Pharmacogenomics: will it change the field of medicine?

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1. Introduction

Tailoring preventive medicine to an individual’s predisposition for specific diseases, predicting a patient’s response to therapeutic agents, designing new therapeutic agents to optimize their effectiveness, and predicting an individual’s response to exercise based on their DNA are only a few concepts that were once thought unlikely but have become reality. It has always been known that an individual’s genetic makeup plays a major role in disease and drug effectiveness, but the exact details have not been known. The near completion of the Human Genome Project and the advanced biotechnology, however, are allowing this mystery to become unraveled through a new applied discipline, pharmacogenomics.

The aim of pharmacogenomics is to decrease adverse responses to therapy through determining new therapeutic targets and genetic polymorphisms that effect drug specificity and toxicity. A gene sequence is considered polymorphic if one or more genetic variations occur in at least 1% of the population. This discipline was originally broken down into two areas: pharmacogenomics, the genes that dictate drug behavior, and pharmacogenetics, the variability in drug response and metabolism due to hereditary differences. These two terms, however, are now used interchangeably and represent an area of research that is expanding rapidly. The majority of genetic polymorphisms to date are found in drug metabolizing enzymes, receptors, and transport proteins. These single nucleotide polymorphisms (SNPs) can have no significant effect, change drug metabolism by >10,000-fold, or alter protein binding by >20-fold [1–3] (Fig. 1). Ultimately, this information will be used to select the most effective therapeutic agent, effective dosage, and drug response for an individual as opposed to making these decisions based on data for the average person.

Pharmacogenetics information is also extremely beneficial in all areas of healthcare. For example, the pharmaceutical industry can develop more effective drugs and bring only those that are not predicted to have serious toxic effects to clinical trials. Similarly, physicians can prescribe medications without trial and error and avoid life threatening drug reactions. Insurance companies may also experience cost savings due to fewer trips to a physician’s office and less prescriptions of non-effective medications. Patient satisfaction and compliance will also likely
Fig. 1. Single nucleotide changes (SNPs) can occur in receptors, drug transport proteins and drug metabolizing enzymes. These SNPs can have no effect or altered protein binding, which leads to a disease state or altered drug metabolism.

increase. This review will give a brief summary of the history of pharmacogenomics and its importance in drug development and patient response to different therapeutic regimes.

2. History of pharmacogenomics

The concept of altered responses based on genetic differences is not new. Fredrich Vogel first used the term pharmacogenetics in 1959 [4]. Prior to that time, pharmacogenetics had its roots in three areas of research: drug metabolism, Mendellian genetics, and chemoreceptors. In 510 BC, Pythagoras recognized that some individuals developed hemolytic anemia with fava bean consumption [2]. In 1914, Garrod expanded this observation to state enzymes detoxify foreign agents so that they may be excreted harmlessly; however, some people lack these enzymes and experience adverse effects [5]. Hemolytic anemia due to fava bean consumption was later determined to occur in glucose-6-phosphate deficient individuals [6,7].

Through the early 1900s, the dawn of pharmacogenetics continued by combining Mendelian genetics with observed phenotypes. In 1932, Snyder performed the first global study of ethnic variation and deduced that taste deficiency was inherited. From his work, he proposed that the phenylthiourea non-taster phenotype was an inherited recessive trait and the frequency of occurrence differed between races. The frequency in American Caucasians was 30%, American Blacks was 2–23%, Chinese was 6% and Eastern Eskimos was 40% [8]. Soon, other genetic differences such as aldehyde dehydrogenase and alcohol dehydrogenase deficiencies were discovered. These deficiencies are common in Asians and cause them to have less alcohol tolerance [9,10]. Similarly, polymorphisms in the N-acetyl transferase enzyme are also segregated by ethnicity and also correlate to the latitude of the country. As the latitude increases, the number of slow acetylators decreases. Thus, the slowest acetylators occur in countries nearest the equator [5].

These genetic differences were originally thought to be caused by genetic variance but it was not until the advent of molecular biology in the 1950s that disease states could be carefully analyzed. The following dogma: gene → protein → biochemical process → disease state became the model for examining human diseases. It was during this period that the DNA double helix was identified, the human chromosome was visualized, and protein polymorphisms were identified. Following this scheme, sickle cell anemia was the first trait to reveal that a single point mutation can change protein structure and lead to a disease phenotype [11,12]. Clinically, some of the common variations or SNPs screened for are those involving the HFE gene for hemochromatosis, the apolipoprotein E (ApoE) gene for risk of coronary disease and Alzheimer’s disease, the factorV gene and prothrombin gene for predisposition to thrombosis, and the methylenetetrahydrofolate reductase (MTHFR) gene for predisposition to venous thromboembolism. Within the past few decades, the advances in genetic technology to detect polymor-
phisms have caused an explosion in pharmacogenetic research and many of these discoveries have been employed into clinical practice.

One polymorphism extensively studied is the use of apolipoprotein polymorphisms to predict the risk of heart disease and response to therapy. Individuals with the ε4 allele have a 40% increase in experiencing a myocardial infarction (MI) than carriers of the ε2 and ε3 allele [13]. Some studies also demonstrate that carriers of the ε4 allele are also more likely to develop coronary lesions and die from coronary heart disease [14,15]. Recently, the Scandinavian Simvastatin Survival Study took this data one step further to analyze whether the benefits of treatment with simvastatin differed between the different genotypes after a MI [16]. The data revealed that the mortality rate was reduced to 5–7% with the treatment of simvastatin in all genotypes. The mortality was reduced 13% in patients who were not ε4 carriers and had low Lp(a) concentrations, 50% in individuals who were ε4 carriers or had high Lp(a) concentrations, and 80% in those who had both risk factors. Thus, individuals with the ε4 allele experience the greatest benefits from simvastatin.

3. Drug metabolism (P-450 enzymes)

Genetic polymorphisms are not only important for determining predisposition to disease but also for determining an individual’s ability to metabolize different therapeutic agents. Recently, several drugs were withdrawn from the market due to adverse reactions. Could these adverse incidents been avoided if pharmacogenetics were taken into account? Adverse drug reactions are the sixth leading cause of death in the United States and result in more than 100,000 deaths/year and US$75 billion in healthcare costs [17,18]. If physicians had a better understanding of an individual’s ability to metabolize different classes of therapeutic agents or which ones would elicit a poor response, an overall decrease in morbidity and mortality could be expected. For this reason, polymorphisms in drug metabolizing enzymes are one of the most extensively studied areas of pharmacogenomics.

Most of the enzymes involved in drug metabolism and the elimination of many therapeutic agents are members of the cytochrome P-450 (CYP) system. These enzymes are mainly located in the liver and gastrointestinal tract and include greater than 30 isoforms [19,20]. Only a few of these enzymes are of clinical significance and need to be extensively characterized during drug development. Several of the enzymes involved in the largest number of biotransformations and exhibit significant genetic polymorphisms are CYP3A, CYP2D6, CYP2C19, and CYP2C9. These enzymes are classified by the name of the enzyme (CYP), followed by the family (2), subfamily (D), and gene (6) associated with the biotransformation. Polymorphisms in these genes can lead to three possible phenotypes: poor, normal, and ultra metabolizers. Poor metabolizers lack an active form of the expressed enzyme due to an inactivating polymorphism. Normal metabolizers have at least one copy of an active gene and ultra-metabolizers contain duplicated or amplified gene copies.

If only one of these CYP enzymes was responsible for the biotransformation and elimination of a specific therapeutic agent, the ability to predict adverse reactions would be simplified. The possibility of CYP interactions can be modeled through in vitro studies with the therapeutic agent and pure CYP enzymes of interest obtained from recombinant DNA or extracted from human liver microsomes [21,22]. In reality, more than one isoenzyme is involved and each individual contains a different combination of polymorphic CYP enzymes. Thus, an individual could be a poor metabolizer for one CYP enzyme and normal to ultra for another. Thus, these in vitro assays can not predict the volume of distribution or clearance rates for clinical doses. The in vivo modeling, however, is a good starting point to determine if certain therapeutic agents are worth pursuing in the pharmaceutical industry.

One drug metabolizing enzyme that has resulted in numerous drugs being pulled from market is CYP3A. CYP3A is involved in the oxidative biotransformation of up to 50% of clinically important therapeutic agents. The key importance of this drug has led to the discovery of a large variation in drug metabolism. The expression of CYP3A is regulated by genetic and non-genetic factors that can result in a 5–20-fold interindividual variability in metabolic clearance [23]. The activity of CYP3A is influenced by multiple drug interactions. Some inducers of this
The CYP3A pathway for degradation. Some of these agents include cyclosporin, terfenadine, atorvastatin and fexofenadine. Rezulin concentrations above 25 mg were toxic as determined by the decrease in CYP3A activity, the reduction in immunoreactive protein, and changes in the morphology of the hepatic cells [30]. Thus, genetic variation in the CYP protein most likely plays an important role in which individuals develop toxicity. These mutations can occur in the CYP3A enzyme or in the P-450 enzymes that metabolize Rezulin by sulfonation, glucuronidation and oxidation to form a sulfate conjugate. It is possible that a pharmacogenetic profile on these individuals could have prevented the deaths, hospitalizations, and liver transplants that occurred.

Propulsid (Cisapride), also pulled off the market, is a drug used to manage gastrointestinal disorders. Structurally, it is a piperidinyl benzamide that interacts with 5-hydroxytryptamine to elicit a response and is metabolized by the P-450 enzymes CYP3A4 and CYP2A6. Based on its $K_m$ (8.6 μM) and peak concentration (0.17 μM) under clinical condition, it is unlikely to cause inhibition of coadministered drugs. However, some drugs are known to inhibit the metabolism of cisapride such as selective HIV-1 protease inhibitors, the calcium channel blocker mibebradil, and a few antimycotics [31]. Recently, several studies have shown that cisapride can induce adverse cardiac events such as a prolonged QT, ventricular dysrhythmias, and syncopal episodes. Until recently, the mechanism behind these adverse reactions was unknown. Experimental evidence indicates that cisapride has antiarrhythmic properties that prolong the cardiac action potential by inhibiting voltage dependent potassium channels [32–34]. Again, the variation in observed responses and the extent of drug interaction changes among individuals are likely to depend on CYP3A4 polymorphisms, interindividual differences in tissue concentrations of CYP3A4, and pre-existing medical conditions.

4. Drug transport proteins

Transport proteins are proteins that allow compounds to be transported across cell membranes. These proteins include Na⁺/K⁺ ATPase, the membrane receptor for cardioactive digitalis glycosides, and the CYP3A pathway for degradation. Some of these agents include cyclosporin, terfenadine, atorvastatin and fexofenadine. Rezulin concentrations above 25 mg were toxic as determined by the decrease in CYP3A activity, the reduction in immunoreactive protein, and changes in the morphology of the hepatic cells [30]. Thus, genetic variation in the CYP protein most likely plays an important role in which individuals develop toxicity. These mutations can occur in the CYP3A enzyme or in the P-450 enzymes that metabolize Rezulin by sulfonation, glucuronidation and oxidation to form a sulfate conjugate. It is possible that a pharmacogenetic profile on these individuals could have prevented the deaths, hospitalizations, and liver transplants that occurred.

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Transport proteins are proteins that allow compounds to be transported across cell membranes. These proteins include Na⁺/K⁺ ATPase, the membrane receptor for cardioactive digitalis glycosides,
and P-glycoprotein. P-glycoprotein is a drug transport protein that originally was thought to transport chemotherapy agents across cell membranes and be involved in drug resistance of cancer cells. The role of this protein, however, has been expanded to transporting a variety of drugs that include digoxin and cyclosporine A. The transport of these drugs are involved in metabolism at multiple anatomic sites including the gut, the lumen, the renal tubules, hepatocytes, and CSF [35,36]. The digoxin MDR-1 interaction is of importance since the therapeutic index is small and drug concentrations need to be continuously monitored. In a recent study, a significant correlation was observed between a polymorphism in exon 26 C3435T and the expression levels of MDR-1. Individuals who were heterozygous or homozygous for this polymorphism had a lower expression rate of the protein. Those that were homozygous 24% had the lowest expression rate of MDR-1 and consequently, the highest digoxin concentration [37]. Thus, this polymorphism would be a valuable tool in determining the therapeutic concentration needed in individual cardiac patients. Also, some drugs such as macrolide antibiotics may inhibit maximal binding of other therapeutic agents. Therefore, drug transport protein polymorphisms and their drug interactions may provide another valuable tool in screening for potential toxic effects of therapeutic agents.

5. Receptors

Most administered drugs have therapeutic targets that elicit the desired effects. These targets can include receptors, enzymes, or proteins involved in various cellular events such as signal transduction. Numerous studies have identified polymorphisms in these targets. Although these receptors do not show dramatic increases and decreases (up to > 1000-fold) in activity as drug metabolizing enzymes, effects up to 20-fold are still observed [1–3]. Some polymorphisms have been identified in ace-converting enzymes, the cystic fibrosis transmembrane conductance regulator (CFTR), ion channels and beta-adrenergic receptors.

Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in Caucasians. While this disease demonstrates a fair degree of clinical heterogeneity, there is a mutation spectrum of over 700 different mutations resulting in many different genotypes and phenotypes. Some of the mutations do not allow the protein to be transported and inserted into the membrane and others insert dysfunctional membranes into the proteins. Thus, a drug that targets one genotypic variant may not work for another.

The most frequent genotype is a homozygous ΔF508 mutation in the CFTR gene, which occurs as single copy gene in approximately 5% of the general population [38]. This polymorphism causes the CFTR to misfold and consequently, fails to traffic efficiently from the endoplasmic reticulum to the plasma membrane. Thus, the protein cannot form the chloride channel and stimulate the cAMP cascade [39]. Knowing how this polymorphism effects the biological process, researchers were able to customly develop the xanthinine drug CPX [40]. CPX binds to the misfolded protein, which reverses abnormal trafficking, allows stimulation of the cAMP cascade, and stimulates the function of mutant CFTR channels. The proposed mechanism of CPX is to down-regulate mutant CFTR expression and convert the mutant gene expression pattern into one more characteristic of wildtype [41].

Receptor polymorphisms do not only help in the development of new therapeutic agents but can also predict outcomes and beneficial therapy regimes. One target that can predict outcome is angiotensin-converting enzyme (ACE). This enzyme is part of the angio-renin system and plays a large role in regulating cardiovascular functions such as blood pressure and cardiac output. There are two genetic variants possible depending on an insertion (I-form) or deletion (D-form) of a base pair at position 287 in the gene. The prevalence of this mutation is equally distributed in the Caucasian population between the I/I (23%), I/D (49%), and the D/D (28%) genotypes. Individuals with the D/D form express ACE levels 25–200% higher than I/I individuals and have a 23% increased risk of a myocardial infarction than I/D and I/I individuals [42]. This increase may be due to characteristics associated with the D genotype such as increased aortic stiffness and arterial wall thickness [43,44].

Another extensively studied area is the association between β₁-adrenergic receptor polymorphisms and
asthma. β₂ receptors are expressed in the lung, as well as other locations such as epithelial cells and immune cells. However, the receptors exert their primary effect on bronchial smooth muscles resulting in relaxation and dilation and are likely important in the treatment of asthma. In vitro experiments with isoproterenol reveal that there are three polymorphisms that alter receptor function: Arg16Gly, Gln27Glu, and Thr164Ile. Arg16Gly displays enhanced promoter down regulation, Gln27Glu is resistant to down regulation, and Thr164Ile displays decreased affinity along with altered coupling to the cAMP cascade [45–47]. These polymorphisms are found in both asthmatic and non-asthmatic populations at an equal frequency [48]. Therefore, these differences do not contribute to the disease state. The differences in expression, however, do correlate well with some asthmatic symptoms. Patients with nocturnal asthma have the Arg16Gly SNP and experience down regulation at night and patients with the Gln27Glu polymorphism are also protected from bronchial hyperactivity [49,50]. These polymorphisms have also been associated with initial responses to albuterol, tachyphylaxis to formoterol, severity of asthma, and elevated IgE levels. Thr164Ile has not been as well studied, but one study indicates that this polymorphism is associated with increased morbidity and mortality in congestive heart failure patients [51].

6. Genetic impact on exercise response

While the clinical applications of pharmacogenomics on the pharmaceutical industry and healthcare system are intense areas of research, another area of research that has not gained as much publicity is the genetic impact on exercise response. This area of research focuses on which types of patients will benefit the most from exercise and which ones will need pharmaceutical intervention to improve their health status?

The genetic impact on exercise is known to alter hormonal concentrations and has both direct and indirect health consequences. After intense exercising for only 5–10 min, the catecholamines increase from 1200% to 1800%, the pancreatic hormones increase from 60% to 75%, and the pituitary and adrenocorticotropic hormones increase from 150% to 200% [52]. These changes can induce both direct and indirect health effects in genetically different individuals. For example, a direct adverse effect can be exercise-induced asthma or sudden death (sudden death usually occurs in individuals that have a genetic defect that leads to heart abnormalities such as hypertrophy). Exercise can also indirectly affect health status by altering gene expression. Two well-known examples are the positive effects of exercise on cholesterol levels and decreasing hypertension.

Now that the correlation between genes, diseases, and therapeutic drug responses are beginning to be understood, the effects of exercise on gene expression/function have become increasingly important and may be involved in patient therapy [53]. For example, cholesterol levels usually decrease with an exercise plan, but the degree of response is varied among individuals. One study compared increases in high-density lipid concentrations to apolipoprotein E gene polymorphisms. The e2 allele was found to have the most significant increase in high-density lipid concentrations when compared to the e3 and e4 alleles [54]. Another study analyzed blood pressure reduction to apolipoprotein E subtype. This study revealed that e2 allele was the least responsive [55]. Thus, no one allele is superior to another in all aspects. Exercise may be sufficient therapy to lower cholesterol levels in one individual, but therapeutic intervention is likely needed to control hypertension and vice versa. Discoveries such as this reinforce the concept that each person is a distinct genetic individual and must be treated differently.

7. Summary

Pharmacogenomics has become increasingly important in healthcare, both from the standpoint of new drug development and primary care. Industry will benefit from the identification of new targets, screening of new therapeutic agents for adverse affects before clinical trials, and tailoring of therapeutic agents to individual patients. Physicians and patients will benefit since the medication and method of therapy can be tailored for the maximum health effects. In the near future, genetic profiles of individual patients, via an electronic medical record, will be
available to clinicians so that therapeutic strategies may be optimized from the time of initial therapy. As the Human Genome Project comes to an end, we must continue to gather information identifying SNPs and genes as well as clinical data to support the medical efficacy of such genetic profiling.

References


