Clinical application of pharmacogenetics

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Pharmacogenetics encompasses the involvement of genes in an individual's response to drugs. As such, the field covers a vast area including basic drug discovery research, the genetic basis of pharmacokinetics and pharmacodynamics, new drug development, patient genetic testing and clinical patient management. Ultimately, the goal of pharmacogenetics is to predict a patient's genetic response to a specific drug as a means of delivering the best possible medical treatment. By predicting the drug response of an individual, it will be possible to increase the success of therapies and reduce the incidence of adverse side effects.

For a drug to be approved for use, it must be shown to be appropriately safe and effective. This evaluation is done on a statistical basis within patient populations. However, it is rare for a drug to be safe or effective for everyone. The inherent variability among individuals has a significant effect on the quality and cost of healthcare. We have analyzed the efficacy of major drugs in several important diseases, based on published data, and the summary of the information is shown in Table 1. The highest percentage of patients responding is 80% for Cox-2 inhibitors, and the lowest is 25% for cancer chemotherapy. Many of the drugs fall in the range of 50–75% response. Of course, some of those deemed non-responders could nonetheless have some benefit, and some of the responders might still suffer symptoms, so a sharp cut-off is useful only for comparison purposes.

The safety of drugs also varies from drug to drug and from disease to disease, but many drugs have some side effects of clinical importance. This is despite the intensive effort of pharmaceutical companies to develop safer drugs and of the regulatory agencies to maintain strict safety guidelines. Of the 1232 chemical entities approved as drugs in the USA, 193 (16%) are associated with adverse events severe enough to require a 'black box' warning on the product label[1]. In an often-cited meta-analysis[2] it was reported that 1.8 million people were hospitalized for adverse drug events in the USA in 1994, with over 100 000 deaths.

The purpose of a clinical pharmacogenetic assay is to distinguish between those patients who are more and those who are less likely to respond to a drug, or conversely, those who are more and those who are less at risk for adverse events. With this information, better choices for drug therapies can be made to maximize the likelihood of efficacious treatment and minimize the risk for adverse reactions. Figure 1 shows schematically how such tests might be applied.

To predict how a patient will respond to a particular drug, it is necessary to have a test that will identify the patient as a responder or non-responder. Such a test would be directed toward one of two types of responses, therapeutic response (efficacy) or adverse side effect (safety). An efficacy test would separate patients into two groups, those who are more likely to show an efficacious response than the population as a whole, and those who are less likely. After being tested, a patient would either be prescribed the drug if in the former group, or prescribed a different drug or alternative therapy if in the latter. A safety test would work in a similar fashion, but in this case the test would divide the population into groups whose risk for the side effect is either lower or higher than the population as a whole. Again, the former group would be better suited to using the drug while the latter group would be better treated with something else. Note that the goal need not be to exclude all non-responders or all patients at risk for adverse events. It is sufficient that the test could change the benefit:risk ratio to a degree that would justify the cost and inconvenience of the test.

Why genetics?

There are many reasons why a patient might or might not respond to a drug or suffer an adverse event. Among these are mis-dosing, drug–drug interactions, drug allergies and medication error. Nevertheless, a patient's individual genetic predisposition remains the major unelucidated reason for inappropriate drug response. Twin studies on drug metabolism showed clear heritability in the rate of drug metabolism[3], and genes have been demonstrated to be the root cause of a number of well-documented cases of adverse drug reactions and therapeutic failure[4] (Table 2). In a study in a major hospital, Classen and co-workers[5] identified 2227 instances of adverse drug events among hospitalized patients, of which the majority (42%) were attributed to mis-dosing. However, 50% of the adverse drug events had no preventable cause and are likely to be related to genetic factors. Causes of treatment failure are more difficult to determine, but it can be assumed that an equivalent fraction is caused by preventable factors. Genetic based patient idiosyncrasy is likely to be a major component of the non-preventable adverse drug events and therapeutic failure, and might contribute to between 25% and 50% of inappropriate drug responses.

Several genes have been shown to be associated with specific drug responses, and have been thoroughly reviewed by Evans and Relling[4], some examples of which are shown in Table 2. Many of these are genes that encode proteins involved in drug absorption, distribution, metabolism and elimination, others are genes that encode drug targets, and some have functions whose relation to the drug is not clear. In each case, however, the genes are polymorphic with a major allele encoding the normal protein, and one or more minor alleles with altered function. These alterations most often result in reduced function or absence of function, in some instances increased function and rarely in altered function.

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Table 1. Response rates of patients to a major drug for a selected group of therapeutic areas[1]

Therapeutic area	Efficacy rate (%)
Alzheimer's	30
Analgesics (Cox-2)	80
Asthma	60
Cardiac Arrythmias	60
Depression (SSRI)	62
Diabetes	57
HCV	47
Incontinence	40
Migraine (acute)	52
Migraine (prophylaxis)	50
Oncology	25
Osteoporosis	48
Rheumatoid arthritis	50
Schizophrenia	60

Pharmacogenetics assays

Providing an assay for clinical assessment of a patient's probable response to a drug is a major challenge in pharmacogenetics. Although it is possible to prepare a research assay to assess a DNA sample, the development of an assay for the use in a clinical setting has considerably higher requirements. Specifically, a useful clinical assay should include the following:
- improvement in a medically important response
- limited false positives (efficacy-based assay)
- limited false negatives (safety-based assay)
- interpretable and clinically useful results
- clinically validated results adequate for regulatory acceptance

Improvement in a medically important response

A test must not only detect a DNA sequence that is indicative of a response, but that response must have medical importance such that a better decision can be made than would otherwise be possible. Examples of situations in which a pharmacogenetic test would not be justified might include one for which an existing, conventional test might provide equivalent information, or one for a minor, reversible side effect.

Limited false positives, or limited false negatives

A false positive in a test for drug efficacy is a non-responder identified as a responder; in a safety test it is

Fig. 1. Clinical application of pharmacogenetic tests. In the general population (center) some individuals taking a particular drug will derive therapeutic benefit (purple) and some will not (blue). Also, some individuals will have a characteristic adverse side effect (yellow). Pharmacogenetic assays will determine whether a patient is more or less well suited to the particular drug based on results from a genotyping assay. In some cases, there might be both efficacy- and safety-based assays.
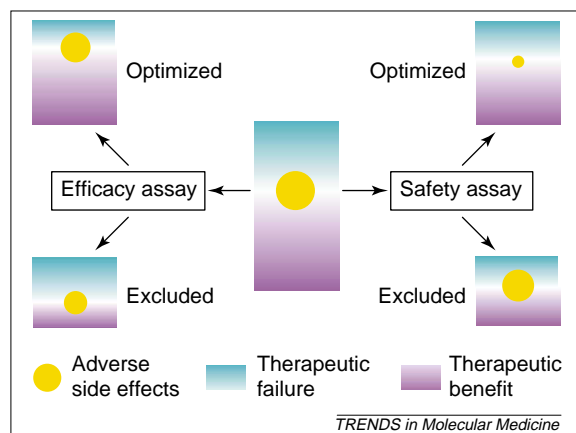
the patient who will not have an adverse event identified as being in the at-risk group. False negatives are the opposite; responders identified as non-responders or patients at risk identified as not-at-risk. An efficacy test must have a low false positive rate but can tolerate a moderate frequency of false negatives. That is, the response rate in the optimized group need not be 100% to be valuable, but on the other hand, the number of potential responders in the 'excluded' category should be minimized. In the case of a safety-based test, the opposite is true. In this case it is necessary to identify most, if not all, patients at risk for the adverse side effect so false negatives must be very low. Because such adverse events are uncommon in most drugs, a high rate of false positives can be tolerated. Eliminating 20% of patients to avoid a serious adverse event in 2% would be a reasonable trade off.

Interpretable and clinically useful results

Genotyping tests are complex, and interpretation of the results requires a high level of scientific knowledge. Clinicians are not molecular geneticists, nor should they be. Therefore, a useful assay must be easy to use in a conventional clinical setting, and should provide results that can be easily understood by the physician and relied on by the patient. The tests must be simplified to the greatest degree possible, and interpretation tools, whether as written materials or computer algorithms, must be available. Highly complex analyses, such as multiple polymorphism analyses (DNA chips) or gene expression analysis will be particularly challenging.

Clinically validated results adequate for regulatory acceptance

For a test to be used for the management of patients, the results must be produced by the use of the Food and Drug Administration (FDA)-approved diagnostic assay or through an in-house validated laboratory 'home-brew' diagnostic assay. In the USA, the validation is included in the license submission to the FDA for a test kit, or is part of a Clinical Laboratory Improvement Amendments (CLIA) or Voluntary Hospitals of America (VHA) laboratory's internal validation documents for a test developed and conducted by that laboratory. In either event, certain requirements must be met, as is the case for any diagnostic test, to assure that the test is safe, efficacious and reliable. In the case of diagnostics, the test must have demonstrated analytical validity; that is, probability that a test will be positive when the specified sequence is present and negative when the specified sequence is absent, and clinical validity; that is the probability that the test will be positive in people who have the condition of interest, and negative in those who do not show the condition. Determining clinical validity for a test that is predictive of a condition, or that assesses a risk of a condition could be problematic owing to the low frequency of many of the conditions among those who are at risk, and the variable timing of appearance of the disease. Although

Table 2. Examples of clinically relevant genetic polymorphisms that influence drug metabolism and effects

Gene	Drug – therapy	Clinical response	Ref.
Drug-metabolizing enzymes			
CYP2C9	Warfarin — anti-coagulation	Dosing in patients with R144C allele (reduced catalytic activity) use lower maintenance dose for anti-coagulation therapy.	14
CYP2D6	Codeine — analgesia	Patients with two inactive alleles do not metabolize codeine to morphine and get no analgesia.	15
Thiopurine methyl-transferase	Thiopurines — leukemia, autoimmune disorders	Patients with two inactive alleles can develop toxic overdose in azathioprise therapy.	16,17
Drug targets			
β-2 Adrenergic receptor	Albuterol — asthma	Patients homozygous for Gly17Arg mutations suffer exacerbation of asthma symptoms with regular use of albuterol.	18
ALOX-5 (5-Lipoxygenase)	Zileuton — asthma	Patients with two non-expressing alleles of *Alox-5* do not respond to 5-lipoxygenase inhibitor.	19

formal evaluation of clinical validity, including peer review, is required for validation of genetic tests, there are no clear guidelines for such assessment. However, a highly informative report from the National Human Genome Research Institute can be found at www.nhgri.gov/ELSI/TFGT.

The requirements for analytical and clinical validation have a major effect on the design of the test, and on the nature of the responses that can be tested. First, the number of patients that use a particular drug and the number demonstrating the characteristic response must be sufficiently high that it will be possible to derive data from an adequate number of patients. For instance, in the case of rare adverse side effects, occurring in less than about 0.5%, it might not be possible to gain samples from enough affected individuals to carry out a statistically meaningful assessment of the assay. Second, the complexity of the results and the magnitude of the results produced by the test will strongly affect the size of the trial needed to validate the assay. As an example, consider two tests to determine the appropriate dosing of a drug based on genetically determined metabolism rates. One test identifies two dosing groups, high and low, and the other test identifies three, high, medium and low. In this instance, the number of trial subjects needed to validate the more complex test would be substantially higher than the less complex. Finally, tests that provide data on a number of sequences in several genes simultaneously – the 'gene chip' concept – will require either independent validation on each of the sequences, or a validated algorithm to determine the meaning of the overall pattern. The process by which such an algorithm would be tested and validated is not clear.

Pharmacogenetic techniques
There are several different testing formats that can be used for the detection of DNA or RNA sequences for the purpose of conducting a pharmacogenetic test. The choice of which technology will be influenced by factors such as the complexity of the target sequences, quantitative *versus* qualitative results, sensitivity requirements, and availability of skilled personnel in the laboratory. For most applications requiring the analysis of genetic information, amplified methods such as PCR are currently the fundamental technology of choice due to the extreme sensitivity of PCR and the widespread familiarity with the method[6]. Numerous detection formats are available for the detection of PCR products or other amplified nucleic acid products, including hybridization and base sequencing methods that are suitable for detection of mutations, single nucleotide polymorphisms (SNPs) and specific sequences. Specific procedures have been developed that make PCR useful for quantitation[7]. Signal-amplified methods are a rapidly developing molecular analysis tool that relies on the detection of a sequence followed by amplification of the detection signal rather than by creating copies of the original DNA target molecule[8–9]. Although signal amplification has not yet approached the sensitivity of amplified methods, signal amplification is generally less complex than target amplification. For more complex levels of sequence information, such as when multiple mutations or sequences need to be efficiently determined in order to produce an effective test result, then technologies such as sequencing, DNA chips or high-throughput-based SNP testing methods might be used[10–13]. Further descriptions of some of the more advanced of the available technologies for pharmacogenetic testing are listed in Table 3.

Currently, all of these techniques are being employed in the analysis of genetic markers, with selection on an appropriate method determined by the needs of each laboratory. Some methods are particularly robust, such as restriction fragment polymorphism (RFP) and allele-specific PCR, whereas others are particularly amenable to high-throughput automation like microarrays and single-base primer extension. No single method works well for all genetic polymorphisms. We can expect to see future systems for DNA analysis that will involve a DNA-purification and processing module, any of several modules for sequence-specific analysis, and a single information read-out that will provide answers in a common format. Calls for a single detection methodology to be shared among all clinical DNA analysis systems are both impractical and unnecessary.

Table 3. Examples of technologies for clinical detection of genetic markers

Technology	Typical genetic markers	Characteristics
DNA sequencing	Sequences, mutations, SNPs, VNTRs, deletions, insertions,	Broad utility for characterization of genetic mutations; Not quantitative
Hybridization based correlated methods: target amplification	Sequences, mutations, SNPs, mRNA	Sequential copying of target sequence followed by signal generation event to presence of initial target. Highly sensitive and specific. Can be used for quantification. PCR is most widely used method.
Hybridization based methods: signal amplification	Sequences, mutations, SNPs, mRNA	Signal amplification event triggered by an initial binding event. Particularly useful for quantification. Sensitivity less than target amplification. Amenable to high throughput screening applications.
Microarray	SNPs, SBH analysis, mRNA expression level profiling	Amenable to high levels of multiplexing. Quantitative and qualitative analysis. Useful for screening broad patterns of sequences. Less well established in diagnostics than sequencing or target amplification
Restriction and conformational analysis	Polymorphism detection and confirmation	Primarily used for mutation detection and analysis. Widely used examples are RFLP and SSCP.
Single base primer extension	SNP detection and confirmation	Adaptable to generic formats. Amenable to high throughput screening

Abbreviations: RFLP, restriction fragment length polymorphism; SBH, sequencing by hybridization; SNP, single nucleotide polymorphism; SSCP, single stranded conformation polymorphism; VNTR, variable number tandem repeat.

Pharmacogenetic testing in the future

Two factors will affect the availability of genetic testing as part of selection of drug therapy: testing technologies and test validation. As seen above, there are already several methodologies available to the needs of pharmacogenetics. These methods can readily detect single base changes, complex rearrangements, and differences in gene expression, and are capable of highly multiplexed analysis. Improvements are necessary in automation, especially sample preparation, speed and cost. Nevertheless, assay technology is not a significant limitation to the expanded use of pharmacogenetics.

The greater hurdle in developing pharmacogenetic tests for clinical use is establishing appropriate test validation. Analytically, a test must be adequately precise, repeatable and reproductive to reliably detect sequences of interest in patient samples. The assay methods described in Table 3 are all capable of delivering analytically valid results.

For a test to be clinically valid it must also adequately predict the association of the test result with a clinical outcome. As genetic tests have inherently high analytical validity, the clinical validity is primarily a function of the relationship of a gene, and sequence variants of that gene, with an expected outcome. This relationship could be difficult to establish; for example, there might be multiple genes that contribute independently to a particular drug-related response, leading to a low positive predictive value for any one of these. Also, for any gene, there might be multiple alleles that give rise to the condition, most or all of which will need to be detected. Furthermore, in genes with low penetrance, the presence of the sequence will correlate to the condition in only some of the cases. Demonstrating that a test has clinical validity, and can deliver a useful result can only be accomplished by careful clinical studies. These studies can be costly and time consuming. In some instances, also, the number of individuals with a particular genotype or characteristic drug response could be low. Nevertheless, the progress of pharmacogenetics from laboratory results to clinical use will depend on how soon medical researchers, pharmaceutical manufacturers, and diagnostic companies can provide the answers.

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