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Mini review

Pharmacogenomic approaches in clinical studies to identify biomarkers of safety and efficacy

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ABSTRACT

Although toxicogenomics originated as a field of primarily preclinical investigation, a variety of genomic approaches can also be employed during or after clinical development to identify biomarkers linked to drug exposure and/or drug safety. Comparing and contrasting the different pharmacogenomic approaches according to their scale (targeted, focused or exploratory) illustrates the potential utility of each type of strategy in characterizing the genetic determinants that may play roles in various aspects of drug activity. Examples of targeted ADME genotyping, focused SNP panels, and exploratory whole genome association studies are briefly reviewed to provide an overview of the range of pharmacogenetic options available to the research community to support the ongoing efforts to identify biomarkers predictive of drug exposure and/or safety in human subjects.

Contents

1. Introduction

Since its inception as a subfield of biomedicine, the science of toxicogenomics has most frequently been applied in the pharmaceutical industry in preclinical models to either characterize the mechanisms of toxic actions, or aid in the prediction of toxic liabilities, of drug candidates being considered for clinical development. From the earliest reviews of this topic, the value of toxicogenomics for evaluating toxic potentials of compounds via assessing chemical effects on gene expression were clearly articulated and in many cases put into practice (Nuwaysir et al., 1999; Fielden and Zacharewski, 2001; Pennie et al., 2001; Hamadeh et al., 2002; Ulrich and Friend, 2002).

The earliest proof-of-concept toxicogenomic studies were initially conducted in a variety of liver-based model systems including human hepatoma-derived cells (Burczynski et al., 2000), ex vivo hepatocyte cultures (Waring et al., 2001a,b), and hepatic tissues from animals treated in vivo (Waring et al., 2001a,b) but since then the field has rapidly expanded to include a plethora of other model systems in many non-hepatic tissues as well. On the basis of several hundred published studies, the tangible benefits of employing toxicogenomics in early drug development have been firmly established. In less than a decade the field has grown increasingly complex as pathway analysis tools and reference databases have evolved in parallel with the field of toxicogenomics (Ganter et al., 2008). Indeed, a retrospective assessment has recently been published and authors from a major pharmaceutical company recently concluded that toxicogenomic evaluations (1) often improved understanding of toxic mechanisms, and (2) in combination with traditional safety assessments also improved the drug attrition rate (Foster et al., 2007).

Despite this initial focus on the application of genomic approaches in preclinical models to evaluate toxicity and safety, a variety of genomic approaches varying in both scope and scale

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Table 1
Pharmacogenomic approaches and strategies (by scale).

Strategy	Scale	Pharmacogenomic approach	
		Pharmacogenetic	Transcriptomic
Targeted	Single or few genes	Highly accurate characterization of genotypes in one or few genes	Highly quantitative measurements of mRNA levels in one or few gene targets
Focused	Dozens to hundreds of genes	Evaluation of multiple alleles in a battery of genes related to a given pathway or cellular activity	Evaluation of expression levels in multiple mRNAs in signaling pathways of interest, or in a gene signature
Exploratory	Whole genome/transcriptome interrogations	Broad screening of several hundred thousand to >1 million SNPs and CNVs in the human genome	Semiquantitative survey of tens of thousands of transcripts in the human transcriptome

can also be applied in human studies to identify novel markers that may be linked not only to efficacy but also to safety and/or adverse reactions. For the most part these types of studies have generally been characterized as pharmacogenomic approaches since these genomic analyses are typically performed on samples obtained from subjects prior to, or during the course of, drug therapy in clinical trial settings.

Pharmacogenomic approaches encompass the analysis of either DNA (pharmacogenetics) or RNA (transcriptomics) for the ultimate purpose of understanding why different persons respond differently to drugs. An additional useful subdivision of these types of pharmacogenomic assessments consists of characterizing the strategies as targeted, focused or exploratory. Classifying pharmacogenomic strategies according to their scale (see Table 1) illuminates different aspects and objectives of these approaches that are required to identify various types of genomic biomarkers associated with specific clinical outcomes of interest.

2. Targeted pharmacogenomic strategies

Targeted pharmacogenomic strategies can be defined as those where defined alterations in the primary sequence, or changes in the expression, of the suspected gene are well-established and hypotheses can be prospectively tested regarding the role of genetic variability (or variable gene expression) in contributing to the clinical outcomes under study. Clinical outcomes of specific interest in these types of studies include but are not limited to measures of drug exposures (pharmacokinetics), drug effects (pharmacodynamics), or drug responses (pharmacoprediction).

Targeted pharmacogenetic approaches (DNA genotyping-based) are commonly employed in early phase clinical studies to aid in determining whether genetic variants in genes will influence whether patients (1) achieve sufficient levels of the compound under a given dosing schedule (ADME-related genotyping) or (2) exhibit the desired/expected response to the targeted therapy (mechanism-related genotyping). In the best-case scenario, much or all of the data influencing a targeted pharmacogenetic profiling strategy for a given drug candidate is accumulated prior to its entry into clinical drug development, as described below.

Prior to a compound's evaluation in human subjects, the anticipated metabolic fate of a drug candidate is characterized in preclinical models and summarized in an investigational new drug (IND) application or an exploratory IND (Robinson, 2008). These drug safety and metabolism studies identify the likely metabolic routes in humans by screening and identifying metabolites of the compound in various fluids and tissues across multiple species. Compounds are screened in human microsomes, cell lines, multiple species and even humanized animal models in order to identify the human isozymes expected to influence the metabolism of the drug. The end-result of this substantial preclinical metabolic characterization package is typically a strong hypothesis regarding the major routes of metabolism expected for a drug candidate that will be tested in human subjects.

A host of literature-based information from studies that have interrogated cytochrome P450 s and other genes involved in phase 1 and phase 2 metabolism have formed the basis for ADME genotyping practices now routinely utilized during clinical drug development (Williams et al., 2008). Polymorphisms in ADME genes are often prospectively assessed in early phase clinical studies in order to understand whether genetic variation in proteins involved in suspected metabolic pathways will influence the metabolism of new drug candidates entering clinical development. For this reason, rigorous analytical validation of targeted genotyping methods used in such studies is of critical importance since clinical data will be interpreted in light of the assigned genotypes (Isler et al., 2007). While an in-depth review of the genetic variants that can be assessed in CYPs and other ADME related genes is beyond the scope of this review article, the reader is referred to excellent reviews regarding the ever-increasing number of alleles of relevance in ADME genotyping with respect to both toxicity and general pharmacokinetics (Cascorbi, 2006; Ingelman-Sundberg et al., 2007).

Targeted strategies can be based on the pharmacogenetic analysis of variants in genes that are already catalogued as "known valid biomarkers" by the US Food and Drug Administration (FDA) (http://www.fda.gov/cder/genomics/genomic_biomarkers_table.htm), which include variants in xenobiotic metabolizing phase 1 enzymes (cytochrome p450 s including CYP2C9, CYP2C19, CYP2D6), xenobiotic metabolizing phase 2 enzymes (N-acetyltransferase and UDP-glucuronyl transferase) and endogenous metabolic enzymes (dihydropyrimidine dehydrogenase, glucose-6-phosphate dehydrogenase, thiopurine methyltransferase) (Frueh et al., 2008). However variants in any other ADME or non-ADME genes for which a sufficient level of preclinical data supports interrogation of frequent and functionally important polymorphisms can also be prospectively evaluated during early clinical development. Such studies lay the foundation for an improved knowledge base regarding the impact of genetic variability on drug activity during the early "learning phase" of clinical development.

At the conclusion of targeted pharmacogenetic studies, the impact of genetic variability in the ADME or target gene(s) can be assessed, by determining whether there are differences in one or more clinical outcomes between different measured genotype combinations (or predicted phenotypes). Clinical outcomes in such comparisons could include pharmacokinetic properties, pharmacodynamic measurements, or efficacy-related assessments. Fig. 1 depicts one such type of targeted pharmacogenetic exercise, in which two compounds compared head-to-head within a trial may exhibit clearly disparate pharmacokinetic sensitivities to CYP2D6 metabolizer status b/c of varying substrate preferences for CYP2D6-based metabolism.

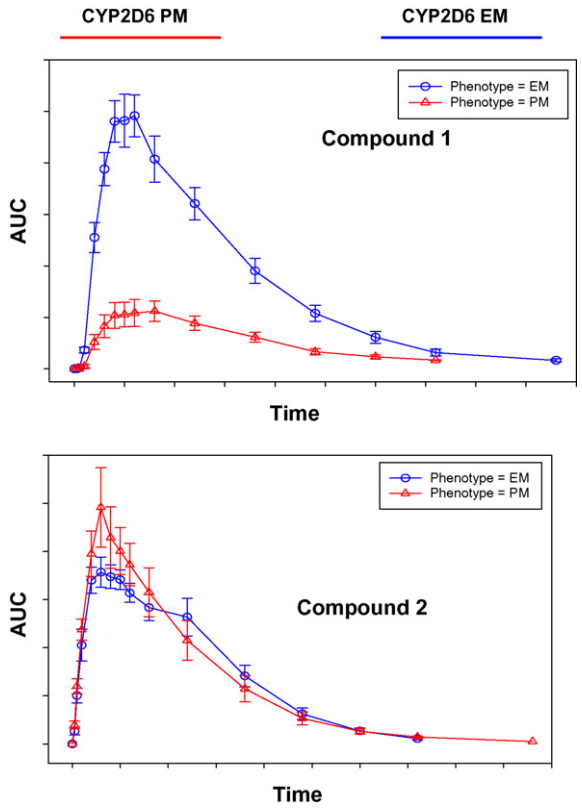

Fig. 1. Example of one type of potential result for CYP2D6 genotyping in a targeted pharmacogenetic study. In this example compound 1 is predicted prior to clinical development to be metabolized by CYP2D6, but compound 2 appears to be metabolized by a non-CYP2D6 pathway. Prospective genotyping of CYP2D6 status to enroll extensive and poor metabolizer subjects in equal ratios enables the assessment of pharmacokinetic properties of each drug in each genetically defined population (CYP2D6 EMs vs PMs).

3. Focused pharmacogenomic strategies

Focused pharmacogenomic strategies are those where a general knowledge base suggests that analysis of the genetic variability, or the expression levels, in a limited battery or subset of genes may provide information regarding drug exposure, drug effects or drug efficacy. These types of studies also require precedent in the literature, but are often based only upon a correlative link between a signaling pathway, route of metabolism or other cellular function and its potential (hypothetical) effect on the disposition, safety or efficacy of an administered drug.

Focused pharmacogenetic studies can be employed when the possibility exists that the preclinical data package may not accurately predict all metabolic routes influencing metabolism of the drug in humans. Reasons for uncertainty can include drug-specific species differences, or less than optimal extrapolation of the model systems employed to characterize the drug's likely metabolic fate in the human organism. In these cases, a non-hypothesis driven approach for identifying genetic alterations in other ADME pathways that could also contribute to unanticipated pharmacokinetic profiles may be warranted.

This type of focused approach, in which the relevant alleles in dozens to thousands of genes are queried at once, is relatively new and not yet routinely adopted. One of the best-characterized examples of an ADME-focused multivariate assay that can facilitate this scale of pharmacogenomic research lies in the technology described by Daly et al. (2007). The Drug Metabolizing and Enzyme Transporter (DMET) assay is based on molecular inversion probe technology originally reported by Hardenbol et al, but focuses on assaying the allelic status of 1227 polymorphisms in 169 different ADME related genes (Hardenbol et al., 2005; Dumaual et al., 2007). The assay is sufficiently rigorous (call rate ~98.5% and accuracy for called genotypes ~99.8% compared to sequencing) and appears to be a viable alternative to employing multiple, labor-intensive targeted strategies for multiple ADME genes that may or may not be involved in metabolism of a drug candidate. Efforts to provide similarly highly parallel but focused genotyping assays for ADME genes are in development on other platforms as well.

In our experience, the DMET approach is useful and appears to be as accurate as originally reported (data not shown) but possesses certain drawbacks, including the complexity of the assay and the difficulty in sufficiently powering such investigations to identify genetic variants of importance during small, early phase clinical studies. It is likely that such focused pharmacogenetic strategies will need to be applied across multiple early phase clinical studies in order to screen sufficient numbers of subjects to identify statistically meaningful genetic associations with clinical outcomes of interest. Nevertheless, the implementation of both ADME-focused and other focused pharmacogenetic strategies (HLA genotyping, etc.) are likely to increase in frequency in the future, as technologies evolve and our understanding of the genetic variability in key signaling pathways influencing drug disposition and toxicity becomes more highly resolved.

4. Exploratory pharmacogenomic strategies

Exploratory pharmacogenomic strategies are the final category. These are large-scale, hypothesis-generating studies that interrogate massive numbers of SNPs or CNVs in parallel in each of many samples (for pharmacogenetic assessments) or the entire known set of mRNA transcripts (for transcriptomic assessments) in order to identify markers or signatures that are correlated with the drug effect of interest. These studies are difficult (but not impossible) to apply during clinical drug development due to size, cost and complexity.

In both of the previous sections, the pharmacogenomic strategies described focused on ADME related genotyping. It has long been recognized that the most common types of adverse drug reactions are those that are examples of exaggerated pharmacology and are therefore dose dependent (Pirmohamed and Park, 2001). Such reactions are augmented and predictable based on the known pharmacology of the drug. In these cases targeted or focused pharmacogenetic analyses of ADME genes may identify the root cause for altered drug exposure that, in turn, may contribute to these types of adverse events.

Idiosyncratic adverse reactions, on the other hand, are far less frequent and are typically not dose-related. The most well-characterized types of these reactions include but are not limited to drug-induced-liver injury (DILI), statin-induced rhabdomyolysis or other forms of myotoxicity, and drug-induced long QT syndrome (Wilke et al., 2007). Because of the low frequency of these idiosyncratic adverse reactions in the general population, they are typically not identified during the clinical development of an otherwise clinically beneficial drug. An international group of experts in the field recently proposed that, given these considerations, the best path forward to identify the genetic basis of these types of adverse reactions in the future would be via the establishment of a global research network (Giacomini et al., 2007).

In September of 2007, a pharmaceutical industry and FDA-led international (510 c3 non-profit) consortium known as the International Serious Adverse Event Consortium (SAEC) was formed, with a purpose of enhancing our understanding of the genetic basis for rare, drug-induced serious adverse events (Holden, 2007). As

defined, the consortium (originally founded by Abbott, GSK, J&J, Novartis, Pfizer, Roche, Sanofi-Aventis and Wyeth) seeks to identify DNA variants that are useful in understanding and predicting the risk of drug induced serious adverse events. The data generated will be made available free-of-charge to qualified members of the scientific community in accordance with the consortium's public data access policy. Through careful selection of biorepositories with well-annotated cases and controls, collaboration with genotyping providers capable of producing high quality data, and identification of a data analysis coordination center capable of analyzing the complex whole genome association datasets generated, the SAEC has already been able to conduct exploratory pharmacogenomic assessments of drug induced liver disease and serious skin rashes (Holden, 2007). Although only in its early phase of implementation, the SAEC appears to represent the best opportunity yet afforded for biomedical researchers to employ a genome-wide, exploratory pharmacogenomic strategy to identify the genetic basis of a variety of idiosyncratic adverse drug reactions.

5. Conclusions

Classifications of pharmacogenomic approaches according to scale highlights the opportunities to impact decision making enabled by different types of pharmacogenomic assays employed during clinical drug development. Targeted pharmacogenomic approaches are most suitable when genetic variability in one or a few genes is suspected to play a role in effects of a drug, and the expanding incorporation of this type of information into drug labels is evidence of the increasingly pivotal role this type of targeted pharmacogenomic information plays in drug development. Focused approaches appear more suitable when pathways and/or multiple genes are suspected to be involved, but it is currently unclear to what extent these approaches will impact drug development in the future. Finally, exploratory pharmacogenomic strategies conducted by the collective efforts of networks and/or consortia appear to afford the best opportunity to discover difficult-to-identify genetic associations that may exist for a variety of idiosyncratic drug reactions. Each of the strategies is capable of shedding light on the genetic variability potentially responsible for different responses in different individuals and is likely to a play substantial role in ushering in the era of personalized medicine in the years to come.

Conflict of interest statement

No conflicts of interest are declared. The author is employed by Wyeth Pharmaceuticals and sits on the Board of Directors for the International Serious Adverse Events Consortium.

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