PHARMACOGENETICS AND PHARMACOGENOMICS

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Abstract

Pharmacogenetics and Pharmacogenomics constitute an especial young field of research in the domain of pharmacology. Both work on genetic variations which occur in individuals resulting reduced drug efficacy and more adverse drug reactions. Pharmacogenetics, emphasizes the diversity of patients and their genetic background, set their response to a given drug therapy, making understood the biological variability whereas pharmacogenomic considers the effects they cause in an individual (patient) different medications. The differences are studied on gene expression induction and repression of genes. The drug metabolism in human body is acted out by the P450 enzymes. In mammals, xenobiotic metabolizing CYPs provide crucial protection from the effects of exposure to a wide variety of chemicals, including environmental toxins and therapeutic drugs. In Phase I reactions (oxidation, reduction, and hydrolysis) and phase II conjugation reactions of drug metabolism in human body (acetylation, glucuronidation, sulfation, and methylation) are influenced by a number of genetic polymorphisms. Because many of the polymorphisms causing adverse effects are the result of single nucleotide changes, then a SNP profile of an individual could be used to guide therapy. If the genotype of an individual was known in advance then better clinical decisions could be made. Currently, more than 30 families of enzyme complexes responsible for drug metabolism have been described in humans and numerous variations exist in the encoding the many enzymes and proteins. The interdependence of the genetic makes up a large pharmaceutical research field today. More new analysis techniques beyond the understanding of the disease is possible and the Pharmaceutical treatment as targeted and individualized therapy. The ultimate goal of studies is to make more effective and affordable (financially) regimens with fewer side effects and greater patient response.

Key words: Pharmacogenetic, Pharmacogenomic, P450 enzymes, Drug metamolism, CYP2C9

Introduction

Historically, pharmacogenetics is the older term and emerged as individual variation in the pharmacokinetic and pharmacodynamic response to drugs became apparent [1-3].The development of the Human Genome Project has coined the new term, pharmacogenomics. This term incorporates pharmacogenetics but has a rather broader meaning, describing the wider influence of DNA sequence variation on phenotype and the effect on drug handling and efficacy. The altar of current technological developments in the field of Biomedical Sciences, is the Pharmacogenetics and Pharmacogenomic. The time when clinicians thought that a drug can be used for all

patients tended to give way to a personalized treatments, consistent with the genomic profile of the patient. By today's standards, the use of new molecular diagnostic studies ever conducted to determine the "uniqueness" of each individual, the overriding sense of polymorphisms and analysis of the human genome.

Currently, more than 30 families of enzyme complexes responsible for drug metabolism have been described in humans and numerous variations exist in the genes encoding the many enzymes and proteins. Several reviews illustrate the ways these variants may be clinically important [4,5-7] but the real clinical significance for most remains unstudied and uncertain. Today, pharmacogenetics and pharmacogenomic open new avenues in personalized treatment, while creating new ethical concerns.

Pharmacogenetics

Pharmacogenetics is the study of how genetic variations influence a person's response to drugs . These variations underlies the response to therapy, including possible adverse effects. It also deals with the assessment of clinical efficacy and the pharmacological phenotype. These are the central tenets of pharmacogenetics. Some health care leaders view pharmacogenetics as providing the potential to create personalized prescriptions; with the opportunity to improve patient compliance, reduce adverse events, and reduce the cost of managing chronic disease. Up to 90% of the variability in drug response between individuals can be explained by genetics. Pharmacogenetic information is now included in the labeling of about 10% of drugs approved by the FDA. Inherited variants in the cytochrome P450 drug metabolism genes contribute significantly to an individual's drug response.

Three of the most clinically important CYP450 drug metabolizing enzymes are CYP2C9, CYP2C19, and CYP2D6. Together, these three enzymes metabolize up to 40% of all currently prescribed drugs. Testing for a panel of genetic variants in each of these CYP450 genes allows the prediction if a person will have impaired or increased metabolism of drugs processed by these enzymes[9]. This knowledge can help to individualize drug selection and dosing based on individual's genetic make-up. The Pharmacological phenotype defined the response of the individual or group of individuals with common genetic characteristics to a particular drug substance. Important though the distinction remains the drug response to drug toxicity observed in patients. Genotypes with dedicated single microarray assay or gene chip variations of genes opened new avenues to study the metabolism of secretion and transport of drugs. In addition, The method of microarrays is most appropriate for the analysis of many polymorphisms simultaneously, which is necessary in pharmacology.

Continuing, Pharmacogenetics, emphasizes the diversity of patients and their genetic background, set their response to a given drug therapy, making understood the biological variability.

Pharmacogenetics need to distinguish between the two branches:

A) The Classical Pharmacogenetics which the analysis of genes, explores

1) Pharmacokinetics on the absorption metabolism activated precursor forms off active drug substances, the formation of biologically active metabolites of the distribution and excretion.

2) pharmacodynamic related to transportation and drug receptors, protein targets of drug action.

B) Ground the Pharmacogenetics and clinical studies that the biological variability that leads to disease and the expression of symptoms and pathology, distinguishing in this way a group, a subset of patients.

Adverse drug reactions (ADRs) are a serious public health issue and occur in an estimated 6.7% of hospitalizations in the U.S., rank as the fourth to sixth leading cause of death.Only an estimated 25%-60% of people respond in the expected way to most currently prescribed drugs.[2] All factors influencing pharmacologic response are likely under some degree of genetic control. Testing for genetic variants that are known to influence drug response will allow the clinician to select the best drug and dose from the start – avoiding some of the trial-and-error traditionally required.

Pharmacogenomics

Pharmacogenomic is the branch of pharmacology which considers the effects they cause in an individual (patient) different medications. The differences are studied on gene expression, induction and repression of genes[10-13]. Genes and their products regulate the transport of drugs to specific tissues, encoding receptors for drugs and protein targets where pharmacological effects exerted. It helps to find suitable compounds for which should be an investigation into the drug discovery design, if a drug is determined by the pharmacodynamic genetic profile of the individual.

It is therefore understood, interdependence of pharmacogenetics to Pharmacogenomic for the conduct results in drug treatment for both the compound (medicine) and the group of patients referred [14].The mutations that occurs in the genetic material is an important factor in genetic diversity may also involve deficits or additions to a large section of DNA (from 2-500kb) or parts thereof (or a few nucleotides).

Division of point mutations
1.Mutations in the wrong sense
2.Meaningless mutations
3.Frameshift mutations reading
4. Mutation in the conservative sequences splicing of introns
5.Mutations in the gene movers
6. Mutations in 3΄and ΄5 untranslated ends
7.Mutations polymorphisms

Table 1. Representation of point mutations

SNPs are defined as the difference in nucleotide sequences in the DNA, comparing it to base basis. Given that the human genome is 3×10^9 basepairs in size and that SNPs occur on average every 1000 nucleotides, thera are over 3 million SNPs in human genome. Mutations polymorphisms or SNPs may takes place on both coding regions influencing the production of proteins, and non-coding regions of the genome which are related to internal structural and operational activities of the gene.

Pharmacogenomic is focusing on the polymorphisms in the regions encoded and can cause modulation of the protein and this action. It is therefore understandable that utility location on chromosomes of both polymorphisms to detect and to treat diseases, for example, the hybrid gene BCR-ABL[15] associated with chronic myelogenous leukemia and in finding the appropriate treatment regimen used for suspension of action. The value of research of polymorphisms, is beyond the diagnosis and study of the metabolism of drugs.

Drug Metabolism in Humans

Altered drug metabolism contributes significantly to drug response variability. Phase I drug metabolism is primarily performed by the cytochrome P450 enzyme family. The cytochromes P450 (CYPs) comprise a vast superfamily (>6000 known members)15 of haem-containing mono-oxygenase enzymes found in virtually all life forms. Members of this ubiquitous superfamily play an important role in the metabolism and biosynthesis of a wide range of exogenous and endogenous compounds16. In mammals, these enzymes are involved, among other things, in the metabolism of xenobiotic compounds—including environmental toxins and therapeutic drugs. One of the most interesting characteristics of the CYPs is their promiscuity. Individual isoforms are capable of interacting with a wide range of chemically diverse substrates, and some CYPs have overlapping substrate specificities. This promiscuity is useful in terms of defence of the organism against potentially harmful xenobiotics, but in some instances can lead to rapid drug clearance/inactivation, production of toxic compounds and/or adverse drug–drug interactions.

In humans, 90% of all of the drugs currently approved for clinical use are metabolized by one of seven CYP isoforms, CYP1A2, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1 and/or CYP3A416,19,20. Of these isoforms, CYP2D6 and CYP2C9 display polymorphisms that can result in the poor metabolism21-27. Having knowledge of the structural features of the active sites of these seven isoforms in particular could lead to a tool that was able to predict whether or not a drug candidate would interact with the CYPs and, if so, which isoform the drug candidate may interact with preferentially. This would impact on the rational design of improved therapeutic drugs and target-specific inhibitors. It would also affect the risk assessment of xenobiotics and the avoidance of adverse drug–drug interactions, whereby one drug modulates the metabolism of another 19 by simple competition for the same active site, and/or by binding in an allosteric region of the same enzyme. Knowledge of the active site structure for these enzymes will significantly reduce the failure rate in clinical trials by identifying any CYP liabilities in the early stages of drug development, and reduce the amount of time and money required to bring a new pharmaceutical to the market.

The CYP2C9, CYP2C19, and CYP2D6 genes are involved in metabolizing a significant percentage of currently prescribed drugs, and variants that impact their action are quite common. Therefore, testing for a panel of genetic variants in these genes is now clinically available and allows a personalized prediction of response to a broad variety of drugs.

Enzyme	% Drugs Metabolized	Examples of Metabolized Drugs[4]
CYP2D6	25%	Beta Blockers: *S-metoprolol, propafenone, and timolol.* Antidepressants: *many of the most commonly prescribed including paroxetine, venlafaxine, amitriptyline, duloxetine, and imipramine.* Antipsychotics: *haloperidol, risperidone, and thioridazine.*Other examples: *codeine, tamoxifen, dextromethorphan, and ondansetron.*
CYP2C9	5%	NSAIDs: *such as celecoxib, naproxen, and ibuprofen.*Oral hypoglycemics: *tolbutamide and glipizide.*Angiotensin II blockers: *irbesartan and losartan.*Other examples: *warfarin, phenytoin, fluvastatin, and sulfamethoxazole.*
CYP2C19	15%	Proton pump inhibitors *such as omeprazole, lansoprazole, pantoprazole, and rabeprazole.*Anticonvulsants: *diazepam and phenytoin.*Other examples: *amitriptyline, clomipramine, clopidogrel, and progesterone.*

Table 2. Percentage of drugs metabolized by cytochrome isoforms

Metabolizer Phenotype	Caucasian/ White	African/ Black	Asian	Special Populations
CYP2C9 Poor Metabolizer	1-3%[1]	Rare[1]	Rare[1]	
CYP2C19 Poor Metabolizer	3-5%[2]	3-5%[2]	13-30%[2,3]	37% Sepik, Papua New Guinea[4]
CYP2D6 Poor Metabolizer	5-10%[2,3]	2-4%[2]	1-2%[2]	
CYP2D6 Ultrarapid Metabolizer	1-3%[3]	1-5% AA[5] 7-29% BA[5]	~1%[5]	29% Ethiopians[2] 28% Subsaharran Africans[5] 14% Tanzanian[5] 21% SaudiArabians[6] Up to 12% East Asians[5] Up to 10% Southern Europeans[2]

Table 3. Specific ethnic groups and metabolizer phrnotype

Genetic testing for the drug metabolism is most necessary for individuals with adverse drug reactions history, particularly to drugs metabolized by these isoforms. Individuals which are up to start treatment with medications known to be significantly

affected by genetic variations in CYP450 enzymes. Last but not least specific ethnic groups with a high prevalence of poor or ultrarapid metabolizers. For example, between 3 and 10% of the Caucasian population fail to metabolize the adrenergic blocking drug debrisoquine and treatment results in severe hypotension. In afro-Americans the frequency of this poor metabolizer condition is 5% and in Asians it is just 1%. Affected individuals are homozygous for a mutant cytochrome P450 gene (CYP2D6) and they also fail to metabolize over 20% of all commonly prescribed drugs, including codeine. The same gene also has alleles that cause an elevated-metabolizer phenotype and this has been correlated with increased susceptibility to cancer.

The main aim of drug metabolism in human body is to render more water soluble and thus more readily excreted by the urine or bile. Drug metabolism involves the alteration of functional groups on the parent molecule (e.g.,oxidation) via the cytochrome P450 enzymes[28-30]. These enzymes are most predominant in the liver but can also be found in the intestines, lungs and other organs.The metabolic pathway consists of 2 phases :

Phase 1 drug metabolism includes processes such as hydrolysis and oxidation reduction which are acted out by P450 enzymes. Polymorphisms in Phase 1 on the CYP 1-4 and having great importance for the metabolism of drugs, classifying patients phenotypically depending on the speed of metabolism using specific indicators of medicines a patient is not receives any other treatment.

Phase 2 drug metabolism includes processes such as conjugation, methylation acetylation and esterification and acted out by NAT-2 TMTn glykopourinotransferases. However, polymorphisms in Phase 2 have been studied in oncology patients, and relate to reduced enzyme activity and adverse reactions to the chemotherapy (breast cancer, colon).

Conclusion

To summarize, polymorphisms are central to the study of pharmacogenetics and pharmacogenomics because they concern:

- Drug metabolizing enzymes DME
- Genes that encode receptor proteins and drug targets
- Group genes and proteins associated with disease and therapy

For many genetic polymorphisms affecting drug efficacy there is no evident phenotype in the absence of a drug challenge. This brings an unwanted element of chance into the selection of appropriate therapies for patients. If the genotype of an individual was known in advance then better clinical decisions could be made. Because many of the polymorphisms causing adverse effects are the result of single nucleotide changes, then a SNP profile of an individual could be used to guide therapy or selection for participation in a clinical trial. The molecular techniques for the detection of SNPs and genotyping include methods such as: *Pyrosequencing, Mass Spectrometry, Molecular Inversion Probe, Golden Gate, Whole Genome Sampling Analysis, SSCA-DNA, Heteroduplex Analysis, Protein Transaction Test, TGGE, DGGE, d-HPLC, Chemical cleavage of Mismatches, Enzyme Mismatch Cleavage, PCR, Multiplex PCR,*

Allele Specific Amplification, RFLPs, DNA sequencing.

From above techniques *Whole Genome Sampling Analysis* used as a diagnostic method, *PCR* is the most appropriate for studying polymorphisms, *d-HPLC* is preferred for genotypes while *microarrays* allow the simultaneous detection of expression of many genes. The better classification of the different subtypes of a disease will result to better diagnoses. The knowledge of the exact cause of each disease allows in the future to develop more specific drugs which they treat the cause of the disease rather than the symptoms as it happens in nowadays.

Ending, more new analysis techniques beyond the understanding of the disease is possible and the Pharmaceutical treatment as targeted and individualized therapy. Ultimately genetic analysis of affected individuals will suggest what drugs could be used and with pharmacogenetic analysis will determine which drugs should be used. This will be the era of personalized medicine, a medicine that is more affordable (financially) for the individuals and has the potential of less adverse drugs reactions with the greater patient response.

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