CHAPTER 31

GENOMIC APPLICATIONS IN PHARMACOGENOMICS

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Introduction

The main goal of pharmacogenomics is to understand the influence of genetic variations between individuals on drug efficacy, metabolism, and toxicity. For some, the terms pharmacogenomics and pharmacogenetics are interchangeable but for others both terms carry different meanings with the term pharmacogenetics having a more limited definition, being reserved for the study of inherited differences in drug response [1, 2]. The study of variations in drug response between individuals can be dated back to Pythagoras at around 510 B.C.E. [2]. More recently, English physiologist, Archibald Garrod, has been the first to propose in 1923 that genetic variants that affect metabolism of endogenous molecules may affect drug metabolism [3]. In 1932, Snyder reported the first inherited trait associated with an exogenous chemical compound (phenylthiourea nontaster trait) in a cohort of 800 families [4, 5]. The pace of progress in the field of pharmacogenomics has accelerated with the completion of the human genome project [6] and at the time of this writing, the Table of Pharmacogenomic Biomarkers in Drug Labels contained 118 entries [7].With the current advances in the fields of -omics, the anticipated benefits of pharmacogenomics are closer to realization than ever before. A better understanding of the interaction between drugs and genetic variants will lead to discovery of drugs that are more powerful, efficacious, and safer. Physicians will be able to prescribe not just the right drug but also the correct dose for a patient, thus maximizing the efficacy while minimizing the adverse effects. Vaccines created making use of genetic information will be able to activate the immune system to a large number of pathogens without exposing an individual to the risk of an infection. With all these and other benefits, the promise is that overall cost of health care will decrease.

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Use of Genomics in Designing Pharmacogenomic Studies

Study Design

The choice between a candidate gene study, genome-wide association study (GWAS), exome sequencing (ES), and whole-genome sequencing (WGS) study is usually dictated by the hypothesis, approach (discovery versus targeted), and available resources. In an ideal setting where resources are not a constraint, a WGS study design is comprehensive and provides more data than are obtainable from the other three study designs. However, obtaining WGS in a sizeable number of patients is prohibitively expensive (although the cost of WGS is coming down rapidly) and analyzing the vast amounts of data generated by WGS requires extensive bioinformatic support.

Candidate Gene Study Designs

Based on the prior knowledge of drug target molecules, metabolism, and excretion, investigators may hypothesize that a certain gene or a group of genes determines the observed effect of a particular drug. Although each drug is likely to have a unique set of genes that determine its response in any individual, certain genes are more likely to be involved in the absorption, distribution, metabolism, and excretion (ADME) of a wide variety of drugs. A group of 32 genes has been designated as the core ADME genes (Table 31.1) by PharmaADME Working Group, a panel of industry and academic experts, and includes genes for several enzymes in the cytochrome P450 system and genes for several proteins that belong to solute carrier family [8]. An extended ADME gene list contains 267 additional genes for proteins responsible for the modification of functional groups of drugs, conjugation of drugs with endogenous moieties, the uptake and excretion of drugs in and out of cells, and those that can either alter the expression of other ADME genes or affect the biochemistry of ADME enzymes [8]. Commercially available gene chips include variants within the core ADME genes such as the DMET™ chip by Affymetrix (containing 1,936 variants across 231 genes) and the VeraCode® ADME Core Panel by Illumina (184 variants in 34 genes). In addition, microarray chips that contain variants from specific gene list can be custom-built.

Table 31-1 **List of ADME (Absorption, Distribution, Metabolism, and Excretion) Genes [8]**

Phase I	Phase II	Transporter
CYP1A1	GSTM1	ABCB1
CYP1A2	GSTP1	ABCC2
CYP2A6	GSTT1	ABCG2
CYP2B6	NAT1	SLC15A2
CYP2C19	NAT2	SLC22A1
CYP2C8	SULT1A1	SLC22A2
CYP2C9	TPMT	SLC22A6
CYP2D6	UGT1A1	SLCO1B1
CYP2E1	UGT2B15	SLCO1B3
CYP3A4	UGT2B17	
CYP3A5	UGT2B7	
DPYD		

Phase I is a type of drug metabolism in which drug chemical structure is modified by addition of polar or reactive groups such as hydroxyl (OH) groups. Phase II is a type of metabolism in which drug chemical structure is conjugated with other charged small molecules such as glycine or glucuronic acid

Whereas this type of assay design can be used for a study with a small number of patients and limited resources, a more likely use is in the drug development process where early identification of drug safety issues may save lives and cost. The biggest challenge with this type of study design is that often our knowledge of a drug's pharmacodynamics and pharmacokinetic pathways is incomplete, making selection of all relevant genes very difficult, if not impossible. Therefore, an association of drug responses with variants that are not included on the chip cannot be discovered.

Genome-Wide Association Study Designs

Whereas in a candidate gene study different sources of information are incorporated to develop a list of genes potentially involved in

a drug's metabolic pathways, in GWAS a comprehensive and unbiased search throughout the whole genome is performed, with the goal of identifying relatively frequent genetic variants that may be associated with drug response [9, 10]. Thus, GWAS allows the discovery of novel genetic variants that are not in the known pathways. As the cost of performing microarrays to conduct a genome-wide scan has come down recently and the currently available microarray chips have good coverage across much of the genome, GWAS designs should typically be used for identifying genomic regions of interest. The identification of a genetic variant responsible for variability in drug response depends on several factors such as the effect size and allele frequency of the genetic variant, as well as the sample size of the study [11]. Genetic variants associated with drug response tend to have larger effect sizes and hence are easier to discover than variants associated with disease phenotypes. However, the often relatively small sample size of pharmacogenomic studies makes it difficult to discover genetic variants. Because of the relatively large effect sizes of genetic variants that are identified in pharmacogenomic GWAS, it is much easier to translate these findings into clinical practice [12]. GWAS can be used to examine the role of different biological pathways and can thus provide important insights into the mechanisms underlying drug response. Several commercially available genotyping arrays provide a wide range of options to choose from, based on one's experimental needs and budget.

GWAS provides an unbiased approach to scanning the whole genome for genetic variants associated with drug response, but there are important limitations. One limitation of GWAS is the penalty for performing a large number of statistical tests. GWAS test the association of drug response with about a million single nucleotide polymorphisms (SNPs) spread across the genome and, due to the large number of tests, result in a very high type I error with a conventional statistical significance threshold. To decrease the risk of inflated type I error, a much lower threshold is often employed, thus requiring a large effect size and/or sample size [13]. Moreover, the effect of variants with a low minor allele frequency (MAF) cannot be studied with currently available sample sizes. Thus, only the effect of relatively common variants (with a minor allele frequency greater than 1 %) on drug response is studied [14]. This limitation is especially evident in studying the cytochrome P450 family of genes that play an important role in drug metabolism. The genes in this family have several isoforms, are polymorphic and have a wide range of allele frequencies including variants with very low MAFs [15]. As a result, there is limited coverage with the currently available GWAS platforms [16]. To summarize, an interesting aspect of pharmacogenomic studies is that an interaction between drug, disease, and genetic variants is potentially possible but a complete examination of this interaction requires very large sample sizes, well beyond those used today.

WES Study Designs

Not only are GWAS limited to examining alleles with relatively higher MAFs, but the association between a variant and a phenotype (such as drug response) is only an *association* and often the identified variants only "tag" the causal variant, requiring further examination of the region around the identified variant. Moreover, whereas heritability estimates of drug response are relatively large, the amount of variability explained by the variants discovered through GWAS is quite small, sending researchers to search for "missing heritability" [17–20]. Some have argued that the drug response may not be determined by common variants (or variants tagged to common variants) but by rare variants. Because GWAS have poor coverage of rare variants, a different method is needed to discover these.

Ideally, sequencing the whole genome should reveal all variants that contribute to drug response. However, despite recent advances in sequencing technology, WGS remains too expensive for large studies. An alternative, in between GWAS and WGS, is to sequence only the exonic regions of the genome, or roughly the 2–3 % of the genome that encodes proteins. By capturing and sequencing only exons, the cost of sequencing decreases significantly, yet our ability to identify rare variants increases markedly [21].

When designing a WES study, a researcher should pay attention to several important

aspects of the study design. Sample selection for WES usually is limited to enrolling unrelated individuals because it is not possible to expose unaffected individuals in a family to a drug. Once a DNA sample is obtained from participants, one of several different methods (such as hybridization, circularization, or PCR) can be used to capture the exonic regions of the genome. Few commercial kits (such as those from Agilent, Illumina, and Nimblegen) are available that employ one of these methods for capture. Of note, these kits differ in their definition of "exome" and cover slightly different parts of the genome. Deciding the depth of coverage is the next step and depends on several factors; for most experiments, coverage between 20× and 50× at each nucleotide will suffice. Once data are available, the first step is alignment to the genome followed by variant calling. Several quality control measures are considered throughout the alignment and variant calling process. Data on called variants are usually saved in a variant call format (VCF), which is then annotated. The annotations may include genomic coordinates, population frequencies, conservation throughout evolution, effect on protein structure, expected severity due to protein change, and any known clinical associations. This is followed by several heuristic filtering algorithms to narrow down the list of variants of interest [21, 22]. Non-synonymous variants are of particular interest for obvious reasons and various statistical and computation methods have been developed to assess the functional impact on proteins [23–32]. Because the majority of the identified variants are rare, statistical methods used for GWAS studies have very low power to detect an effect. An alternative is to analyze a group of rare variants within a defined region, usually a gene, and several statistical methods have been proposed to use this approach [22, 33–40]. Although we are still waiting to see a report of a pharmacogenomic study utilizing WES, it is likely that such studies will provide new and important insights into drug response.

WGS Study Design

Exome sequencing focuses on those parts of the genome about which we have better knowledge, the exons from the known coding regions, but leaves out regulatory components that may control whether a gene will be expressed and the extent of its level of expression. Furthermore, it is very likely that our current knowledge of the protein-coding regions of the genome is incomplete and, therefore, undiscovered but important genes would not be captured by commercial exome capture kits. In contrast to WES, WGS provides information about all identified variants irrespective of the location in the genome and provides the opportunity to discover a large number of genetic variants important to drug response. The study design issues as well as analytical issues are similar to WES except that the volume of data is much bigger and the ultimate number of variants obtained per sample much larger. If GWAS are any guide, WGS is likely to identify a large number of rare variants in the noncoding regions or in pseudogenes, forcing us to further understand how these variants control gene expression.

Other Study Designs

Other genomic methods, such as RNA sequencing (RNA-seq), DNA methylation studies and other epigenetic methods, can be used to study the effect of genomics on drug response, as well. For most drugs, our current knowledge of molecular targets is limited at best. This is true even for drugs that have been in common use for many decades and have been studied extensively, such as aspirin. For example, RNA-seq may identify genes that are upregulated or downregulated in those individuals who respond to a drug as compared to those who do not [41].

Choice of Study Population

Perhaps the most economical way of conducting a pharmacogenomic study to identify genetic variants is in the setting of a clinical trial. In clinical trials, patients are already enrolled to receive a particular drug at (a) certain dose(s), or an alternative drug or placebo, and are being followed for outcomes. Genotyping all or a subset of patients may provide an opportunity to study the effect of genetic variants on drug response phenotypes. Patients for genotyping can be selected at the

end of the study when it is known which patients did or did not have a particular outcome during the clinical trial. Clinical trials are a good setting for studying candidate genes, but a single clinical trial may not provide a large enough number of participants to conduct a pharmacogenomic GWAS. Therefore, results from several clinical trials may need to be combined to get to the needed sample size. While combining clinical trials, usually in a meta-analysis framework, certain issues may arise, however. These include the use of different doses of the drugs, use of concomitant drugs, or underlying differences in study populations. In these instances, decisions may need to be made as to which studies should or can be included.

An example of the use of a clinical trial cohort for conducting a pharmacogenomic study is the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38) trial. This trial compared two antiplatelet agents (clopidogrel and prasugrel) in patients with acute coronary syndromes who were scheduled to have percutaneous coronary interventions [42]. The drug response phenotype was a composite of clinical outcomes (cardiovascular deaths, myocardial infarction, or stroke). While the overall study enrolled 13,608 patients, 1 candidate-gene pharmacogenomic study examined 1,477 subjects who were in the clopidogrel arm and found that carriers of a reduced function allele in the *CYP2C19* gene were associated with a 53 % increased risk of the composite clinical outcome [43]. Another candidate-gene pharmacogenomic study from the same clinical trial included 2,932 patients (1,471 from the clopidogrel arm and 1,461 from the prasugrel arm) and found that the TT genotype of the c.3435C>T variant in the *ABCB1* gene was associated with a 72 % increased risk of a composite clinical outcome in individuals treated with clopidogrel but not with prasugrel [44].

An example of the use of GWAS in a clinical trial setting is a study by Ramsey et al. This study was conducted to identify genetic variants determining methotrexate clearance in children with acute lymphoblastic leukemia and used data from two studies (P9904 and P9905). Investigators found that methotrexate clearance was associated with polymorphisms in the organic anion transporter gene *SLCO1B1* and further confirmed their findings in independent cohorts [45]. Phase I/II clinical studies also provide an opportunity to perform candidate-gene pharmacogenomic studies (for examples see [46–48]).

Another important source for pharmacogenomic studies are clinical cohorts in which information is collected from electronic health records (EHR) [49]. In these studies, data are collected from hospital or other clinical records and DNA is collected for research at the time of contact with the patient. This model has several advantages. The data are collected on patients who are receiving regular medical care and are neither self-selected nor selected on the basis of some criteria. Thus, these subjects provide a "real world" opportunity for pharmacogenomic studies. As the data are extracted from already existing medical records, the cost of acquiring data is minimal although the cost of DNA isolation genotyping remains the same. However, the genotype information obtained for one study can be used for additional studies on the same patient cohort (especially if genotyping is performed using a genome-wide scan) and by doing so can potentially reduce costs further. The disadvantages include that data are not collected for the purposes of research and as a result data may not be of high quality. However, certain types of data are likely to be of reasonably good quality. This would include parameters such as vital signs, laboratory data, medication records, and billing information. Perhaps the most prominent example of the use of EHR is in the Electronic Medical Records and Genomics (eMERGE) network [50].

Application of Genomics in Determining Drug Efficacy

Finding the Right Drug

Some patients respond poorly to typically very effective medicines and often the underlying reason for this is based on differences in our genome. Poor responsiveness may stem from the effect of genomic variations on drug

pharmacodynamics (such as changes in drug receptors) or on pharmacokinetics (such as drug metabolism). For example, β2 agonists, such as albuterol and salmeterol, are quite effective for the treatment of asthma but may not be as effective in a small group of individuals with a certain SNP in the *ADRB2* gene [51]. Identification of such asthmatic patients and treatment with alternative therapies may improve treatment response and decrease morbidity. Similarly, while clopidogrel is very effective in reducing adverse cardiac events in patients who undergo percutaneous coronary intervention with stent implantation, individuals with the *CYP2C19**2 allele remain at an increased risk of future events even with clopidogrel therapy [52]. Identifying individuals with this allele may result in the prescription of alternative anti-platelet agents for an adequate inhibition of platelet function.

Finding the Right Dose

As currently practiced, physicians look at certain features in determining the right dose of drugs for their patients. These include a patient's age, sex, and body weight. This becomes especially an issue when the therapeutic window of a drug is small, such as is the case with warfarin. Warfarin is the most commonly prescribed anticoagulant drug for the prevention and treatment of venous thromboembolism and for the prevention of stroke in atrial fibrillation and with mechanical heart valves. Variants in the *CYP2C9* and *VKORC1* genes have been shown to determine warfarin metabolism and a warfarin dosing regimen based on the genotype has been developed and validated [53, 54].

Application of Genomics in Minimizing Adverse Drug Reactions

Use of almost all drugs is associated with some adverse reactions ranging from very mild ones to very serious ones resulting in severe illness or even death. Identification of genetic variants that may predict these adverse events and choosing alternative therapies for patients with these variants may result in more optimal drug responses.

The earliest examples of the use of pharmacogenomics are studies of severe adverse drug reactions such as seen with the use of mercaptopurine, succinylcholine, and perhexiline [55]. More recent examples include studies of statins, which are a group of lipid-lowering agents that have been consistently shown to reduce cardiovascular morbidity and mortality in patients with coronary artery disease or in patients at risk of developing coronary artery disease [56]. However, some patients develop myopathy while taking statins. A GWAS identified rs4363657, a SNP in the *SLCO1B1* gene (gene product responsible for hepatic uptake of statins), linked to the development of myopathy. Using an alternative lipid-lowering agent or using lower doses of statins in patients with this SNP may avert the development of statin-induced myopathy [57, 58]. Similarly, polymorphisms in *CYP2D6* and *CYP2C19* genes affect the efficacy and safety of tricyclic antidepressants and presence of these variants may require either reduced dosing or alternative therapies [59].

Application of Genomics in Vaccinomics

The application of pharmacogenomics to vaccine design has been labeled as "vaccinomics" and this new field is using the tools of genomics and bioinformatics to develop novel vaccines. Some of the recent approaches in vaccinomics include the use of epitope determination and prediction algorithms for exploring the use of peptide epitopes as immunogens [60]. In addition to developing new vaccines, genomic applications can help to identify individuals who are likely to develop an adequate immune response with a particular vaccine and who will develop adverse effects [61].

Genomic Applications in Research

Drug Discovery

Genomic applications can help the pharmaceutical industry from the very beginning of the drug-discovery process [62]. Genomic

approaches can identify suitable gene targets and may identify potential molecules that can be evaluated as drugs [63]. Furthermore, knowledge of genetic variants may allow for more appropriate and ultimately safer inclusion and exclusion criteria, resulting in a more successful passage of drugs through the pharmaceutical pipeline. Lastly, the interaction of drugs that are currently in clinical use with newly discovered gene targets can be examined and we may discover new uses of previously approved drugs whose safety has already been shown [64].

Discovery of Biological Mechanisms

The use of genomic applications in studying drugs is improving our understanding of biological mechanisms in two main ways. First, whereas drugs are exogenous molecules that are introduced from outside, these molecules may have certain similarities with endogenous molecules of the body. These similarities may include aspects of drug/ligand receptors and metabolic pathways. Identification of receptors and enzymes involved in the metabolism of a drug may provide insights into the metabolism of endogenous molecules. For example, the cytochrome P450 family of genes was initially discovered as encoding detoxifying enzymes, but subsequent studies have highlighted the importance of these enzymes in the metabolism of endogenous molecules [65]. Second, because our understanding of the pathophysiology underlying most diseases is incomplete, historically the presence or absence of signs and symptoms has been used to classify diseases. It is quite possible that different pathophysiological mechanisms may culminate in a similar set of signs and symptoms and hence become defined into one disease process. The use of genomics tools to increase our understanding of drug responses and biological mechanisms will deepen our understanding of the pathophysiological basis of disease and is likely to result in better classification of diseases and more appropriate and targeted therapy for patients.

Conclusions

Whereas there are several drugs with pharmacogenomic warnings from the Food and Drug Administration (FDA), challenges abound regarding the identification of relevant genetic variants, and in every step on the road to clinical implementation (Table 31.2). The full benefit of genomics in clinical practice can only be realized when drug therapy for each individual can be personalized to his/her lifestyle and genome. Advances in several fields are needed before this dream of personalized medicine can be realized [66]. At the same time, application of genomics principles in pharmacogenomics holds promise for not only personalized medicine but also new drug discovery and development and novel insights into the biological mechanisms.

Table 31-2 Challenges in Pharmacogenomics Research

Identification of genetic variant
– Issues with current technology
– Need for large sample sizes
– Requirements for validation studies
– Cost of WGS
– Variable definitions of drug response
– Large number of potential hits
– Need for better statistical tests and algorithms
– Bioinformatics support
Demonstration of Efficacy and Effectiveness of Pharmacogenomic Approaches
– Defining drug response
– Need for large sample size especially studies with hard clinical outcomes, such as death or myocardial infarction
– Need for large validation studies
– Need for technology that can be applied at the point of use
– Statistical methods
– Genotype-guided studies
– Concomitant use of other drugs
Implementation in Clinical Practice
– Patient and physician education
– Privacy and ethical concerns
– Regulatory and legal issues
– Bioinformatics support

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