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Review Article

Pharmacogenetics and Pharmacogenomics: Applications in Drug Development and Therapeutics

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Abstract

Pharmacogenetics and pharmacogenomics is an emerging science that has a great application in drug development and therapeutics. It improves individualized therapy and gives crucial approach individuals with variability in drug response due to heredity thereby promotes personalized medicine. Pharmacogenomics intends to develop rational means of optimizing drug therapy, with respect to the patients' genotype, to maximize efficacy with minimal adverse drug reactions. It has the potential to revolutionize the practice of medicine, and promises to usher in an area of personalized medicine, in which drugs and drug combinations are optimized for each individual's unique genetic makeup. On the other hand, it is the uses 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 context of drug discovery and development, program or as part of an individual's diagnosis and treatment regimen.

Keywords: *Genetics; Pharmacogenetics; Pharmacogenomics; Personalized Medicine; Drug Development; Therapeutics*

Introduction

Pharmacogenetics and pharmacogenomics are two major emerging trends in medical sciences, which influence the success of drug development and therapeutics. There is a diversity of opinion regarding definitions and benefits of pharmacogenetics and pharmacogenomics. Pharmacogenetics involves the study of single gene mutations and their effect on drug response. Polymorphic variation in the genes that encode the functions of transporters, metabolizing enzymes, receptors, and other proteins can result in individual differences in the dose-plasma concentration response relationships for many important therapeutic agents. In contrast, the term pharmacogenomics is much broader and it involves surveying the entire genome to assess several determinants of drug responses. Pharmacogenomics is thought to be the application of genomic technologies to the study of drug discovery, pharmacological function, disposition, and therapeutic response. In this paper, the term 'pharmacogenetics' is used to refer to the study of inter-individual variations in the DNA sequence that are related to drug response, efficacy and toxicity. The related term 'pharmacogenomics', is employed in a broader sense that includes genome-wide variations and potential complex inter-actions as well as alterations in gene expression and post-translational modifications (e.g. proteomics) that correlate with drug response [1,2].

The development of pharmacogenomics is a natural sequela to the success of the initial human genome sequencing project. The promise of that project was that an understanding of all human genes would create the opportunity for new diagnostic, prognostic, and therapeutic technologies. The variation in response to medications across patients can be large, and the occurrence of side effects and adverse events limits the success of many therapeutic strategies. A systematic understanding of the gene systems that modulate response to medications may therefore change the way medications are prescribed [3].

Pharmacogenomics intends to develop rational means of optimizing drug therapy, with respect to the patients' genotype, to maximize efficacy with minimal adverse drug reactions. It has the potential to revolutionize the practice of medicine, and promises to usher in an area of personalized medicine, in which drugs and drug combinations are optimized for each individual's unique genetic makeup. The goal of the personalized medicine is to get the right dose of the right drug to the right patient at the right time. Moreover, pharmacogenomics is exploited as an essential step for target discovery and drug development in the pharmaceutical industry. There are however many obstacles (technological, regulatory, social and ethical) that have to be overcome before (or if ever) the potential benefits are realized [4,5].

Genetic Basis of Variable Drug Response

There are often large differences among individuals in the way they respond to medications, whether the endpoint is host toxicity, treatment efficacy, or both. Potential causes for variability in drug effects include the nature and severity of the disease being treated, the individual's age and race, organ function, concomitant therapy, drug interactions, and concomitant illnesses. Although these factors are often important, inherited differences in the metabolism and disposition of drugs, and genetic polymorphisms in the targets of drug therapy (e.g., receptors), can have an even greater influence on the efficacy and toxicity of medications [Table 1]. These genetic factors are reported to account for 20 to 95 percent of the variability seen in drug therapies [6,7].

Enzyme	Variant Phenotypes	Drugs	Modified Response
Plasma pseudo cholinesterase	Slow ester hydrolysis	Succinyl choline	Prolonged apnea
Acetyl transferase NAT2	Slow, rapid acetylators	Isoniazid Sulfamethazine Procainamide Dapsone Sulfasalazine Paraminosalicylic acid Heterocyclic amines (food mutagens)	Slow toxic neuritis, lupus erythematosus disease susceptibility, bladder cancer Rapid: colorectal cancer
Thiopurine methyltransferase	Poor TPMT methylators	6-Mercaptopurine 6-Thioguanine Azathioprine	Bone marrow toxicity, liver damage
Dihydropyrimidine dehydrogenase	Slow inactivation	5-Fluorouracil	Possible enhanced toxicity
Aldehyde dehydrogenase, ALDH2	Fast, slow metabolizers	Ethanol	Slow: facial flushing Fast: protection from liver irrhosis
Catechol O-methyl transferase	Levodopa Methyldopa	High, low methylators	Low: increased response
CYP 2D6	Ultra rapid* Extensive* Poor metabolizers	Debrisoquine Spartein Phenformin Nortryptilin Dextromorphan, etc.	Poor: increased toxicity Extensive: lung cancer? Rapid: drug resistance

CYP 2D9	Poor metabolizers	Tolbutamide, S-warfarin, Phenytoin nonsteroidal anti-inflammatory agents, imipramine	Increased response or toxicity
CYP 2D19	Poor, extensive hydroxylators	Mephenytoin Hexobarbital Omeprazole Proguanil, etc.	Poor: increased toxicity; poor efficacy (proguanil)
Receptors			
β_2 Adrenoceptor	Enhanced receptor downregulation	Albuterol Ventolin	Poor control of asthma
5-HT2A Serotonergic receptor	Multiple polymorphisms	Clozapine	Associated with variable drug efficacy
HER2	Overexpression in breast and other cancers	Trastuzumab (Herceptin)	Overexpression associated with therapeutic efficacy
Transporters			
Multiple drug resistance transporter	Overexpression in cancers	Vinblastin Doxorubicin Paclitaxel, etc.	Drug resistance

Table 1: *Examples of inherited or acquired variations in enzymes and receptors that affect drug response [8].*

Hyperactivity can result from activating mutations or gene duplications.

History of Pharmacogenomics

The history of pharmacogenetics stretches as far back as 510 B.C. when Pythagoras noted that ingestion of fava beans resulted in a potentially fatal reaction in some, but not all, individuals. Certain Mediterranean populations developed red blood cell hemolysis by eating fava beans. This is owing to a deficiency of glucose-6-phosphate dehydrogenase (G6PD), the commonest human enzyme deficiency in the world, affecting approximately 600 million people. Since then there have been numerous landmarks (Table 2) that have shaped this field of research, and have led to the current wave of interest [9,10].

Year	Individual(s)	Landmark
510 BC	Pythagoras	Recognition of the dangers of ingesting fava beans, later characterized to be because of deficiency of G6PD
1906	Garrod	Publication of 'Inborn Errors of Metabolism'
1932	Snyder	Characterization of the 'phenylthiourea nontaster' as an autosomal recessive trait
1956	Alving., *et al.*	Discovery of glucose-6-phosphate dehydrogenase deficiency
1957	Kalow and Genest	Characterization of serum cholinesterase deficiency
1957	Vogel	Coined the term pharmacogenetics
1960	Price Evans	Characterization of acetylator polymorphism
1962	Kalow	Publication of 'Pharmacogenetics – Heredity and the Response to Drugs'
1977/79	Mahgoub., *et al.* and Eichelbaum., *et al.*	Discovery of the polymorphism in debrisoquine hydroxylase

1988	Gonzalez., *et al.*	Characterization of the genetic defect in debrisoquine hydroxylase, later termed CYP2D6
2001–2003	Public–private partnership	Completion of the initial draft and complete sequence of the human genome
2003	The International HapMap Project	Completion of map of human genome sequence variation
2007	Wellcome Trust Case–Control Consortium	Genome-wide association in 14,000 cases in seven diseases

Table 2: *Historical overview of important advances which have either had, or are likely to have, an impact on identifying genetic factors in determining drug response [10].*

The conceptual basis for pharmacogenomics can be traced to Sir Archibald Garrod's "Inborn Factors of Disease". However, the earliest experimentally validated examples of an effect of inheritance on drug response were first reported half a century ago, in the 1950s and 1960s. Those examples grew out of clinical observations indicating that there were large differences among patients in their response to "standard" drug doses, often coupled with individual variations in plasma or urinary drug or drug metabolite concentrations [11].

In 1932, the link between the inability to taste phenylthiocarbamide (PTC) and an autosomal recessive trait, demonstrating that certain chemicals react differently based on genetic differences was reported. This was one of the first known examples of genetic polymorphism, described formally by Ford in 1965 [7]. The concept of familial clustering of unusual drug responses was strengthened during the 1940s with the observation of a high incidence of hemolysis on exposure to anti-malarial drug primaquine among individuals with glucose-6-phosphate dehydrogenase deficiency [12]. G6PD deficiency is still important with respect to prescribing drugs; the recently introduced uricosuric drug rasburicase contains a warning about G6PD deficiency in its label. Also, the combination ant malarial chlorproguanil-dapsone (Lapdap) drug had to be withdrawn owing to a higher risk of anemia in G6PD deficient patients in Africa [10].

Two classical examples that are still taught in medical school pharmacology classes involve genetic variation in the enzymatic hydrolysis of the short-acting muscle relaxant succinylcholine by the enzyme butyrylcholinesterase (BCHE, pseudo cholinesterase), which was described by Kalow in the 1950s, and the enzymatic acetylation of drugs such as the ant tuberculosis drug isoniazid. Both behaved as monogenic traits and both involved pharmacokinetic variation, variation due to inherited differences in drug metabolism [7,11].

Some patients treated with succinylcholine experienced prolonged muscle paralysis, a serious and potentially lethal adverse response that resulted from the inheritance of an "atypical" form of *BCHE*. Subsequently, it was demonstrated that the BCHE allele encoding the most common atypical form of the enzyme included a non-synonymous coding single nucleotide polymorphism (cSNP), G209 > A, resulting in an Asp70 > Gly alteration in the encoded amino acid that affected the active site of the enzyme. Atypical BCHE was relatively unable to catalyze the hydrolysis of succinylcholine and was resistant to inhibition by the compound dibucaine [7,11].

In the 1950s, Price-Evans and colleagues identified N-acetylation as a major route of isoniazid elimination. Although individuals varied substantially in the extent to which a single dose of the drug was acetylated, variability between monozygotic twins was found to be small compared with that between dizygotic twins, laying the groundwork for studies that have now defined the clinical consequences and genetic basis of the fast and slow acetylator phenotypes. Now, it is known that the individual variation in the extent of isoniazide acetylation is due to genetic polymorphisms in the *NAT2* gene [11,12].

Phenotype-driven assessment of variation in drug metabolizing enzyme genes was the hallmark of research undertaken from the end of the 1950s to the end of the 1980s. This usually requires the administration of a probe drug and the measurement of the ratio between the probe drug and its metabolite, the ratio being used to depict whether the individual had an absolute or partial deficiency of an enzyme. Such techniques were used to define an individual's N-acetylation capacity as slow or fast acetylators (an example of a phase II enzyme), whereas debrisoquine hydroxylation was used to define the activity of the phase I cytochrome P450 enzyme, later named as CYP2D6.

Phenotypic assessment of drug metabolizing enzyme capacity is still used as a research tool, for example defining the relationship between genotype and in vivo phenotype, and through the use of a cocktail of probe drugs that enables simultaneous assessment of multiple P450 enzymes. There is an advantage to understanding the phenotype of a particular gene because it enables the identification of many polymorphisms, even those that have not been discovered, and determination of phenocopy (where there is no functional polymorphism in the gene, but the function is decreased because of the co-administration of a drug that inhibits that enzyme). However, disadvantages include the labour intensive nature of the techniques, the associated cost, the low throughput and the fact that in some cases, the probe substance might not be specific for the one enzyme [10].

The advent of molecular biological techniques enabled pharmacogenetics to enter a new era where the phenotypic assessments could be directly related to nucleotide substitutions (and other variants) in the causative genes. Leading the way here was the molecular characterization of the defects underlying the debrisoquine hydroxylase or CYP2D6 polymorphism. At present, over 80 variants have been described in the CYP2D6 gene. Interestingly, the gene comprises variants that lead to both deficient and reduced activity, in addition to the amplification of the gene that can lead to individuals with between 3 and 13 copies of the gene.

This leads to the ultra-rapid metabolizer phenotype, which shows an interesting north–south geographical distribution with the highest incidence of CYP2D6 ultra-rapid metabolizers being found in Ethiopia. CYP2D6 is responsible for the metabolism of approximately 25% of drugs, with poor metabolizers being at risk of toxicity (e.g. metoprolol causing bradycardia) or lack of efficacy (e.g. through the reduced formation of active metabolites as seen with codeine leading to poor analgesic efficacy and tamoxifen resulting in higher breast cancer recurrence rate) [10].

The advent of pharmacogenomics truly began this century following the completion of the human genome in 2003, and the ready availability of new genotyping and sequencing technologies, which have enabled the assessment of the whole genome [10].

More generally, the past half century has seen developments in the understanding of the molecular basis of drug disposition and drug action, and of the mechanisms that determine the observed variability in drug actions. Hence, the concept of a familial component in drug action initiated the field of 'pharmacogenetics', even before the discovery of DNA as the repository of genetic information. The term pharmacogenetics was coined in 1959 by Friedrich Vogel to describe the scientific practice of examining inherited differences in the response to drugs [7]. Pharmacogenetics involves the study of single gene mutations and their effects on drug response. With the increased understanding of the molecular, cellular and genetic determinants of drug action has come the appreciation that variants in many genes might contribute to variability in drug action; the concept of using whole-genome information to predict drug action is one definition of the more recent term, 'pharmacogenomics' [13-15].The term pharmacogenomics (PGx) has been used in recognition that the genome is more than the aggregate of genes, and that genomic technologies are used to identify disease susceptibility, drug discovery, pharmacological function, drug disposition, and therapeutic response [7].

Current Status of Drug Development and Drug Therapy

The process of drug development includes pathway identification and target selection, screening of chemical compounds, drug development, preclinical and clinical studies and finally, drug marketing. It has become increasingly expensive and inefficient, with fewer new drugs being approved and heightened concerns about the safety of marketed drugs [16,17].

A study on the cost of drug development done by Dimasi., *et al*. [17] in 2003 gave an estimate of US $ 802 million in the year 2000, for an NCE development, right up to its marketing. This indicates the resource cost for a firm and not the effective cost. The capital investment has also been addressed by Dimasi., *et al.* [17] in this study. The estimated capitalized phase cost for an NCE was given as US $1.6 million for animal model testing, 15.2 million for phase I trial, 16.7 million for phase II and US $ 27.1 million for phase III trials [17].

Drug development is also time consuming process. The average time taken from synthesis of a new chemical entity (NCE) to its marketing has increased from an average of 7.9 years in 1960 to an average of about 9 to 12 years in 1990.The increase in time duration can be

attributed to the complexity of the clinical trials and rigid regulations. In addition, there is a high attrition rate with only one out of every 5000 chemical compounds considered to have a therapeutic potential being successfully developed for clinical use [18].

It has been estimated that around US$60 billion, of the US$85 billion spent globally each year on R&D, are wasted owing to failures during the drug development process. Further, if we look at the output from the pipelines in the different pharma and biotech companies it has been declining over the past one or two decades, whereas costs have increased during the same period. The flat rate of drug approvals by the U.S. Food and Drug Administration (FDA) is shown in Figure 1below. The number of new molecular entities and biologic license applications approved by the FDA during the period from 1996 to 2010 has dropped from 56 to 21. This statistic further supports the statement about the decreasing productivity in the industry [19] [Figure 1].

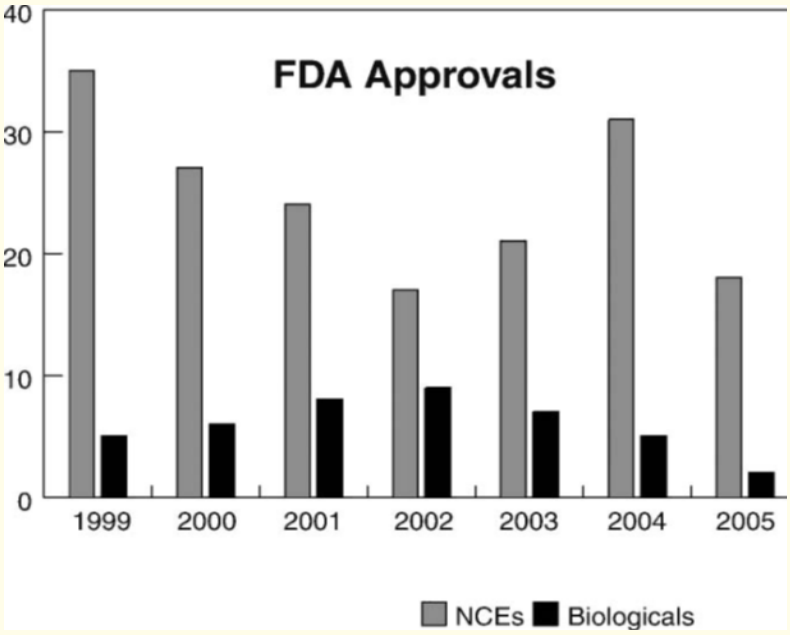

Figure 1: *Trends in FDA approvals of NCEs and Biologicals from 1999-2005 [20].*

The most common reason for termination of a clinical trial or failure of a compound is lack of efficacy, which is followed by safety concerns. This occurs mostly in phase II and phase III of clinical trials. Phase III study, being done on a larger scale and on a larger study population, utilizes a very large share of resources. When a trial is thus ended, the loss incurred financially as well as in terms of time is unacceptable for a pharmaceutical firm [1].

Before the advent of pharmacogenomic studies, efficacy and safety concerns were poorly predictable in a clinical trial. In current times, the predictability of safety and efficacy of a drug has increased to a significant level, as both are influenced by the genetic status of the individual, which can be assessed by pharmacogenomic studies. The incorporation of pharmacogenomics into the drug development process has the potential to improve target identification, accelerate the development process and reduce the attrition rate [18].

The problems for both pharmaceutical companies and healthcare systems do not stop after a drug has been marketed. It is becoming increasingly clear that there is marked variability in the way individuals respond to drugs, in terms of both efficacy and toxicity. For example, there is a 20-fold variation in the dose of warfarin required to achieve optimal anticoagulation across patients [6].

Adverse drug reactions are also a major problem: A recent study shows that a total of 56 (10%) drugs approved between 1975 and 1999 were removed from the market in US, which has enormous financial implications for the industry and undermines public trust. Ad-

verse drug reactions account for 5 per cent of all hospital admissions and increase the length of stay in hospital by 2 days at an increased cost of approximately US$2500 per patient. A meta-analysis in the US suggested that adverse drug reactions killed over 100,000 patients in 1994, making them the 4th most common cause of death [21-24].

A recent systematic review attempted to quantitate the role of polymorphisms in drug metabolizing enzyme genes in predisposing to adverse drug reactions. Of the 27 drugs most frequently cited in adverse drug reaction studies, 59 per cent were metabolized by at least 1 enzyme with a variant allele associated with reduced activity, compared with 7 - 22 per cent of randomly selected drugs. This provides circumstantial evidence that dose alteration through knowledge of the patient's genotype may have prevented some of these adverse reactions. However, it is important to note that the design of the study (relating published adverse drug reaction studies with review articles of drug metabolizing enzyme gene polymorphisms) demonstrates an association and may not necessarily be causative. Furthermore, it does not take into account the fact that adverse drug reactions are likely to have more than one genetic predisposing factor [25].

It is difficult to calculate the likely cost savings in terms of reduced drug toxicity and/or improved efficacy because there are relatively few practical examples and little evidence based on actual clinical practice. Additionally, adverse reactions and efficacy are invariably the outcome of both genetic and non-genetic factors. Nonetheless, the potential benefits, in both health and economic terms, are considerable. However, therapeutic intervention based on individuals' genetic variation will not be applicable to all drugs and careful evaluation of cost effectiveness will be needed on a case-by-case basis [26].

The incorporation of pharmacogenetics into clinical practice therefore has the potential to improve efficacy and reduce toxicity, by allowing the choice of the right drug for the right patient in the right disease at the right dose. For example, understanding how over expression of the HER2 oncoprotein predicts the response to the monoclonal antibody trastuzumab (Herceptin®; Genentech) enables clinicians to tailor trastuzumab treatment to individual breast cancer patients and avoid the morbidity and costs associated with adverse drug reactions or lack of effectiveness to this agent. This also represents a culture change in clinical practice: currently the practice of evidence based medicine is dependent on data from randomized controlled trials and meta-analyses, with the choice of appropriate treatment being dictated by an analysis of the whole population. Successful incorporation of pharmacogenetics will therefore lead to greater consideration of the individual rather than the whole population in the choice of drug [16].

Pharmacogenomics and the Drug Development Process

In the 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 context of drug discovery and development, program or as part of an individual's diagnosis and treatment regimen [27].

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 [27].

The development of recombinant DNA technology (1), the polymerase chain reaction (2), high-throughput DNA sequencing, and related molecular biology techniques have enabled gene-specific drug development. This represents a major paradigm shift from older drug development strategies, which utilized whole-animal physiology-based testing [20].

Pharmacogenomics may have a potentially beneficial effect on all aspects of the drug development process.

Target Identification

The process of drug discovery starts with the identification of a potential target at which the drug can act. The target can be an enzyme in a vital pathway, a receptor, a transporter, a protein in signal transduction or any protein produced in a pathological condition. Drugs currently on the market act on less than 450 of the estimated 10,000 targets in the human genome [20,27]. Target diversity is also limited, with 75 of the top 100 drugs acting on 4 families of molecular targets; G-protein coupled receptors are the commonest site of

action. These targets are known to exhibit variations owing to genetic polymorphisms. Drugs which are based on targets showing wide polymorphisms can have variations in their effect. For example, polymorphism of B2 adrenoceptor gene has produced responders and non-responder phenotypes [28]. This can lead to inconsistent results in those preclinical and clinical studies that would follow if such a drug compound is to be pursued. Thus, at an early stage, the targets can be characterized based on pharmacogenomic studies combined with proteomics and suitable drug compounds selected for further investment [18].

Clinical observations could lead to candidate gene studies that can identify polymorphisms associated with particular disease phenotypes. This, in turn, can lead to new drug targets being identified. This is illustrated by the discovery of CCR5 antagonists for the treatment of HIV infection. It was observed that certain human chemokines can prevent HIV from entering T lymphocytes and that some individuals repeatedly exposed to HIV-1 remain uninfected. Moreover, CD4+ lymphocytes and macrophages from these individuals were shown to be relatively resistant to HIV-1 infection in vitro. Genetic analysis showed that this was associated with a homozygous defect (32 base pair deletion) in their CCR5 gene, leading to a lack of expression of the CCR5 (C-C chemokine receptor-5) receptor on the cell surface of the CD4+ T lymphocytes. Subjects who are heterozygous for this deletion have partial resistance to infection and individuals who are HIV-1 seropositive have a slower decrease in their CD4 T-cell count and a longer AIDS-free survival relative to those with the wild-type CCR5 gene. These findings led to further work to characterize the role of the human CCR5 receptor, and resulted in a new class of anti-HIV therapy. The search for a selective CCR5 antagonist resulted in the development of maraviroc, a new compound that is safe and efficacious and is now in late-stage development for HIV with encouraging results to date [29].

Pre-clinical Drug Development

Pharmacogenetics has already had an impact on this phase of drug development. It has been known for many years that individuals vary in their ability to metabolize certain drugs. The identification of the molecular defects underlying phenotypic variability has led to the development of *in vitro* screens. For example, a major advance has been the development of cell lines expressing drug metabolizing enzymes, such as the cytochrome P450 enzymes. These are the most versatile group of biological catalysts known to exist in nature and are involved in the metabolism of many of the currently used drugs. This allows assessment of the interaction of a drug with a particular enzyme such as a P450 enzyme at an early stage of development, and the subsequent prediction of polymorphic metabolism in man and the possibility of drug-drug interactions. The finding that a drug is a substrate for a polymorphically expressed drug metabolizing enzyme often leads to abandonment of further development. However, if the drug is developed, it also provides an opportunity to warn prescribers through appropriate warnings in the Summary of Product Characteristics (SPC) [21].

A further development in pharmacogenomics has been the use of gene expression profiling in order to predict toxicity; indeed, a large amount of money is being spent on developing databases of gene expression profiles with known toxicants in the hope that this will allow future candidate selection and reduce attrition rates later in the development process. Although this may help in certain situations, where the adverse effect depends on an idiosyncratic feature found only in a small proportion of patients, it is unlikely that the gene profiling patterns developed through animal studies will be of use in humans. It is also important to note that such screens will not be absolutely predictive, and therefore will not replace animal experimentation. However, it is possible that these screens, because of their high throughput nature, will allow more focused animal experimentation, leading to a reduction in the total number of animals tested, and thereby savings in time and cost [30].

Clinical Trials

Clinical studies, which provide the basis for regulatory approval, range from "first in man" kinetic and tolerability studies (phase I) in small numbers of healthy volunteers to the large randomized clinical trials designed to assess the efficacy of a compound (phase III). The typical cost of a phase I study is $7 million, but jumps to $43 million for a phase III study. Pharmacogenetics may lead to refinement of phase I studies by focusing on individuals with known genotypes defined through pre-clinical testing. An earlier identification of problems may lead to the compound being dropped during phase I rather than in phase III, with considerable savings in development costs. In

phase II, there may be further refinement of the pharmacogenetic determinants of drug response, which may provide information necessary for design of the phase III studies.

The net effect may be a reduction in sample size for phase III studies, which may in turn result in more efficient and quicker drug development, and a net reduction in cost [31].

The primary concern in clinical trial is efficacy of the candidate drug. The efficacy of a drug, to a greater extent, is determined by appropriate target selection, which can be guided by pharmacogenomic methods. To cite an example, the drug trasuzumab, an anti-HER2 monoclonal antibody against metastatic breast cancer, was found to be effective only in women who were over expressing the HER2 protein during early clinical trials. In the subsequent trials, studies were done only on women found to be over expressing the HER2 protein. The drug got approval for marketing with the requirement of testing for HER2 over expression, before starting therapy. Had this drug been tested in a whole population without genetic stratification, the efficacy of the drug would not have been brought out [1,18].

The second major concern in a clinical trial is the safety profile of the drug. Data provided by preclinical studies can be applied to humans only to a certain extent. During a clinical trial, the occurrence of a serious adverse event could jeopardize the drug status. Usually such an event would culminate in the termination of the trial. Drug toxicity occurs mainly due to increased plasma levels of the drug, which may be the result of poor metabolizing capacity, owing to genetic polymorphisms. Many of the genes (CYP2C9, CYP2C19, CYP2D6, etc) have been intensely studied in various populations and characterized for various groups. The drug metabolizing enzymes have been identified to exhibit single nucleotide polymorphisms and hence pharmacogenomic approach has a better predictability of the safety of a drug [18].

With the availability of high throughput genotyping methods, pharmacogenetic testing can be incorporated into inclusion criteria for selecting a subject for the trial. Those who are identified as poor metabolizers of the drug tend to attain a higher plasma concentration of the drug, and hence the higher incidence of toxicity. When poor metabolizers are avoided in the study, the occurrence of serious adverse events is reduced. For example, polymorphisms of UGT1A determine the toxicity response to irinotecan due to its effect on the plasma levels of irinotecan. Thus, the study population can be stratified into groups based on their drug metabolizing capacity and those with diminished capacity can be avoided in the study or given lower doses of the drug. The drugs can be thus marketed with pharmacogenetic details along with the product [18].

Polymorphisms in thiopurine methyltransferase and dihydropyrimidine dehydrogenase have been associated with altered drug metabolism and increased risk of severe toxic effects from the anticancer agents 6-mercaptopurine and 5- fluorouracil (5-FU). As a result, the FDA recently required the inclusion of pharmacogenetic testing into the approval label of 6-mercaptopurine for the treatment of pediatric leukemia [32].

Phase IV Studies

Phase IV refers to the period after the drug is licensed; and it involves collecting information regarding drug safety reported to drug companies or health authorities as case reports. Studies may take several forms ranging from hypothesis-generating spontaneous reporting to hypothesis-testing pharmacoepidemiological studies, and can continue for the whole period the drug is on the market. However, evidence about the cause of these adverse events in case-reports is usually poor and more robust methods for obtaining these data are needed. Cohort studies or other designs might not be feasible in the case of rare severe adverse events; but including pharmacogenetics and even genome-wide approaches, in such analyses might eventually provide important new insights into drug safety and adverse reactions. Knowing whether a certain genotype predisposes an individual to an adverse drug reaction (ADR) could protect those individuals from future drug reactions. The application of appropriate genotyping could be a real alternative to the complete withdrawal of a drug from the market, if it becomes possible to genotype patients for a high risk of adverse events [33,34].

Since phase IV involves exposure of large numbers of patients to the drug, detection of rare adverse events usually occurs in this phase. Storage of DNA samples from patients treated with the drug in this phase may allow pharmacogenetic testing and identification of genetic predisposing factors, which will further allow an improvement in the risk-benefit ratio. This is best exemplified by abacavir hypersensitivity, where studies post-marketing have identified a major genetic predisposing factor in the MHC locus [35]. However, a note of caution needs to be added here: since detection of adverse events is a function of the power of the studies, any reduction in the total number of patients studied in phase III may lead to the statistical need for larger, more structured phase IV studies in order to identify rare and long-term toxicities. Prospective collection of DNA samples is a possibility in phase IV, but would be expensive. The cost of this may have to be borne by the pharmaceutical industry, but whether this may result in a more expensive product, and hence a shift in cost to healthcare, is unclear at present [36].

Challenges in Incorporation of Pharmacogenomics in Drug Development Process

After years of hesitation a larger number of pharmaceutical and biotech companies are now supporting the idea of a more individualized pharmacotherapy. At the Cleveland Clinic's Medical Innovation Summit in October 2009 David Brennan, the CEO of AstraZeneca, said, 'We cannot solve today's problems with a model designed to deliver one-size-fits-all drugs developed by individual companies within an ageing system of regulation. Instead, the future is one of personalized therapies developed by collaboration within a modern, fit-for-purpose, regulatory framework'. This statement seems to show that the pharma companies have realized that changes are needed, not only in the way drugs are being developed but also when it comes to collaborations and reforms of the regulatory system. AstraZeneca is not the only company that now supports the ideas about a more individualized pharmacotherapy, companies such as Amgen, Astellas, Bristol-Myers Squibb, Eli Lilly, GlaxoSmithKline (GSK), Johnson & Johnson, Merck, Novartis, Pfizer, Roche/Genentech, Sanofi-Aventis and others also do [19].

Although the pharma and biotech companies are now paying increased attention to personalized medicine, it will still take some years before a large number of new drugs guided by companion diagnostics reach regulatory approval and, hence, benefit the patients. The companies that are now involved in this research-demanding area will have to face several challenges.

Disease Heterogeneity

The models and hypotheses suggested in the past with regard to the mechanisms of action of many drugs have often been too simple. We have long since passed the point of 'one disease, one target, one drug'. Today, we know that most diseases are heterogeneous and that they can be divided into biological subgroups based on molecular profiling, and that each of these diseases will require a specific therapeutic intervention.

For example: Breast cancer is a disease where heterogeneity exists and, based on either gene-expression profiling or immunohistochemistry, at least three subtypes can be indentified: (i) HER2-positive tumors; (ii) ER/ PR-positive tumors; (iii) triple-negative tumors when ER, PR and HER2 are all negative [19].

The challenges to the industry created by pharmacogenetics and pharmacogenomics are significant and include the following: Genotyping will identify many new disease-related genes and provide an explosion of new targets to pursue; and Pharmacogenomics profiling will lead to patient stratification, and these new targets, as well as existing targets, will be divided into subsets [19].

It is estimated that genotyping will identify new disease related genes that will lead to between 5,000 and 10,000 new potential targets. Because the current amount of targets is approximately 450 and is comprised of mainly four target classes, such as G-protein-coupled receptors (GPCRs), ion channels, nuclear hormone receptors and enzymes, these new targets will add genomic and medicinal diversity [19].

Pharmacogenomic profiling of patients will increase the amount of drugs that we will need to design to target a more segregated patient population. Thus, blockbuster drugs (or flagship products) will be replaced with subsets of compounds that, together, comprise a blockbuster drug class. Many benefits to patients will follow from this change. It has been estimated that, currently, as few as one-third of

patients taking prescription medicines actually derive the intended benefit. Adverse drug effects among the remaining two-thirds could be as high as two million per year, with up to 100,000 of these being fatal [19].

The significant burst in new targets from genotyping and the sub-setting of these targets as a result of patient stratification will lead to increased demands for lead optimization. Biotechnology companies such as ArQule (Medford, MA, USA), which specialize in the use of eADMET (early-ADMET) bioassays and predictive tools for designing drug-like molecules, can help to fill this gap by partnering with large pharmaceutical companies on lead optimization collaborations [37].

Pharmacogenomic Approaches

There are generally two approaches to pharmacogenomic research, which are summarized as the "genotype-to-phenotype" and the "phenotype-to-genotype" approaches.

Genotype-to-Phenotype Approaches

In this approach, the investigators start with a set of genes that are known (or strongly suspected) to be important in modulating the response to drugs, and then they search for variation in their sequences (that is, their genotype). Given an understanding of genetic variation, they can search for the phenotypic consequences. Examples of approaches amenable to genotype-to- phenotype analysis might include gene families known to be important for pharmacokinetics (the study of how medications are absorbed, distributed, and cleared from the body), such as phase I metabolism enzymes (the mixed function oxygenases of the cytochrome p450 system), phase II metabolism enzymes (the conjugation system), and membrane transporter molecules. Other systems amenable to the genotype-to-phenotype approach are those that are involved in pharmacodynamics (the study of how medications have their therapeutic effect) and those whose mechanisms are well understood at the receptor and pathway level. Examples might include the well described pathways of inflammation in asthma, the purine/pyrimidine biosynthetic pathways that are targeted by some anticancer agents, or the enzyme cascade that controls blood clotting [38,39].The steps of a genotype-to-phenotype approach can be summarized in this simplified way: Identify the genes that belong to the system that is involved in modulating drug response; Catalog the variation in the DNA sequences across the population; Search for phenotypes associated with the sequence variation; and Confirm clinical relevance of the genotype-phenotype associations.

Phenotype-to-Genotype Approaches

Phenotype-to-genotype approaches toward pharmacogenomic discovery are different from genotype-to-phenotype approach in that, instead of identifying a family of genes in which to characterize genetic variation, investigators search for a phenotypic measure that shows significant variation. This measure can be a clinical measure (such as the rate of clearance of a drug or the peak level of the drug for a given dose), a cellular measure (the rate of cellular uptake of a drug or the profile of gene expression), or a molecular measure (the enzymatic turnover rate of an enzyme or a substrate binding constant). In any case, it is the phenotypic variation that first draws attention and then follows a search for the genes that are responsible for this variation [38,39]. The steps of a phenotype-to-genotype approach, therefore, can thus be summarized: Identify a phenotype that shows significant variation; Search for genes that may explain this variation; Characterize genetic variations and check for association with the phenotype; and Confirm proposed genetic basis for the variation and its clinical relevance.

Methods of Studying Genetic basis of Drug Response Variations

Candidate Gene Approach

Candidate gene approach for identifying genetic determinants of drug response variability involves identifying association between various allelic variants or SNPs within the candidate gene and the drug response. The method of candidate gene approach starts with identification of candidate genes. For a drug response, candidate genes could be the genes coding for the drug metabolizing enzyme, the proteins involved in the drug transport, the proteins involved in the cellular mechanisms, the receptor proteins etc. The candidate gene is studied for allelic variants. A candidate gene can have more than one allelic variant or SNP. Candidate gene effects can be studied in case (people with altered drug response) and controls (people with normal drug response) [40,41].

Candidate gene studies have provided valuable data in the areas of pharmacogenetics and pharmacogenomics. This is especially the case for adverse drug reactions attributable to alleles of a single gene, often one that encodes an enzyme contributing to metabolism of the drug. For example, thiopurine drugs (such as azathioprine), which are used as immunosuppressant and in cancer treatment, can cause bone-marrow suppression associated with inactivating polymorphisms in thiopurine S-methyltransferase (TPMT). The US Food and Drug Administration (FDA) now recommend testing for TPMT variants in patients being treated with azathioprine [42].

Susceptibility to hypersensitivity reactions induced by the anti-HIV drug abacavir was also shown to relate to a single gene in several candidate studies. The gene involved is not a metabolic gene but the class I human leukocyte antigen gene *HLA-B*; most hypersensitivity cases carry the *HLA-B*5701* allele, so genotyping for *HLA-B*5701* before commencing abacavir treatment is recommended by the FDA and other drug regulators worldwide [42,43].

Although there are several examples of the successful use of candidate-gene approaches for investigating drug toxicity, there are fewer examples of the successful use of such approaches for understanding overall drug response, possibly because several genes, not necessarily obvious candidates, such as the metabolic genes, may contribute to response. An exception to this is the response to coumarin anticoagulants; here, there is extensive literature from candidate-gene studies on genetic factors that affect coumarin dose requirement, with only minor variations in findings among studies. Other examples of drugs for which candidate-gene studies have provided insights into response include clopidogrel, the *CYP2C19* genotype affects the response directly and also influences the treatment outcome [44].

Candidate gene studies are less expensive than linkage disequilibrium studies and genome wide scans. This approach, however, ignores much of the genome, and thus is likely to miss many causal regions or genes and instead find many false-positive associations. Moreover, a level of understanding of the drug response pathway is required to identify the candidate genes unlike genome wide scans [40,41,45].

Genome Wide Scan

Although the approach of analyzing candidate genes has considerable intuitive appeal, it suffers from the criticism that it fails to consider a potential contribution of other genes, including those whose function is not yet well understood [39].

Genome wide scan is a very extensive and elaborate study method for effects of various allelic variants occurring throughout the genome and the drug response in a disease condition. This method involves identification of all the allelic variants in the entire human genome and the creation of an SNP map. This is tested for association with drug response variation. The advantage of genome wide scan approach, as compared with the other methods, is that, it can identify the polygenic determinants of drug response. By casting a wide net of genetic markers across the entire genome, this approach does not require one to pre-specify particular candidate genes for study and examines much of the common variation across the human genome. This approach increases the translation level of genetic testing in clinical response. It is estimated that the human genome has approximately three million SNPs and it is not cost effective to screen all the SNPs. As a result, only representative SNPs that are distributed evenly across the genome are selected for testing. This may be in the range of 200000 to 300000 SNPs per human genome [41,45- 47].

A clear advantage of this method is that it is hypothesis-free and that this may reveal unexpected SNPs related to drug response. Hence this method does not rely on current knowledge with regard to the metabolism and mechanism of action of the drug. Genome-wide association studies have presented novel associations of SNPs with drug response. Moreover, GWAS have convincingly detected hundreds of variants associated with a large number of diseases. Many of these findings are novel; associated SNPs in genes or chromosomal regions were not previously implicated in disease. For example, novel information about the pathogenesis and progression of complex diseases, like RA and Crohn's disease, could be revealed using the genome-wide SNP approach. These results are especially exciting in light of the previous difficulties replicating genetic findings for many diseases. For example, linkage and candidate gene studies of prostate cancer have had limited successes in replicating findings across studies, whereas GWAS have detected more than a dozen highly replicated genetic variants associated with this disease [41,45].

Until recently, GWA studies on drug response have been mainly concerned with drugs for which the dose needs to individualized (coumarin anticoagulants) or for which failure to respond presents an important clinical problem (for example, interferon-α treatment in hepatitis C infection or antitumour necrosis factor (TnF) treatment in rheumatoid arthritis). In some cases, GWA has also been applied to phase III clinical trials of new drugs for which DNA samples and detailed data on response are already being collected as part of the study (for example, iloperidone [42].

The disadvantages of genome wide scan approach are that it is very expensive to perform and since a large number of SNPs are mapped, they need to be further evaluated, as it is not hypothesis driven like that of the candidate gene approach [48].

Haplotype Analysis (Pathway Gene Method)

Haplotype analysis approach combines the advantages of the candidate gene approach and the genome-wide approach. It involves the study of clusters of SNPs occurring in linkage disequilibrium in a chromosome and their association with the drug response. This is different from genome wide scan approach in that only selected haplotypes are analyzed and not the entire genome. Haplotype blocks are created by clustering selective SNPs and their linkage disequilibrium is tested with family studies. The haplotype blocks are then tested for association with clinical outcomes [18,41].

Human leukocyte antigen (HLA) matching is a clear example of how haplotypes can be used in the clinic to improve outcome. In this scenario, transplant recipients and donors are genotyped at several markers along the major histocompatibility complex. The HLA haplotypes are then determined by ordering the alleles along the chromosomes. Patients who match the donor haplotypes closely are predicted to have a better transplant outcome than patients who do not. The development of HLA haplotype matching has proved to be crucial in making transplantation between unrelated patients and donors a success [49].

Haplotype analysis provides more information than pharmacogenetic study of single nucleotide polymorphisms and is cost effective. Based on these studies, the various genetic determinants of a drug response can be identified and drug development can be customized accordingly.

Examples of haplotypes used in studies to predict drug response include the β-2 adrenergic receptor (*ADRB2*) haplotypes related to β-agonist response in asthmatics and major histocompatibility haplotypes related to abacavir hypersensitivity in HIV-infected patients [28,43].

Genetic Tests Currently in Clinical Practice

Currently one of two general treatment approaches is typically employed in the pharmacological management of disease. The first is a trial and error approach, employed for drug treatment of diseases such as hypertension, diabetes, depression, schizophrenia, arrhythmias, esophageal reflux and others. For these diseases, there are several drugs that are reasonable first line therapy. Finding the drug(s) that is most effective in a given patient is often through trial and error, and can often take months to accomplish. The other approach to drug management of disease is a per protocol approach, where the treatment for a given disease is essentially the same for everyone with that diagnosis. Examples of diseases treated in this way include most cancers, heart failure, myocardial infarction and post-transplantation patients. In both scenarios, a certain percentage of patients will obtain no benefit from a given drug, or will experience serious adverse effects [50].

The promise of pharmacogenomics in reconfiguring approaches to drug use has considerable currency. Pharmacogenomics is expected to improve, even to overturn, current approaches to drug treatment by reducing adverse reactions, increasing drug efficacy and refining prescribing practices. Indeed, drugs are designed and prescribed on a population basis, but each patient is an individual. Current therapies are based on a trial-and-error method of matching patients with the right drugs and right dosage. In the future by understanding of pharmacogenomics principle, doctors will be able to analyze a patient's genetic profile, define his/her appropriate patient group for a particular medicine and prescribe the best available drug therapy from the beginning. Current methods of basing dosages on weight

and age will be replaced with dosages based on a person's genetics. This will maximize therapeutic value and decrease the likelihood of adverse drug reaction [51].

Pharmacogenetic tests are potentially useful tools for making a therapeutic decision by identifying patients who should or should not receive a particular drug, or guiding individual drug-dosing. There are some pharmacogenetic tests already being applied in therapy. The most commonly applied pharmacogenetic testing for patient care is detection of polymorphisms of genes coding for drug metabolizing enzymes and assisting in dosage selection or modification. A recent study has shown that 59% of the drugs that cause adverse effects are metabolized by polymorphic enzymes [18].

Genotyping and phenotyping are used to categorize an individual as a poor or extensive metabolizer, and sometimes as an intermediate or ultra-rapid metabolizer. Poor metabolizers may be predisposed to toxicity as a result of elevated drug concentrations whereas ultra-rapid metabolizers may be more likely to achieve inadequate plasma concentrations for therapeutic effect. In the case of a prodrug, poor metabolizers may lack response and ultra-rapid metabolizers may be at greater risk of toxicity [52].

The FDA approved the AmpliChip, which is the world's first pharmacogenetic microarray-based test approved for clinical use. The AmpliChip CYP450 Test provides comprehensive coverage of genetic variations for the CYP2D6 and CYP2C19 genes. These genes account for the metabolism of an estimated 25% of all prescription drugs. The AmpliChip will help physicians make better decisions about drug treatments and dosages. Physicians can order AmpliChip test to find out if the patient has mutations in a gene that is active in metabolizing many types of drugs, including beta-blockers, antidepressants, antipsychotics, and some chemotherapy drugs [4].

Pharmacogenetic test information is currently included in over 200 drug labels among those approved in the United States. The information is classified into three categories that guide the clinical use of pharmacogenetic tests for reaching a therapeutic decision 1) test required, 2) test recommended and 3) information only. The majority of drugs with labels containing pharmacogenetic test information do not require pharmacogenetic testing. For example, the label for the anticancer drug irinotecan recommends testing for the presence of a variant of UDP-glucuronosyl transferase 1A1 (*UGT1A1*) to prevent drug toxicity. However, pharmacogenetic testing for UGT1A1 has many challenges for its appropriate clinical use, similar to most pharmacogenetic tests [53].

Some of the major challenges to the clinical application of pharmacogenetic tests – incomplete knowledge of the extent of human genetic variation, availability of alternative biomarkers, and the lack of a model of delivery for pharmacogenetic information [53].

Thus, far, only four drugs – cetuximab, trastuzumab, maraviroc and dasatinib – require a pharmacogenetic test before they are prescribed.

Erbitux (cetuximab) is an EGFR inhibitor for treating metastatic colorectal cancer and head and neck cancer. EGFR immunostain performed on tumor tissue is used to predict patient response to Erbitux. The drug was discovered by ImClone, and its U.S. distribution is conducted by ImClone and Bristol-Myers Squibb while ex-U.S. distribution is done by Merck KGaA. It was approved by the FDA in 2004. The drug label requires the EGFR test [7].

The monoclonal antibody, trastuzumab, was developed by Genentech and Roche to target human epidermal growth factor receptor 2 (HER2), which is overexpressed in 20 - 30% of metastatic breast cancers. It was approved in the United States by the FDA as Herceptin in 1998 and in 2000 through the EMEA's centralized procedure, for use in individuals who have tumors that overexpress HER2. Commercial diagnostics for HER2 have been available for several years using immunohistochemistry (HercepTest, Dako, Glostrup, Denmark) and fluorescent *in situ* hybridization (PathVysion, Abbott, Abbott Park, IL, USA) to distinguish between normal expression and overexpression of HER2 [54].

In the absence of selection, the overall response rate of breast cancer patients is approximately 10% However, overall response rate increases to 35 - 50% for patients selected on the basis of HER2 amplification. Therefore, HER2 selection is critical for the use of Herceptin

in the treatment of breast cancer patients. Testing for gene amplification or overexpression of HER2 is now well established, required by FDA, and offers theragnostic value for treatment of women with breast cancer with Herceptin [4].

Maraviroc is a chemokine receptor type 5 (CCR5) co-receptor antagonist, which is indicated for the treatment of patients infected with CCR5-tropic HIV-1, and those who are resistant to multiple antiretroviral agents. In the labeling for this drug it is stated that a tropism testing to identify patients infected with CCR5-tropic HIV-1 must guide the therapeutic use of maraviroc [19].

Thiopurine drugs such as 6-mercaptopurine (6MP) and Azothioprine have been used since the 1950s as immunosuppressant for a range of autoimmune conditions as well as Leukaemia. Thiopurine drug use is associated with potentially fatal myelosuppression in individuals who poorly metabolize the drug. An enzyme associated with thiopurine metabolism, named TPMT was isolated in 1980.TPMT testing for patients with acute lymphoblastic leukaemia (ALL) has been provided in the UK, Germany, The Netherlands and Ireland as part of research programmes, often with financial support from national cancer charities. Owing to the small market for thiopurine drugs and generic competition there has been little commercial interest in TPMT testing [55].

Warfarin is a widely used oral anticoagulant, which has a narrow therapeutic index. Individual daily dose requirements vary from 0.5 mg to 20 mg/day, with over-anticoagulation, as measured by the international normalized ratio, predisposing to bleeding. Although there are many clinical factors that lead to the variability in daily dose requirements, most studies worldwide have now shown that CYP2C9 genetic polymorphisms, particularly the *2 and *3 variants, which are associated with reduced catalytic activity of CYP2C9, account for approximately 15% of the variability in dose requirement. This is consistent with the fact that CYP2C9 is the main P450 isoform responsible for the metabolism of S-warfarin, the more active enantiomer of warfarin; VKORC1 genetic polymorphisms account for approximately 25% of the variability in dose requirement consistent with the fact that warfarin inhibits VKORC1 to inhibit the vitamin K-dependent activation of clotting factors II, VII, IX and X [10].

This has led to the development of many dosing algorithms, including the IWPC algorithm, which represents a collaboration of approximately 21 groups worldwide, and a change in the warfarin drug label by the US Food and Drug Administration (FDA) in 2007, and the subsequent introduction of dosing tables in 2010. However, despite the consistency of the evidence, and the label change, genotype guided prescribing for warfarin is not reimbursed in the USA, and has not been recommended in clinical guidelines [10].

Challenges in Inclusion of Pharmacogenomics in a Clinical Setting

Pharmacogenomics has the potential to radically change the way health care is provided. But, it is still in the stage of infancy. There are many challenging issues to be overcome to implement pharmacogenomics vision in clinical practice.

Providing Scientific Evidence for Improvement in Patient Care by Pharmacogenomic Testing

First and fore most among the challenges we face as we attempt to transfer pharmacogenomics to the bedside is the science itself. Unless there is strong scientific evidence in support of the value of pharmacogenomic testing for patient care, there is no reason to make that testing part of the therapeutic encounter [56].

Identification of an association between a clinical phenotype, such as drug response, and a genetic variant or a set of genetic variants is an increasing theme in the medical literature. However, many such associations were not reproduced in subsequent studies; false-positive associations are one common reason. Another reason may be subtle differences in patient study groups, such as ethnicity or definition of end points. Thus, similar to other diagnostic approaches, pharmacogenetic testing will be implemented only when its predictive value is established [39].

First of all, it is difficult to conduct definitive clinical pharmacogenomic studies to prove that individualization of drug therapy on the basis of genetics improves clinical outcomes. Given the multigenic nature of most drug effects, the difficulty in controlling for non-genetic confounders (for example, drug interactions, diet and smoking) and the lack of funding for large-scale pharmacogenomic studies with adequate follow-up, it is not surprising that there is a lack of evidence available to catalyze a change in clinical practice [57].

Development of New Genomic Technologies

Rapid and reliable automated methods must be developed to efficiently conduct whole genome sequencing to examine the influence of genes to the susceptibility to disease and individual drug response [4].

There are remarkably few examples of genetic technology that have been validated to the point where they can be used in clinically licensed and regulated laboratories. The diverse range of mechanisms that account for genetic polymorphisms (SNPs, insertions/deletions, splice variants and so on) means that providing definitive results for even a single gene is a tremendous challenge, as has been demonstrated for *BRCA1* and *BRCA2*, which are implicated in breast cancer, and *CFTR*, which has a role in cystic fibrosis. Ultimately, testing for pharmacogenetic polymorphisms must overcome the same technical hurdles that govern other molecular diagnostics. The good news is that a given genotype needs to be determined only once, unlike a measure of renal function [57].

Healthcare Professionals' Education

The clinical application of pharmacogenetics is likely to have a significant impact on the professional requirements of primary health care providers. Currently most physicians and pharmacists receive little training in genetics or genetic counseling. They might be subject to liability if they lack sufficient knowledge of genetics to adequately interpret diagnostic tests, prescribe appropriate pharmacogenomic-based drug therapy in proper dosages, consider pharmacogenomic- based drug interactions, or properly dispense pharmacogenomic-based prescriptions and maintain privacy and confidentiality of genetic information. If pharmacogenomics is to be translated into individualized drug therapy, a concerted effort will have to be directed to the 'genomic' education of all healthcare professionals, including physicians, dentists, nurses and pharmacists. With greater knowledge comes greater responsibility. While both physicians and pharmacists play important roles in enabling patients to access to this technology, they alone are unlikely to be able to cope with the volume of knowledge with which they will need to be familiar. A practical solution may require an expansion of the role of clinical genetics services instead of educating primary health care providers about clinical application of pharmacogenomics. While physicians and pharmacists will still need to be educated about these treatments, the development of independent specialists may be a useful way to ease the burden. Clinical geneticists have the background to understand and interpret genetic tests, and genetic counselors have the requisite knowledge and training to advise patients of genetic tests available to them, obtain informed consent and counsel individuals both before and after testing. Considering the complex nature of genetic information relating to disease development and drug metabolism, the creation of genetic information specialists is probably the best method for ensuring that pharmacogenomics and pharmacogenetics can be applied to clinical medicine without compromising the autonomy and health care of patients [56,58].

Patient Acceptance

Finally, patients will also have to be educated and will have to understand and accept pharmacogenomic testing. Furthermore, significant social and ethical issues must be addressed if the science underlying pharmacogenomics is to have its full potential impact on the clinical practice of medicine and if patients and physicians are to embrace this new science enthusiastically. In some ways, the ethical issues in pharmacogenomics are simplified because, in this area of genomic medicine, the data are generally non-stigmatizing and the physician can 'do something' in response to a test result, such as raise or lower the dose of a drug, or select a different drug. For example, in the case of the *TPMT* genetic polymorphism, a genomic test result might even dictate that the physician lowers the drug dose. Administration of a standard dose of 6-mercaptopurine to a patient homozygous for the *TPMT*3A* variant allele would clearly endanger the patient [56].

The ethics of genetic analysis is currently under avid discussion and debate. Previously, a system of trust and internal control was utilized to prevent inappropriate use of genetic information. This approach has been very successful, with breach of trust being a rare event. However, the field of bioethics is now focusing on prevention of potential or theoretical abuses of genetic information against individuals. This has led to questions about what information is needed, who should have access to the data, and how they should be used. Issues such as these are deeply challenging, as the insurance carrier paying for genetic testing is the same entity that could use the information to

identify disease or therapy risks that could be used to restrict future coverage. However, the great potential gains from pharmacogenomics, in terms of both patient well-being and cost of healthcare, heavily outweigh the risks. Putting such powerful information in the hands of knowledgeable healthcare providers and those involved in the discovery of new approaches to disease treatment or prevention offers so much promise that society must find a way to ensure that inappropriate exploitation does not preclude the vast public good that will emerge from the burgeoning field of pharmacogenomics [59].

Ethical Issues

The therapeutic applications of pharmacogenomics may also raise some ethical questions. One fact which may have ethical implications is that many genetic variants cluster in racial groups. As a result, it is inevitable that some fairly small racial populations have genetic variants that make them particularly vulnerable to some drugs. This can create stigmatization and discrimination and, in some cases, drug manufacturers may not find it economical to develop a new drug to aid a potentially small market. The risk of creating inequities when developing drugs to avert problems caused by natural genetic differences linked to race is an important one [51].

Identification of genetic variants associated with altered pathophysiology has raised questions of whether individuals or groups enriched for such genetic variants could be stigmatized (for example, by being denied insurance) [39].

Cost

One reason for slow adoption of pharmacogenetic testing is that data on cost-effectiveness are limited. This is obviously a complex issue that relates not only to the cost of genotyping itself but also to competing costs, such as those of caring for a patient with a catastrophic and predictable complication of drug therapy. Of note, however, a genotype needs to be established only once in a life time and the costs of currently available tests are often less than those of the drugs themselves [39]. Cost for whole genome sequencing, SNP analysis and expression profiling are still expensive even though the cost is plummeting [4].

Regulatory Issues in Genetic Testing

Questions regarding regulatory issues include mechanisms for regulating genotyping tests, the extent to which pharmacogenetic analyses should be incorporated into new drug development before or after large clinical trials, and whether and how pharmacogenetic information can be incorporated into the product labels that inform clinicians and patients. The U.S. Food and Drug Administration has launched an initiative to collect pharmacogenetic information during drug development that may help address some of these issues; in addition, as already discussed, some drug labels have been changed to include pharmacogenetic information [39].

The clinical application of genomics will require new labelling and prescription guidelines. In pharmacogenomic era of clinical practice drug regimen will be based on the genotype, which is going to be decided by pre-therapeutic pharmacogenetic test. This will require formulation of new prescription guidelines. Even if the results of pharmacogenetic tests suggest that an individual may respond adversely to, or derive no therapeutic benefit from, a drug, the physician and/or patient may still wish to use the drug 'off label'. Regulations may therefore need to cover the permissibility of 'off label' uses. Pharmaceutical companies are required to consider the new labeling requirement for consumer about the risk and limitation of pharmacogenomic drugs [58].

Drug	Therapeutic area	Biomarker	Referenced subgroup	Labeling sections
Abacavir	Infectious Disease	HLA-B	HLA-B*5701 allele carriers	Contraindications
Amitriptyline	Psychiatry	CYP2D6	CYP2D6 poor metabolizers	Precautions
Carvedilol	Cardiology	CYP2D6	CYP2D6 poor metabolizers	Drug Interactions
Celecoxib	Rheumatology	CYP2C9	CYP2C9 poor metabolizers	Use in Specific Populations
Chloroquine	Infectious Diseases	G6PD	G6PD deficient	Precautions
Chlorpropamide	Endocrinology	G6PD	G6PD deficient	Precautions

Codeine	Anesthesiology	CYP2D6	CYP2D6 ultra-rapid metabolizers	Use in Special Populations
Clozapine	Psychiatry	CYP2D6	CYP2D6 poor metabolizers	Use in Specific Populations
Dexlansoprazole	Gastroenterology	CYP2C19	CYP2C19 poor metabolizers	Drug Interactions
Esomeprazole	Gastroenterology	CYP2C19	CYP2C19 poor metabolizers	Drug Interactions
Fluoxetine	Psychiatry	CYP2D6	CYP2D6 poor metabolizers	Warnings, Precautions
Glipizide	Endocrinology	G6PD	G6PD deficient	Precautions
Metoclopramide	Gastroenterology	CYB5R1-4	NADH cytochrome b5 reductase deficient	Precautions
Sodium Chloride	Gastroenterology	G6PD	G6PD deficient	Precautions
Phenytoin	Neurology	HLA-B	HLA-B*1502 allele carriers	Warnings
Primaquine	Infectious Diseases	G6PD	G6PD deficient	Precautions, Adverse Reactions
Isoniazid	Infectious Diseases	NAT1-2	Slow acetylators (inactivators)	Precautions
Trastuzumab	Oncology	MS4A1	CD20 antigen positive	Indications and Usage, Clinical Pharmacology

Table 3: *Pharmacogenetic biomarkers in drug labeling [60].*

Pharmacogenomics and its Future

Pharmacogenomics receives increasing attention and is becoming an integral part of drug discovery and development although there are still many challenging issues to be overcome for the implementation of pharmacogenomics in clinical practice. Potential solutions are evolving rapidly. Genotyping costs are plummeting. It is possible that the era of a thousand dollar for whole genome sequencing will be coming in the near future. The cost of a thousand dollars seems a trivial cost considering that the whole-genome sequencing data are useful for the whole life [4].

Perhaps in the future we may all carry a "gene chip assay report" that contains our unique genetic profile that would be consulted before drugs are prescribed, but at present chips allowing the genetic profiling of patients is still science fiction [51].

Conclusions

Pharmacogenomics has become an integral part of modern drug development and a large number of pharmaceutical companies are using this information to identify novel drug targets, identify patient subpopulations that are likely to benefit from the therapy under development or for other screening purposes.

Pharmacogenomics can greatly improve clinical decision-making of drug therapy by providing information as to how the genetic makeup influences a patient's reaction to drugs. However, the effective translation of this information into clinical practice is still missing owing to various obstacles (technological, regulatory, social and ethical). Those obstacles have to be overcome before the potential benefits are realized.

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