Review

A perspective on Pharmacogenetics and Pharmacogenomics

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Abstract

Pharmacogenetics is the study of variability in drug response and toxicity due to genetic factors. Pharmacogenomics applies the information gained by pharmacogenetics to the development of medications. The field of pharmacogenomics has led to the development of genotyping technologies which has allowed for wider screening for inherited causes of variable outcomes following drug administration.

Keywords: Pharmacogenetics, pharmacogenomics, genotype and therapy.

INTRODUCTION

Pharmacogenetics is the study of variability in drug response and toxicity due to genetic factors. Pharmacogenomics applies the information gained by pharmacogenetics to the development of medications. The field of pharmacogenomics has led to the development of genotyping technologies which has allowed for wider screening for inherited causes of variable outcomes following drug administration (Khatri et al. 2012).

Pharmacists will use a patient's genetic profile to select the most appropriate medication for the treatment or prevention of a disease to which the patient is genetically predisposed. An individual's genetic profile may be used to choose a medication with minimal side effects by accounting for genetic differences in the production of cytochrome P 450 enzymes(CYP450).In other words,the newly emerging fields of pharmacogenetics and pharmacogenomics deal specifically with genetic determinants of drug therapy. (Sadee, 1999; Evans and Relling,1999; Mancinelli et al. 2000) While pharmacogenetics largely focuses on candidate genes, such as drug-metabolizing enzymes, pharmacogenomics broadens the scope of study to include all genes (i.e., the genome). The latter approach takes into account that a patient's response to any given drug depending on numerous proteins in the body, such as metabolizing enzymes, transporters, receptors, and entire signaling networks mediating the response. Furthermore, genetic defects that underlie the disease process could determine the drug's effects even if they have no direct bearing on its mechanism of action.

What Can We Expect for Drug Therapy?

Genomics has brought about a new era in therapy. Until now, genetic research has progressed on a case-by-case basis, in response to questions about the role of genetic variants in a given disease or treatment outcome. For drug therapy, genetic information will also have profound impact in a number of ways:
•   Disease genotype determines the optimal therapeutic approach.
•   Genetic variants inform selection of effective drug and proper dosage.
•   Genetic variants affecting disease susceptibility serve to initiate early treatment or prevention.
•   Genomics directs the design of novel drugs that avoid or exploit specific genetic variants.
     Treatment failures are probably far more common than Adverse Drug Events(ADE). Therefore, prospective genotyping may prove most valuable for enhancing drug efficacy rather than avoiding ADEs—for example, in neurodegenerative disorders where evaluation of drug

response is difficult and negative outcomes are severe. Preventing ADEs also has the potential to improve therapy.
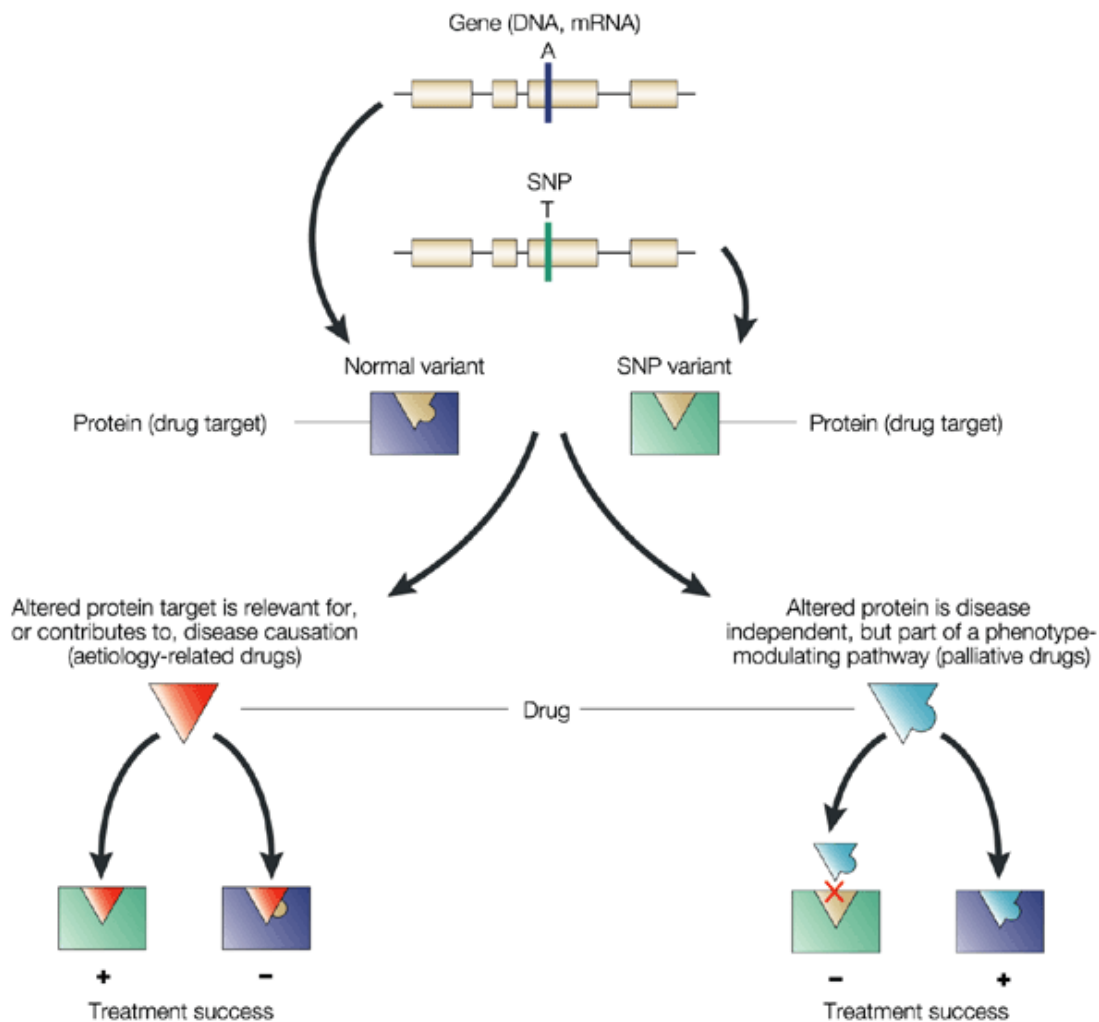
Therefore the dawn of genetic medicine and its role in pharmacotherapy heralds a new era of pharmacists and drug manufacturing corporations. The Human Genome Project (HGO), which is an effort to map the entire sequence of human DNA, is already providing the tools for developing better diagnostic and therapeutic options for clinical medicine. The ultimate goal is to learn how to prevent disease or treat it with gene therapy or a drug developed specifically for the underlying defect. (Collins, 1999) HGP is on its way to map the complete set of genetic instructions that make up the human genome. These instructions consists of approximately three billion base pairs that code for 80,000 to 100,000 genes located on 23 pairs of chromosomes. (Collins, 1999) It is hoped many more single-gene disorders will be identified. This will prove useful particularly in conditions that are not normally considered to be congenital or hereditary. An example is Lenegre-Lev disease in which there is progressive conduction system defect (PCSD)in which sodium channel defect becomes evident only in the fifth or sixth decade of life(Priori et al.1999) Such a wide spectrum of possibilities opens up the clinical chemist's education to the understanding of molecular biology, its applications and the tools involved in genetic studies.

Gene Mapping (Collins, 1999)

A genome map describes the order of genes or other markers and the spacing between them on each chromosome. At the coarsest resolution are genetic linkage maps, which depict the relative chromosomal locations of DNA markers (genes and other identifiable DNA sequences) by their patterns of inheritance. Physical maps describe the chemical characteristics of the DNA molecule itslef. Genetic linkage maps show the relative locations of specific DNA markers. These markers must be polymorphic to be useful in mapping; that is, alternative forms must exist among individuals so that they are detectable among different members in family studies. Polymorphisms are variations in DNA sequence that occurs usually on an average once every 300 to 500 base pairs. Variations within exons can lead to observable changes, such as differences in eye colour, blood type, and disease susceptibility. Variations in intron regions have little or no effect on an organism's appearance or function. Yet at DNA level can be detected and used as markers. Examples of these types of markers are a) restriction fragment length polymorphisms (RFLPs), which reflect sequence variations in DNA sites that can be cleaved by DNA restriction enzymes, and 2) variable number of tandem repeats units and, therefore, in length (a characteristic easily measured). The human genetic linkage map is constructed by observing how frequently two markers are inherited together. On the genetic map, distances between two markers are measured in terms of centimorgans (cM). Two markers are said to be 1 cM apart if they are separated by recombination 1% of the time. A genetic distance of 1cM is roughly equal to a physical distance of 1 million bp (1 Mb). The value of the genetic map is that an inherited distance can be located on the map following the inheritance of a DNA marker present in affected individuals. Genetic maps have been used to find the exact chromosomal location of several important disease genes, including cystic fibrosis, sickle cell disease, Tay-Sach's Disease, fragile X syndrome, and others. Genetic mapping includes the application of recombinant DNA technology involving in vitro radiation-induced chromosome fragmentation and cell fusions (joining human cells with those of other species to form hybrid cells) to create a panel of cells with specific and varied human chromosomal components. Restriction enzymes isolated from bacteria act like microscopic scalpels cutting DNA at specific palindromic sites creating sticky or blunt ends. For example restriction enzymes with 4 bases recognition sites will yield pieces 256 bases long, 6-base recognition sites will yield pieces 4000 bases long, 8-base recognition sites will yield pikes 64,000 bases long. Since hundreds of different restriction enzymes have been characterized, DNA can be cut into many different small fragments.

Physical Maps vary in their degree of resolution. The lowest resolution physical map is the chromosomal (sometimes called the cytogenetic) map, which is based on the distinctive banding patterns observed by light microscopy for stained chromosomes. A cDNA map shows the locations of expressed DNA regions (exons) on the chromosomal map. The more detailed map cosmid contig map depicts the order of overlapping DNA fragments spanning the genome. A macro restriction map describes the order and distance between enzyme cutting (cleavage) sites. The highest- resolution physical map is the complete resolution of the DNA base- pair sequence of each chromosome in the human genome. In short, genome maps at the coarsest level, measures recombination frequency between linked markers (genes or polymorphisms), restriction fragments of 1 to 2 Mb can be separated and mapped, ordered libraries of cosmids and Yeast Artificial Chromosomes (YACs) have insert sizes from 40 to 400 kb and the base sequence is the ultimate physical map. In the early 1980s, the first generation of the human genome map was based on restriction fragment length polymorphisms (RFLPs) identified by Southern analysis. Linkgage analysis by RFLP became a very powerful tool for molecular diagnostics. The early 1990s saw the rise of the second generation of mapping, based on microsatellite markers and polymerase chain amplification. The third generation of genome mapping is based on single-nucleotide polymorphisms (SNPs), and analyzed by PCR and chip-based micro arrays (Vilain, 1998; Swenn et al., 2011).

Gene (DNA, mRNA)
A

SNP
T

Normal variant   SNP variant

Protein (drug target)   Protein (drug target)

Altered protein target is relevant for,   Altered protein is disease
or contributes to, disease causation   independent, but part of a phenotype-
(aetiology-related drugs)   modulating pathway (palliative drugs)

Drug

+   −   −   +

Treatment success   Treatment success

mRNA, messenger RNA; SNP, single-nucleotide polymorphism

The practical details of these procedures and other methodologies are beyond the scope of this review.

Examples of Genetic Variants Affecting Drug Response and Toxicity Cytochrome P450

This is by far the best-documented series of polymorphisms, with profound effects on drug therapy. A very large gene family comprised of numerous isoforms, cytochrome (CYP) enzymes oxidatively metabolize xenobiotics, including many drugs. Specializing in the removal of lipophilic foreign chemicals, these enzymes rank among the most abundant proteins in the liver, but they also play key roles in other tissues, such as the kidneys and adrenals (where they serve to synthesize endogenous hormones) (Vilain, 1998). Once ingested,

lipophilic compounds tend to accumulate to toxic levels, were it not for CYP enzymes with broad substrate selectivity. Because most CNS-active drugs must be lipophilic to cross the lipophilic blood-brain-barrier, these drugs largely depend on CYP metabolism for elimination from the body. When CYP mutations result in null alleles (no catalytic activity), drugs may reach toxic levels if given in regular doses. Indeed, a number of defective CYP genes have accumulated to high allele frequencies, whereas deleterious sequence variants in most other genes are rare. Perhaps one might consider that CYP enzymes play a dual role. One the one hand, they inactivate the drug/xenobiotic and prepare it for excretion. On the other hand, they are also capable of activating foreign chemicals to highly reactive toxic intermediates that might act as carcinogens or mutagens. Hence, the accumulation of null alleles may convey some as yet
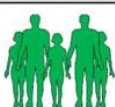
unspecified advantage to heterozygous carriers (having only 1 defective allele) (Nebert, 1997).

A number of remarkable examples have emerged where genotype can be clearly associated with drug effect. Ironically, this includes addictive drugs, such as codeine and nicotine. Through oxidative demethylation, CYP2D6 converts codeine to morphine, the active analgesic metabolite (Sellers and Tyndale, 2000); therefore, poor CYP2D6 metabolizers do not respond well to codeine. By contrast, the strongly addictive nicotine is inactivated by CYP2A6. Individuals with CYP2A6 mutations smoke fewer cigarettes and can quit more easily, (Sellers and Tyndale, 2000) possibly because failure to metabolize nicotine rapidly might lead to enhanced tolerance and ADEs. These findings have led to the concept of mimicking gene defects to treat drug dependence, for example, using CYP2A6 inhibitors to combat smoking. (Sellers and Tyndale, 2000)

Most antidepressants are mainly metabolised by CYP2D6 and/or CYP2C19 enzymes. For example, fluoxetine is mainly metabolised by the CYP2D6 enzyme whereas sertraline is metabolized by the CYP2C19 enzyme.

The current availability of pharmacogenomics enables the separation of individuals into the following categories based on their metabolizing capacity of both CYP2D6 and CYP2C19 enzymes with Pharmacogenomic-driven prescriptions are personalized taking an individual's genetic metaboliser status into account; (Klaus Lindpaintner, 2002; Swenn et al., 2011).

	Poor Metaboliser (PM)	Intermediate Metaboliser (IM)	Extensive Metaboliser (EM)	Ultrarapid Metaboliser (UM)
Segmented Population				
CYP2D6 (Caucasian)	10%	40%	48%	2%
CYP2C19 (Caucasian)	4%	20%	46%	30%
Metabolism Status	Slow	Reduced	Normal	Very Fast

After: Pharmacogenomic-Driven

Enzymes that inactivate or activate Anticancer Drugs

Much of cancer chemotherapy employs maximally tolerated doses to inflict the greatest damage to the tumor while sparing normal tissues. Cancers are genetically highly variable because genetic/genomic instability is a hallmark of nearly all cancers. Thus, we must expect significant genetic differences between normal and tumor tissue, each raising different issues in therapy. Mutations of drug-inactivating enzymes in normal tissues potentially increase the risk of severe toxicity and even death. Documented examples include the enzyme thiopurine methyl transferase, TPMT, and dihydropyrimidine dehydrogenase (DPyDH). Homozygous carriers of TPMT null alleles (0.3% frequency) are at high risk for severe toxicity from thioguanine (against childhood leukemias) and azathioprine (immunosuppressant) therapy unless the dose is drastically reduced. (Krynetski and Evans, 1998; Lennard et al., 1989) Yet, much work remains before anticancer therapy can be optimized with the use of genotyping.

Drug Transporters

Numerous genetic diseases are associated with membrane transporters, (Rosenberg and Short ,1994) encoded by hundreds or even thousands of genes. It is therefore surprising that relatively little is known about genetic variability of transporters affecting the drug response. This may soon change as intense efforts are aimed at clarifying the role of transporters in drug absorption, disposition, and targeting. Disorders involving defects in solute transporters, such as severe diarrhea in glucose/galactose malabsorption and primary bile acid malabsorption, may be associated with pronounced general changes in drug absorption. The first prominent example with particular relevance to drugs was the finding that multidrug resistance protein MDR-1 serves as a transporter that extrudes numerous drugs out of cells (Roninson et al. 1986).

Overexpression of MDR-1 in tumors has been associated with resistance to adriamycin, paclitaxel, and many more anticancer drugs allele, suggesting broad implications for drug disposition.

The transporter-like sulfonylurea receptor (SUR) regulates ATP-sensitive K+ channels and insulin secretion. Two gene splice site mutations that lead to disruption of the SUR protein segregate with familial persistent hyperinsulinemic hypoglycemia of infancy (Thomas et al.1995). While these mutations are rare, any possible consequences for treatment with sulfonylureas need to be addressed. Similarly, the transporter-like Na+/H+ exchanger (NHE1) serves as one of the target receptors for the antihypertensive diuretic, amiloride. Mutations in NHE1 confer amiloride resistance upon toxic effects of chronic acidosis (Counillon et al., 1993). Such genetic variants in a multitude of transporters or transporter-like proteins could modulate the activity of a broad variety of drugs.

Drug Receptors

Polymorphism of the beta-2 adrenergic receptor emerged as a primary example of genetically altered drug effects at neurotransmitter and hormone receptors that couple via G proteins (GPCRs) (Liggett, 1998). An SNP resulting in an R16G substitution was shown to cause enhanced beta-2 receptor down regulation in response to therapy with beta-adrenergic agonists (eg, albuterol). This is associated with reduced efficacy and duration of action, requiring a switch to alternative medication for asthma treatment in children. However, few, if any, GPCR variants have been shown to affect therapy in such a way to recommend genotyping before therapy.

A number of functional variants in the class of steroid hormone-nuclear receptors have also shown dramatic changes in their response to drugs and hormones. Glucocorticoid resistance in asthma has been associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform (Sousa et al., 2000). Lastly, dominant negative mutations of PPAR gamma (peroxisome proliferator-activated receptor gamma) are associated with severe insulin resistance, diabetes mellitus, and hypertension (Barosso et al. 1999). PPAR gamma belongs to a group of nuclear receptors that may be involved in a broad spectrum of disorders. These receptors are the targets of antidiabetic thiazolidinediones and fibrates. Barosso and associates (1999) raise the possibility that treatment with a thiazolidinedione in combination with an RXR agonist ligand (the retinoid RXR forms a heterodimer with PPAR gamma) may overcome mutant PPAR gamma dysfunction in affected patients.

Cholesteryl ester transfer protein (CETP) mediates the exchange of lipids between lipoproteins and may promote the atherogenicity of low-density lipoprotein. (Kuivenhoven and colleagues (Kuivenhoven et al., 1998) studied the relation between a frequent CETP polymorphism (presence or absence of a TaqI restriction site; alleles B1 and B2) and progression of atherosclerosis, as well as cholesterol-lowering therapy. The B1 variant was associated with higher CETP plasma concentrations and lower HDL levels. This CETP marker was also associated with disease progression. Moreover, progression of coronary atherosclerosis slowed upon pravastatin therapy in B1/B1 carriers but not B2/B2 carriers (16% of treated male patients). Therefore, this common DNA variant appears to predict therapy outcome with pravastatin in male patients.

Genotyping Infectious Agents

Pharmacogenomics clearly extends to genetic variability of viruses, bacteria, fungi, and parasites. Numerous examples exist in the literature describing the molecular mechanisms of drug resistance. In particular, a large portion of intensive multidrug therapy of HIV/AIDS is guided by genotyping of the virus to detect variants that render specific drugs ineffective. (Perrin and Telenti, 1998) Because HIV therapy is long-term, drug failure has serious consequences, and the drugs are exceedingly expensive, HIV-genotyping may well become standard clinical practice. Similarly, therapy for hepatitis C may benefit from genotyping to identify the more virulent strain (genotype 1), with enhanced response to interferon and ribavirin (McHutchinson et al., 1998). Following the complete sequencing of the Mycobacterium tuberculosis genome, the genetics of tuberculosis resistance may offer another promising area for enhancing drug therapy (Telenti, 1998).

In each of these cases, current therapy requires long-term drug treatment, and therapeutic failure can have disastrous consequences. This could propel genotyping into clinical practice at a fast pace. The most obvious role for the pharmacist will therefore be the education of patients on the use of medications using genetic information. As the knowledge base concerning disease and genetic factors affecting disease continues to grow, it will become more important for pharmacists to become knowledgeable about different aspects of genetics-based pharmacothereapy as well as pharmacogenomics to meet the challenges ahead as well as to be a part of the futuristic medicine.

Many drug corporates have initiated pharmacogenomics and transcriptomic services including innovative diagnostics. The goal such companies is to provide services by clinical pharmacogenomics analysis, which enables optimization of clinical trial design, is to facilitate evaluation of clinical pharmacological parameters such as dose response, treatment efficacy, safety-toxicity (adverse effects).

Skuldtech is specialized in the discovery of new Biomarkers and the development of Diagnostics focused on personalized medicine applications. This expertise enables the company to provide genomic, pharmacogenomic and transcriptomic services, as well as innovative diagnostics associated with new treatments, especially in cancer, neurodegenerative and infectious diseases. Meeting the FDA recommendations, Skuldtech a Biotech has developed pharmacogenomic services based on NGS technologies and its extensive experience able to support pharmaceutical industry to optimize clinical trials.

CONCLUSION

Personalized medicine is based upon your age, lifestyle and health which influence your response to medications. But so do your genes. Therefore, scientists are working to match specific gene variations with responses to particular medications with the hope of tailoring treatments to individuals. That is what the field of

pharmacogenomics does and forms the very basis of personalized medicine and therapy.

Future implications

Deeper insights are required into how physical and functional networks of interacting DNA, non-coding RNAs including microRNAs, proteins and other molecules drive cells behavior in health and disease states. Hopefully, step-by-step advances in transcriptome, proteome, epigenome and interactome analysis will lead to patients stratification and personalized health care through systems science-based integrative genome profile.(Khatri et al.2012)

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