

# Pharmacogenomics: A Step Towards Personalized Medicine

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**ABSTRACT:** Pharmacogenomics (PGs) refers to the role of individual's genetic make up in response to the drug treatment. Since its inception in the last decade or so, PGs has come a long way to help us in understanding that the era of blockbuster drugs (one size fits all) may eventually become history and be replaced by tailor-made drugs based on genetic profile of the individual. As it is beginning to unravel the functioning of the human genome, the impact of PGs in drug discovery and development is becoming increasingly apparent with the discovery of new targets and associating clinical outcome with the individual's genotype. However, there are unanswered questions with regard to its risk-benefit analyses and the adaptability of the patient population and the view of the pharmaceutical industry towards this discipline. This review article covers the basic aspects of PGs, current trend with its applications and benefits, and the road map for future.

**KEYWORDS:** Pharmacogenomics, Cyp2D6, SNPs, FDA, VGDs.

## INTRODUCTION

Pharmacogenomic (PG) studies exhibit how genotypic variation is responsible for variability in drug response and applies concepts about variations in hepatic drug metabolism enzymes to the rest of genome. It is an idea that has been brewing for years, waiting for the rapid and accurate sequencing tools that could make it a reality. The term PGs, coined in 1997 (Mehr 2000) comes from the words pharmacology and genomics and is thus the intersection of pharmaceuticals and genetics. It combines traditional pharmaceutical sciences such as biochemistry and pharmacology with annotated knowledge of genes, proteins, and single nucleotide polymorphisms. Although environment, diet, age, lifestyle, and state of health all can influence a person's response to drugs, understanding an individual's genetic profile is thought to be the key to creating personalized drugs with greater efficacy and safety.

## Basic Aspects of Pharmacogenomics

Before embarking on explaining how PGs can revolutionize drug discovery and development, it is worth going back in time in order to understand the events that led to the birth of this discipline. The genetic variations were first studied in relation to ABO blood group frequencies at the end of First World War by two Polish scientists Ludwik and Hanka Hirsfeld (Stoneking 2001). This seminal work was published in Anthropologie journal in 1919 after refusal from Lancet (premier medical journal at the time and even now), as the editor did not find any relevance of the work. The data generated by these two scientists was later used by Felix Bernstein who proposed in 1924, using the Hardy Weinberg's principle, that ABO blood group frequencies result from a single gene with three variants (alleles) and not two genes with two alleles as was the prevailing belief then. The study of genetic variations

has really come the age since then with better appreciation of human genetic differences and diversity being the accepted norm. The discovery of DNA structure in 1953 by Watson and Crick was an important milestone that paved the way for the genetic engineering era two decades later followed by the launch of human genome project in 1990. The plethora of inventions and discoveries in biological sciences for studying genetic variations led to coining of the term, "single nucleotide polymorphisms or SNP's" in early 1990's by Michael Boyce-Jacino and his group that forms the basis of the PG studies. Over the last decade, Dr. Boyce-Jacino's group has developed leading technologies for SNP analysis and conducted pioneering research in human SNPs.

Single nucleotide polymorphisms or SNPs (pronounced "snips") are DNA sequence variations that occur when a single nucleotide (A, T, C, or G) is altered in the genomic DNA sequence. For a variation to be classified a SNP, it must occur in at least 1% of the population (Montgomery and Louie 2001; Veenstra et al. 2000). SNPs make up about 90% of all human genetic variation that occur every 100 to 300 bases along the 3-billion-base human genome. Two of every three SNPs involve the replacement of cytosine (C) with thymine (T). SNPs can occur in both coding (exons) and non-coding (introns) regions of the genome. Although many SNPs have no effect on cell function, certain others could predispose people to disease or influence their response to a drug.

The year 1999 saw the establishment of a SNP consortium that was a conglomeration of ten large pharmaceutical companies and the U.K. Wellcome Trust philanthropy under the leadership of Arthur L. Holden to find and map 300,000 common SNPs. This, however, led to the identification and mapping of 1.4 million SNPs in the human genome (Stoneking 2001), with over 60,000 of these residing in the coding region of the human genome. Over 1.4 million SNPs have been identified so far and the number is increasing everyday as more humans are being studied (Evans 2005). The application of knowledge about these SNPs is already beginning to make its way into drug discovery, clinical development and molecular diagnostics. A SNP in a drug metabolism enzyme leads to a poor clinical outcome by causing variability in drug response while a SNP in a drug target can change the pharmacodynamics of drug response. Examples of the latter include polymorphisms in beta-2 adrenoreceptor

and 5-Lipoxygenase that have shown to change the sensitivity of the patients to their respective medications, beta agonists and Zileuton (Evans 2003). These individual gene polymorphisms and how they affect the drug response is often referred to as pharmacogenetics (Hedgecoe 2003). PGs is a broader term that encompasses the interplay of the variations in the whole genome governing the pharmacokinetics and pharmacodynamics of different drugs. However, both these terms have been used interchangeably and it may seem befitting to say that pharmacogenomics is essentially similar pharmacogenetics with two single polymorphic changes, as shown.

It is becoming increasingly clear, with the advent of modern state of the art technologies and access to individual genotypic data, that the era of one drug alleviating the disease of entire population may soon be history and will be replaced by tailor-made drugs based on the genetic profile of an individual. Surprisingly, all humans are 99.9% genetically identical. The 0.01 per cent difference in genome sequences accounts for difference in our susceptibility to, or protection from all kinds of diseases, severity of illness and the way our body responds to different classes of drugs (Falcone 2004). Targeting the individual out rightly may be an ambitious attempt and therefore, it seems more plausible to start profiling a group of individuals belonging to a particular race or residing in a designated geographical area to understand the common genetic basis before refining the process for individualized therapy.

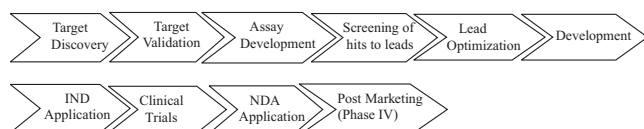
A considerable number of drugs in the market today have problems not only due to their lack of efficacy on the entire population but also their ability to cause serious adverse reactions (SARs). Most drugs are efficacious on a small percentage (say 20-30%) of the population. These patients are referred to as "responders" as they respond well to the particular drug in question as opposed to "non-responders" where the drug is not effective at all. Another group of patients are "toxic responders" where the drug causes toxicity leading to SARs. A typical example could be a drug that is metabolized by the enzyme CYP 2D6. Responders will possess a wild type form of the enzyme while non-responders carry the polymorphic form, which causes extensive metabolism of the drug before it takes effect. The toxic responders, on the other hand, possess an allele that leads to diminished enzyme activity thereby leading to drug accumulation and toxicity.

Although, the drug is usually made the “scapegoat” in all the cases, it is the individual's genotype that is primarily responsible for such an outcome. In addition, there is an enormous debate about the role of environment, diet, age, lifestyle, and state of health in body's response to drugs (Mancinelli et al. 2000). However, a careful analysis suggests that all the above factors impinge and cause changes at the genetic level of an individual, thereby making genetic understanding, of utmost importance in studying drug response variability.

Genotypic analyses of patient populations provide useful insights in predicting the clinical outcome of a drug, thus playing an essential role in clinical development of the drug. The information gathered about over-expression of certain disease specific genes helps in the identification of novel drug targets, thereby serving as a basis for rational drug discovery. The data generated by these PG studies is already impacting modern day drug discovery and will, in future, lead to the development of population based and/or individualized therapies. The day is not far from the horizon when genotyping a patient will become a reality (in terms of costs and public acceptance) and drug prescription will be based on his/her genetic profile, thus maximizing the benefits and minimizing the risks involved.

## CURRENT TREND WITH APPLICATIONS AND BENEFITS

The discipline of PGs has an immense potential for application in all stages of the process of drug discovery and development. A typical process of drug discovery has been outlined in Fig.1



**Fig. 1:** Steps involved in drug discovery

Current trend is towards integrating the knowledge acquired and the technology developed using principles of PGs with the traditional drug discovery workflow. With the advances made in the field of genomic research, an enormous amount of data has been generated and well documented regarding DNA and protein sequence information, genes, mutations, polymorphisms and protein structure and function. A

majority of this information is still an untapped territory and holds immense potential for identifying novel drug discovery targets. For example, a large number of institutes and pharmaceutical companies have already ventured into target based drug discovery in the field of cancer. Presently, 48% of the cancer therapeutics in pipeline is based on the novel targets identified. (Therapeutic Insight 2005). Validation of these targets seems to be currently the biggest challenge for the scientific community. A comparison between the normal and diseased state based on genotyping analysis has played a vital role in the development of assays for validating gene targets. Examples of such assays include RNAi studies, microarray, and gene knock-in and knockout studies.

Studies on the mechanism of action of drugs have resulted in a plethora of information regarding pharmacokinetics and pharmacodynamics of the drug. Certain drugs are known to bind to specific receptor binding sites on the cell surface and inactivate the downstream pathways to produce the desired therapeutic effects. Mutations in these receptor genes may result in lack of receptor-drug interaction leading to failure of the therapeutic effect of the drug. For example, in some individuals, mutations in the genes coding for beta 2 receptors on the surface lining of bronchiolar smooth muscle cells cause them to become non-responders to salbutamol, a beta-adrenergic agonist and bronchodilator used for treatment for asthma (Emelien et al. 2000). A prior knowledge of these mutations in a patient population can avoid serious complications arising from ineffectiveness of the drug. PGs can play a significant role in identifying such patients so that an alternative treatment regimen can be prescribed to them.

Majority of drugs are xenobiotic molecules that undergo modification or alteration in their chemical composition within the body leading to an ineffective principle or conversion into a toxic metabolite for ultimate elimination from the body. This process is known as drug metabolism or biotransformation. Metabolism proceeds in two phases: Phase I reaction where the drug is biotransformed to a more polar metabolite by oxidation, reduction or hydrolysis reactions. Oxidation reaction is the most common phase I process and is carried out by Cytochrome P450 enzymes (Weinshilboum 2003). These enzymes are mixed function oxidases that collectively form catalytically the most versatile and important Phase I

enzyme group. In Phase II reaction, the drugs or phase I metabolites which are not sufficiently polar for excretion by the kidneys, are converted to more hydrophilic forms by conjugation reactions with endogenous compounds in the liver. These conjugated metabolites are then readily excreted by the kidneys (Montgomery and Louie 2001). Genetic polymorphism among these enzymes leads to a variability in drug metabolism (Srivastava 2003).

An example of Phase I enzyme with genetic variation that is responsible for variability in drug response is Cytochrome P450, CYP 2D6, the first and probably the most well characterized enzyme for genetic polymorphism. A number of SNPs and gene deletions and duplications relating to CYP 2D6 have been identified in different populations, which are responsible for the inherited differences in drug effects. In case of CYP 2D6 deficiency, the drugs that are inactivated by CYP 2D6 metabolism pathway such as tricyclic antidepressants and fluoxetine have an exaggerated effect and the ones, which are activated by this pathway like codeine, have a sub-therapeutic effect (Evans 2003).

A very well studied example of phase II metabolism is the enzyme TPMT, responsible for the inter-individual variability in metabolism of thiopurine drugs. TPMT or thiopurine methyltransferase is a cytosolic enzyme that catalyzes methylation of the thiopurine drugs, such as thioguanine, mercaptopurine, and azathioprine and converts them into their respective metabolites. Mercaptopurine, a drug originally developed to treat certain forms of leukemia, is converted by TPMT into its inactive metabolite methylmercaptopurine. The TPMT gene has three polymorphic types that are responsible for variable activity of the enzyme in different individuals and races. Approximately 90% of individuals are homozygous for wild type allele of the enzyme and metabolize the drug i.e. mercaptopurine normally. In this population the toxicity is low, but relapse of the disease symptoms is high. Around 10% of the individuals (1 in 10 people) are poor metabolizers and have high toxicity as a consequence (Evans 2003). Approximately 0.3% of the population (i.e. 1 in 300 people) is homozygous for the variant alleles and lacks the enzyme activity and hence has a high risk of hematopoietic toxicity, myelosuppression and development of secondary tumors. As a result, there

exists an inter-individual variation in detoxification mechanism of thiopurine drugs (Weinshilboum 2003; Lee et al. 2005). A prior knowledge of the enzyme levels in a population using genotyping studies can serve as an indicator for administration of these drugs in appropriate doses or find an alternative therapy for patients with enzyme deficiency. Currently, the pharma companies marketing these drugs recommend the use of PG testing on their labels to guide drug dosing depending on inter-individual variation in the TPMT activity.

PG profiling plays a significant role in the design of clinical trials of a drug. An established example is that of Tacrine, a drug developed for patients suffering from Alzheimer's disease. These patients are known to overexpress APOE gene that has three isoforms E2, E3 and E4 and is responsible for production of apolipoprotein E, an essential protein, which guides cholesterol through the bloodstream. It is also known to modify the development of Alzheimer disease by interfering with the production of beta-amyloid protein. Accumulation of the beta-amyloid protein causes blockage of nerve cells in the brain cortex leading to their deterioration. E3 is most common form of the gene while individuals possessing E4 isotype are more predisposed to develop the disease. Patients of Alzheimer's disease with E2 and E3 form of the gene are known to respond well to Tacrine treatment while the ones having E4 are poor responders (Evans and McLeod 2003). Thus knowledge of the APOE genetic make-up of an individual with Alzheimer disease is important in not only selecting appropriate patient population for clinical trials but also serves as an important biomarker for identifying individuals predisposed to developing the disease.

A recent example where PG studies have played an important role is regarding the failure of certain drugs or their sudden withdrawal from the market due to ADRs (Adverse Drug Reactions). Vioxx, a Cox (cyclooxygenase)-2 inhibitor compound from Merck, used to relieve signs and symptoms of arthritis, acute pain in adults, and painful menstrual cycles had to be withdrawn from market 3 years after its launch due to its cardiac toxicity which became evident in 0.1% of the population in its post marketing phase (the product was launched in 1999 and voluntarily withdrawn in 2004). Also the company now has to face a number of lawsuits from the patients who after being administered Vioxx suffered due to its cardiac toxicity. An existence

of a database of genetic profile of people with different ethnic and racial background would have made possible to utilize this information for the screening of compounds in relation to different populations and in predicting any untowardly side effects. This, however, may limit the chances of a pharma company to come out with a blockbuster drug, one that fits all populations and age groups, but on the other hand, definitely increases the chances of the drug becoming successful for a particular patient population.

PG studies, thus, is leading to the concept of individualized therapy, whereby the patients or the consumers would gain the maximum benefit, as they will no longer have to take the medicines unsuitable for them and bear the adverse effects. Pharma companies and regulatory authorities are already encouraging the exploitation of these technologies that would result in the discovery of tailor-made drugs. Herceptin, an engineered monoclonal antibody from Genentech, used for the treatment of metastatic breast cancer is the first step towards the era of tailor-made medicines. It is only prescribed for patients in whom the tumor is found to over express the ERBb2/HER2 receptor protein (Mancinelli et al. 2000). Approximately 25 to 30% of the breast cancer patients with metastatic disease are known to abundantly over express HER2 protein and hence make ideal cases for Herceptin therapy (Phillips et al. 2004). Although the test conducted measures the protein overexpression using immunohistochemical method and not the genetic profile of the patient, making it not a PG profiling in its true sense; still it proves the significance of such knowledge and its potential application and advantages. Development of Herceptin also proves the fact that PGs finds application in more than one stages of drug discovery development. The link between HER2 overexpression and metastatic mammary carcinoma was established before development of this molecule and it was specifically engineered keeping the target identified as the focus of the whole program, thus providing an excellent example of rational target based drug discovery where the right medicine is chosen for the right disease. This led to the recruitment of selected patients, over expressing HER2 protein, for Phase III clinical trials. The efficacy of the drug was established based on two Phase III clinical trials on a small number of patients (469 and 222) that led to the approval of the drug. Global herceptin sales were of the order of US \$ 276 million in the year 2000 (Chem. and Engg. News

August 2001).

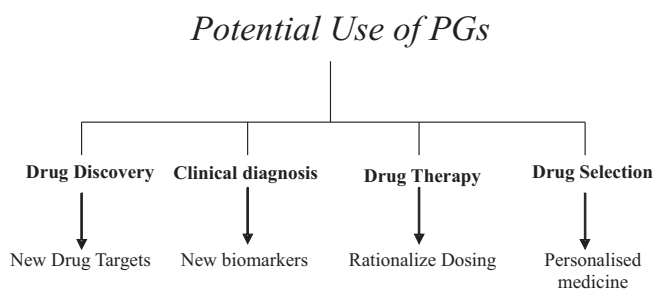


Fig. 2: The potential use of PGs

## Road Map for Future

The science of PGs has not only impacted upon drug discovery in Western countries, but is starting to play a role in Asian countries as well. There are considerable efforts underway in six Asian countries (China, India, Japan, Republic of Korea, Singapore and Taiwan) to exploit PG related activities including examination of guidelines and establishment of projects to establish foundations of this field in order to derive maximum benefit from such studies. China has agreed that they, in principle, would conform to the "International Declaration on Human Genetic Data" adopted by UNESCO in 2003. The Chinese Ministry of Health is funding disease genomics and PGs research work (Tamaoki et al. 2004). India is now emerging as the future centre for Global Clinical Trials due to access to a large naïve patient population with diverse ethnic background. In this regard, DBT (Department of Biotechnology, Government of India) has identified PGs as high potential area and has drafted guidelines to develop proposals in this field. Companies like Bioserve have already started the PGs initiative with collaborative funding from Technology Development Board (TDB) under the Department of Science & Technology, Govt. of India allied with APIDC Venture Capital Ltd. In Japan, Japan PGs Consortium (JPCG) was set up in June 2003, comprising of 10 pharma companies whose goal is not only to improve the basic conditions and establish a Japanese standard for promoting PG study in development and post-marketing of the drug but also act as a support center to help companies in performing PG clinical studies with genetic analysis. In June 2004, Japanese Ministry of Health, Labor and Welfare has issued a draft proposal entitled, "Submitting clinical trials information in which PG approaches were used, to the regulatory

agency for making a guidance document for PG approaches on pharmaceutical developments". SNP analyses among CYP 450s have revealed that out of the 452 SNPs identified so far, 244 could be considered unique to Japanese. In the Republic of Korea, the "Research Guideline for functional analysis of the Human Genome" was published in June 2002 and outlines the responsibilities of the Institutional Review Board along with protecting patient privacy rights and the right to self-determination. The governments of Singapore and Taiwan are also ready to accept the challenges posed by PG testing by establishing guidelines and norms for conducting such studies.

The Asian continent thus seems to be well poised for applying the potential of PGs along with their Western counterparts.

Although PGs holds a lot of potential and promises a fair deal equally to patients and drug manufacturer's alike, it raises a lot of questions that need adequate attention before yielding practical results. Generation of individual's genotypic data poses problems about ownership of such data and whether it infringes on the privacy of the concerned individual(s). Since most of the SNP data generated so far is in public domain, policies regarding the use of such data need to be transparent enough to avoid any legal implications. Who should be given access to such data and for what purpose? Will PGs alter the role of patent in drug development, as a huge amount of SNP data mentioned above can be used by pharma companies to make better safe and efficacious drugs? Will the existence and use of genetic data jeopardize the rights of an individual to be covered by medical insurance companies (if the companies know that the genetic data available predisposes the individual to a particular disease)? The laws governing medical insurance policies need revisiting lest genetic data is used for generating tailor-made drugs. Drugs for personalized therapy may become expensive in the absence of billion dollar blockbuster drugs and therefore, are patients willing to disburse more for drugs that have been individualized to cause maximum benefit and minimal side effects? Who pays for the cost of the genetic tests; the patient or the pharma company? The regulatory agencies such as the US FDA have to come up with new/modified guidelines to accept novel drugs discovered using PG principles. In this regard, FDA had come up with guidelines in Nov. 2003 for voluntary genomic data submission (VGDS) that may become

mandatory in a few years from now (FDA Guidance for PG data Submission Nov. 2003). An Interdisciplinary PG research group (IPRG) has been set up to review the VGDS filings. FDA has stated clearly that this data will not be used for making regulatory decisions. The intent, however, is to build expertise and foundation in this area for developing scientifically sound regulatory policies. A VGDS provides an opportunity to have informal, scientific meeting with FDA PGs experts and help build consensus around PG standards, policies and guidance's.

In addition to the above concerns, the pharma industry has to swallow the bitter pill in grasping the idea that each novel drug discovered may not bring in billions of dollars that were seen in yesteryears but have to content with generation of lesser revenues (hundreds of million dollars, may be) with a specific drug working on a selective patient population (Murphy 2000).

The above mentioned concerns however, outweigh the advantages PGs can bring in future and there is no doubt that it will eventually become an established scientific discipline leading to personalized medicine; a dream we hope will be realized in our life-time.

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