Review Article

Pharmacogenomics: The Concepts Behind Personalized Medicine

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The rapidly growing field of genomics holds the promise of radically changing many aspects of medicine and medical practice. As the current era of genomics advances, the amount of genetic information available to clinical researchers is rapidly expanding. Pharmacogenomics is an emerging discipline, critical for assessing the genetic basis of drug response and toxicity in patient populations. It is known for years that individuals can vary widely in their disease susceptibilities and in response to drug action. Recent findings indicate linkage between genetic polymorphisms and functional changes in proteins that are responsible for the pharmacokinetics of medications. Likewise, polymorphisms in genes encoding the targets (e.g. receptors) can alter the pharmacodynamics of the drugs. New insights into the molecular pharmacology and the functional elucidation of polymorphisms are paving the way from genomics to personalized medicine.

Introduction

Despite of major improvements in therapeutics and medical interventions optimal therapy remained relatively elusive for almost all the diseases. There is clear evidence of significant heterogeneity in ADME (Absorption, Distribution, Metabolism and Excretion) of drugs, which results in variable efficacy and toxicity of therapeutic agents, when viewed across the population[1]. It is known for years that biochemical diversity of individual patients is responsible for variable response against drugs. Pharmacogenomics is an emerging discipline tries to address the genetic basis of biochemical diversity and critical for assessing heterogeneous drug response and toxicity in patient populations[2]. It takes advantage of genomic techniques such as high-throughput DNA sequencing, gene mapping, bioinformatics and chemoinformatics to allow researchers identify the actual genetic basis of interindividual and interracial variation in drug efficacy[3,4].

The field started in 510 BC when Pythagorous observed that some people develop hemolytic anemia after consumption of fava been[5]. In early 1900 the people connected drug related disorders with Mendellian genetics. In the 1950s and 1960s, the field was boosted by observations that there are relevant polymorphisms within a few drug-metabolizing enzymes that are responsible for metabolizing the majority of the drugs and a common source of adverse reactions. The term 'Pharmacogenetics' was coined by Fredrich Vogel in 1959[6]. Pharmacogenomics was born in early 2001 with all other -omics word during the near completion of draft human genome sequence. Primary analysis of human genome sequence revealed that there is significant variation between inter-individual DNA sequences through out the genome. Most common variation is a discrete single nucleotide variation, polymorphism, which might causes variation in protein sequences and expression level[7].

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Basics of drug metabolism and toxicity

From the site of administration to the target site, and further during its clearance from the body, a drug interacts with various protein including transporters, receptors, targets, effectors and metabolic enzymes, all encoded by various genes[8].

Absorption: Among the various mechanisms of drug absorption one of the mechanisms is carrier mediated transport system of the drug (facilitated transport & active transport)[9]. Endogenous molecules that are transported actively includes riboflavin, niacin, pyridoxin, ascorbic acid, cyanocobalamin, ions like sodium, potassium calcium, iron, glucose, amino acids etc. Drugs having structural similarity to such agents are absorbed actively, particularly the agents useful in cancer chemotherapy[10]. Examples include absorption of 5-flurouracil and 5-bromouracil via the pyrimidine transport system, absorption of methyldopa and levodopa via an L- amino acid transport system and absorption of ACE (Angiotensin converting enzyme) inhibitor enalapril via a small peptide carrier system, others diffuse passively through plasma membrane.

Distribution: After absorption drug is distributed to various parts of body through blood which contains certain proteins that aid in distribution of drug. These include, serum albumin binds to large variety of all kinds of drugs. a1-Acid glycoprotein binds to basic drugs such as imipramine, lidocane, qunidine etc. Hemoglobin binds to phenytoin, pentobarbital and phenothiazines.

Metabolism: Drug is metabolized at various sites in body. The drug metabolizing ability of various organs in decreasing order is liver>lungs>kidney>intestine>placenta>skin. Majority of enzymes involved in drug metabolism are the members of Cytochrome P450 monooxygenase system. Six major families of cytochrome P450 enzymes are important metabolizing enzymes in the liver[11]. Activity of these enzymes can be affected by presence of exogenous substances. Drugs can

inhibit or induce the effectiveness of these enzymes. Likewise, the variability of P450 isozymes can lead to drug underexposure or overexposure. In some people these isoforms may under express or completely lacking. In one specific instance, a patient in a clinical trial who lacked the CYP2D6 isoform was identified after hypotensive fainting-spells, which was caused by drug overexposure[12]. Although commonly serving to detoxify xenobiotics, these enzymes are also principally responsible for the activation of procarcinogens and promutagens in the human body.

Table1. Drug metabolism by the major families of CYP450 enzymes[13]

CYP450 isoform	% of drugs metabolized
CYP3A4	55
CYP2D6	20
CYP2C19	15
CYP1A2	5
CYP2E1	1
Others	4

Excretion:- It is the process by which drugs and/or their metabolites are irreversibly transferred from internal to external envoirnment. Principal organs for excretion are lungs, biliary system, intestine, salivary glands, sweat glands and kidney. In renal excretion principal mechanisms are

a) Glomerular filtration

b) Active tubular secretion which is carrier mediated process and required for excretion of organic anions like penicillins, salicylates,sulfates, glucuronides etc or organic cations like morphine, mecamylamine,choline,histamine, etc

The quality and quantity of proteins involved in those processes and their interaction with drugs influences the absorption, distribution, metabolism and efficacy of the drugs as well as the their adverse effect profiles. As a result, multiple polymorphisms in many genes may affect drug response and the downstream secondary events triggered by these drugs, requiring a genome -wide search for the responsible genes. Genetic polymorphism in DMEs gives rise to three distinct subgroups that have measurable differences in their ability to metabolize drugs to either inactive or active metabolites[14]. Individuals capable of efficient drug metabolism are called extensive metabolizers (EMs) and individuals with deficiencies in metabolism, which typically requires mutation or deletion of both alleles of a gene, are termed poor metabolizers (PMs). Conversely, gene amplification and subsequent over-expression results in ultra rapid metabolizers (UMs). Standard doses of drugs with a steep response curve or a narrow therapeutic range could produce adverse drug reactions, toxicity or decreased efficacy in PMs. However, when taken by UMs, the standard dose might be inadequate to produce the desired effect, or, if the active agent is a metabolic product, could result in an effective overdose[6].

Moving from "One Drug Fits All" to personalized therapy

Till date therapeutics still relies on statistical analysis of population's response and formulate drug dosage based on information derived from population averages for individual patients. This "one drug fits all" approach leads to 100,000 deaths per year and US $75 billion in health care costs in US alone[15,16]. Unavailable data for rest of the world will be more horrifying. Pharmacogenomic research promises to evolve into an individualized approach to therapy where optimally effective drugs are matched to a patient's unique genetic profile (Fig 2). This involves classifying patients with the same phenotypic disease profile into smaller subpopulations, defined by genetic variations associated with disease, drug response, or both. The assumption underlying this approach is that drug therapy in genetically defined subpopulations can be more efficacious and less toxic than in a broad population.

Thus, individualizing drug therapy with the use of pharmacogenomics holds the potential to revolutionize medical therapeutics, by challenging the "one drug fits all" approach[17]. After human genome sequencing slowly emerging correlation of pharmaceutical sciences with the human genome thus has given rise to the promising new field of pharmacogenomics. (Fig 1) The characteristic of proteins interacting with drugs is determined by naturally occurring genetic variation. DNA loci that vary in nucleotide sequence from one individual to another are referred to as polymorphic. Naturally occurring polymorphism in human population can be found in form of insertions, deletions, amplifications and rearrangements of bases in the genetic material as well as single nucleotide polymorphisms (SNPs)[18].

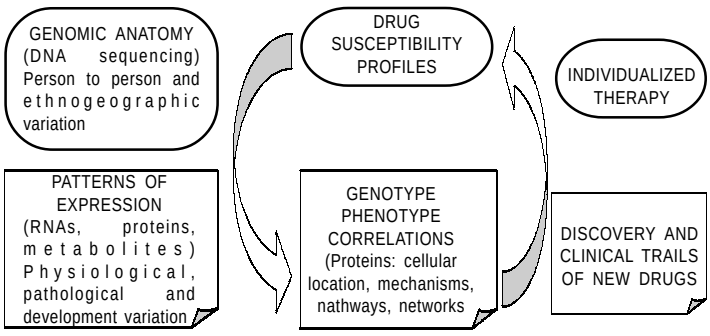

Fig. 1 Pharmacogenomics At A Glance

Mama, What is a SNP?

SNP is a stable substitution of a single base with a frequency of more than 1% in at least one population[14]. SNPs are distributed throughout the human genome at an estimated overall frequency of one in every 1900 bp. At the level of the chromosome, the density of SNPs appears to be relatively constant across the genome with the exception of the sex chromosomes. The widespread occurrence of genetic variation implies that any individual is likely to be polymorphic at many different gene loci, which may result in[19] Fig. 2.

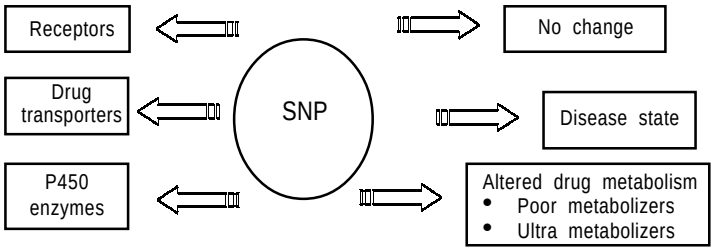

Fig 2 SNPs and their significance

- 'Silent' polymorphisms in coding sequences, no amino acid change, hence no change in properties.
- Formation of a variant protein, which might have altered properties as a consequence of change in structure.
- Polymorphic sequences within both exon and intron regions, which can also result in differential splicing, protein truncation and additional functional anomalies.
- Polymorphisms in regulatory regions that can alter gene expression, RNA levels and stability, and consequently protein expression levels.

Presence of SNP can alter drugs interaction with receptors, transporters, metabolizers and disease causing genes. Thus change in a single nucleotide can have vast effect in many aspect of drug metabolism.

It is clear in this context that conventional approaches of genetic analysis are insufficient to correlate presence of SNP with multifactorial world of drug response. It is also likely that a single-gene approach is liable to have a limited predictive power. Therefore, a number of candidate genes are selected for analysis in a typical study[20].

Experimental strategies for SNP-based approach

Do we need to sequence the whole genome for each individual? No, that level of detail will not be necessary. In contrast to the number of bases in our genetic code (6 billion) the number of proteins encoded by genome is a modest number, probably 35,000 or so[3]. Majority of those has no significant role in drug metabolism. Enzymes that are important for each patient's drug metabolism profiles can be tested using modern techniques like DNA microarray[21]. So by using large arrays of miniaturized tests called biochips, we will eventually have profile of several thousand key proteins and enzymes for each individuals (Fig 3)[22,23].

Two experimental strategies, linkage analysis and association studies, are being used by geneticists, to investigate genetic variants in human disease[24]. The former, LINKAGE ANALYSIS seeks to define a physical relationship between two or more genetic markers, identifying the location of a disease gene, whereas ASSOCIATION ANALYSIS correlate a sequence variant with a well-defined phenotype. Linkage analysis compares inheritance patterns of predefined genetic markers with gene of interest. The approach pinpoints a region of a chromosome based upon a non-random pattern of co-inheritance of markers, although usually it does not uncover the specific genetic variation responsible for particular phenotype. Multigenerational family pedigrees are required to facilitate tracing segregation patterns of genetic markers. Linkage studies have been employed to map hundreds of highly penetrant disease loci. Association studies test whether a SNP or MICROSATELLITE is enriched in patients with disease compared to suitable controls[25].

Information gained from such studies is pooled in genome sequence databases (GSdb) and serves as a reference point for assembling and interpreting pharmacogenomics information in drug discovery, development and prescription. From such cumulative database mining it has been found that there are relevant polymorphisms within a few DMEs that are responsible for metabolizing the majority of today's marketed drugs[26,27].

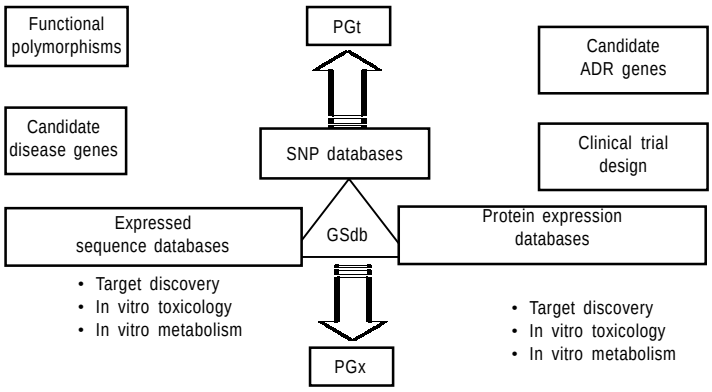

Fig. 3 Pharmacogenomics (PGx), Pharmacogenetics (PGt), And Drug Discovery

Anticipated benefits of pharmacogenomics

Better, Safer Drugs the First Time: Instead of the standard trial-and-error method of matching patients with the right drugs, doctors will be able to analyze a patient's genetic profile and prescribe the best available drug therapy from the beginning. This will speed recovery time and increase safety as the likelihood of adverse reactions is eliminated[12,28].

More Accurate Methods of Determining Appropriate Drug Dosages: Current methods of basing dosages on weight and age will be replaced with dosages based on a person's genetics --how well the body processes the medicine and the time it takes to metabolize it. This will maximize the therapy's value and decrease the likelihood of overdose[29].

Advanced Screening For Disease: Knowing one's genetic code will allow a person to make adequate lifestyle and environmental changes at an early age so as to avoid or lessen the severity of a genetic disease. Likewise, advance knowledge of particular disease susceptibility will allow careful monitoring, and treatments can be introduced at the most appropriate stage to maximize their therapy[30,31].

Target Specific Drug Discovery: Pharmaceutical companies will be able to create drugs based on the proteins, enzymes, and RNA molecules associated with genes and diseases. This

will facilitate drug discovery and allow researchers to formulate a therapy more targeted to specific diseases. This accuracy not only will maximize therapeutic effects but also decrease damage to nearby healthy cells[22,32].

Improvements in the Drug Discovery and Approval Process: Pharmaceutical companies will be able to discover potential therapies more easily using genome targets[26]. Previously failed drug candidates may be revived as they are matched with the niche population they serve. The drug approval process should be facilitated as trials are targeted for specific genetic population groups --providing greater degrees of success. Targeting only those persons capable of responding to a drug will reduce the cost and risk of clinical trials[33].

Decrease in the Overall Cost of Health Care: Decreases in the number of adverse drug reactions, the number of failed drug trials, the time it takes to get a drug approved, the length of time patients are on medication, the number of medications patients must take to find an effective therapy, the effects of a disease on the body (through early detection), and an increase in the range of possible drug targets will promote a net decrease in the cost of health care[34,35].

Clinical Trials: Pharmacogenetics can be used in analyzing clinical trial data to determine if a drug is more efficacious for a selected group based on genotype. This could speed up the clinical trial process and drug approval because no longer would a clinical trial be abandoned if a drug showed minimal effectiveness in a large population. Effect in a smaller subset of the trial--linked to a specific genotype-could yield some salvageable component of the trial and yield valuable therapy for a small population likely to be overlooked in large trials[12,13].

An example of pharmacogenetic applications assistance in drug development and clinical trials is amonafide[44]. Initial clinical safety trials identified two different safe doses. One trial recommended a dose of 250 mg/m^2, while another recommended 400 mg/m^2. Later trials split the difference and used 300 mg/m^2. This resulted in patients either receiving too low a dose to be effective or a dose that resulted in withdrawal from the trial. In genotyping studies, two major sub-populations based on NAT2 polymorphisms have been identified; one group that metabolized the drug well and developed toxic levels of the metabolite, and one group that did not. It was also found out that the population could be phenotyped using caffeine as a metabolic substitute to determine NAT2 genotype.

Pharmacogenetics has the potential to increase the speed and amount of data collected from a clinical trial that can ultimately increase the efficacy of drug treatment[36].

Hurdles to overcome

Ethical considerations

Using pharmacogenomics to understand the genetic basis of drug response requires individuals or members of a particular ethnic community as study subjects. Despite the obvious scientific value of using individuals and families in pharmacogenomic studies, it raises serious ethical concerns. Genetic information is, by its nature, inherently personal, familial and communal. Disclosure of information about genomes might cause research subjects personal, psychological, familial, or economical problems[37,38]. There are serious potential risks for discrimination and loss-of-privacy, which need to be addressed. The need to integrate molecular data with clinical data implies that clinical phenotype and genotype information may be accessible by various persons and organizations. Publication of patient related data in journals as well as in databases (such as Genebank) also poses a serious threat to personal privacy and may lead the loss of study-subject confidentiality. It is crucial, however, that the privacy of patient identity, identifying information and other demographic information should be protected. Pharmacogenomics studies may also affect particular ethnic communities, as exemplified recently by genetic research on Ashkenazi Jews[39] or the association of apolipoprotein E genotypes with Alzheimer's disease[40]. We need a firm policy to prevent discrimination or stigmatization against a particular group of people and to develop mechanisms to protect rights of ethnic communities.

Regulatory Perspectives

Uncertainties based on new genetic information, which have not yet been formally addressed. The regulatory agencies need to actively engage in internal discussions, and in an open dialogue with the pharmaceutical industry and to identify the new implications, questions and issues related to drug (and device) approvals. The following questions and issues need further discussion, and are amongst the major concerns of the industry with regard to drug development programs using various elements of pharmacogenomics:[41]

- What are the regulatory implications of genetic profile screening of patients during investigational drug therapy?
- Is it acceptable to the drug approval authorities (e.g. FDA) to stratify patients entering into a clinical trial on a priori based genomic test?
- What are the statistical ramifications when using genomics tests to define patient subsets?
- What are the performance and statistical requirements for the pharmacogenomic diagnostic that would be used for the purpose?
- What use would the agency allow for a post hoc subset analysis based on a pharmacogenomic diagnostic test in a clinical trial that failed to demonstrate efficacy or had an unacceptably high rate of adverse effects?

Successful documentation and implementation of such and other relevant guidelines may help pharmacogenomic based drug discovery programs to gain impetus.

Besides the regulatory aspect, these programs should also be evaluated in light of their potential cost effectiveness before

investments in research, development, and health care resources are made.

Cost-Effectiveness Analysis

The cost-effectiveness of health care technologies is driven by several primary factors. Below, we review these factors in relation to pharmacogenomics.

The cost of a genetic testing strategy includes more than just the cost of the test itself. Induced costs such as additional clinic visits, genetic counseling, and further diagnostics are potentially of greater magnitude and should be evaluated. Tests that have direct implications for patient care will be more efficient than those requiring additional follow-up. In general, interventions with a one-time cost that offer long-term benefits, such as immunizations, are often cost saving or cost-effective[42]. Pharmacogenomics will sometimes fall in this category. Indeed, one of the benefits of genetic testing to predict drug response is that the information can be used throughout the lifetime of the patient. Thus, other potential uses of the genetic information obtained from a test may further offset the cost of the test[38]. This is most likely to occur when the genetic variation affects more than one drug as with the P450 metabolic enzymes, for example.

The effectiveness of pharmacogenomic tests in clinical practice will be determined by several factors in addition to the accuracy of the test. Genetic tests for detection of variant genes are typically quite accurate, with sensitivities and specificities near 99% when direct sequencing or restriction site assays are used. However, the degree of association between genotype and clinical phenotype will be equally as important. For example, if 50% of patients with a certain gene variant experience a severe adverse side effect from a drug, avoiding the use of the drug in all patients with the polymorphism would unnecessarily deprive half of the patients (the "false positives") of medication. The issue of "false-positives" will be important for almost all applications of pharmacogenomics, and the consequence of labeling patients as having a genetic variation despite the fact that not all of them will have clinically relevant effects must be considered[43]. The degree of phenotypic expression of genetic variation is known as gene penetrance. Thus, genes with high penetrance will be better candidates for cost-effective pharmacogenomic strategies. Note that the term "false positives" does not refer to patients who were falsely identified as having a variant gene, but rather to patients with a variant gene who do not express the clinical phenotype[33].

Conclusions

Thus, pharmacogenomics has great potential to improve the effectiveness and safety of pharmaceutical care. However, pharmacogenomic strategies will be cost-effective only for certain combinations of disease, gene, drug, and test characteristics. Further research and innovations are required before pharmacogenomics can become affordable for a larger section of the society. The thrust areas should include:

- More knowledge of sequences and polymorphisms
- Better knowledge of enzyme mechanisms
- More efficient genomic screens
- Improved functional assays
- Validation of bioavailibility models
- Application to clinical trials and epidemiology
- High throughput production scale instrumentation

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