of drug-development time by demonstrating efficacy in specific populations;
- optimization of clinical utility by demonstrating linkage between subtypes and efficacy;
- reduction of time-to-market by demonstrating specificity to the predicted population;
- explanation of response and identification of groups at risk; and
- increased reimbursement by differentiated responder and non-responder populations.

The banking of genetic material from late-phase clinical trials provides the opportunity to identify responder, non-responder and adverse reactant populations. Retrospective studies using collections of DNA that supply medical information on specific disease types, drug response and ethnic composition could build a foundation for the continuing evolution of medicine, from diagnosis and treatment towards prediction and prevention.

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