SBV AHEAD

BRINGING PHARMACOGENOMICS AND PERSONALIZED MEDICINE INTO CLINICAL PRACTICE

Adithan C, *Director*
Central Inter-Disciplinary Research Facility and Professor of Pharmacology,

Sri Balaji Vidyapeeth Mahatma Gandhi Medical College and Research Institute Campus
Pillaiyarkuppam, Puducherry - 607403, India
Email: director@cidrf.res.in

Abstract ▶ Pharmacogenomics and personalised medicine are emerging as important tools in individualised drug therapy. Many of the genes which encode drug metabolising enzymes, transporters and receptors are polymorphic. Individuals with polymorphic genes are likely to experience therapeutic failure or drug toxicity. This is clinically important for drugs with low safety margin such as oral anticoagulants, anti-epileptics and anticancer drugs. Prior genetic testing may help to predict responders and non-responders to the above mentioned drugs, besides avoiding drug toxicity. CIDRF is planning to introduce CYP2C9, CYP2C19 and CYP2D6 genetic testing shortly, which shall help to integrate pharmacogenomics with clinical practice.

Key Words: Pharmacogenomics, Personalized medicine, Genetic testing, Drug toxicity.

Introduction

The present scenario in drug therapy is not satisfactory. In spite of advances in the understanding of pathophysiology of diseases and introduction of newer drugs, the optimal therapy for major diseases is still elusive. For example, 30 % of schizophrenic patients do not respond to drug therapy, 27 % of hypertensive patients are poorly controlled and many anticancer drugs fail to bring remission. Further, adverse drug reactions (ADR) remain a problem. A USA study reported an annual mortality of about 1 lakh patients due to ADR[1]. Most of these problems are due to variability in drug responses seen in patients. Hence, there is a need to improve the outcome of pharmacotherapy. One of the ways to achieve this is by —selecting drugs and posology based on individual genetic profile of patients. Thus, pharmacogenomics and personalized medicine is a new, promising and emerging area of medical science.

Pharmacogenomics

Pharmacogenomics is the study of the effect of genetic variability of an individual in drug response. It is known that DNA sequence of all human beings is 99.9% identical and we differ by 0.1%. Since human genome has about 3 billion bases, the 0.1% difference (genetic variations) amounts to around 3 million spelling differences in the human genome. Majority of genetic variations are due to single nucleotide polymorphism[2]. The genetic polymorphism of an individual can influence the susceptibility to disease, and the absorption, distribution, metabolism and excretion of drugs. Besides, the function of receptors and response to drugs also gets modified. The presence of genetic polymorphism may result in poor drug response or drug toxicity.

Personalized Medicine

It has been observed that genetics can account for 20–95% of variability in drug disposition and effects[3]. The

environmental factors, concurrent drug intake, disease process etc. can further contribute to the existing variability. Therefore, it is preferable that personalised medicine be used for a more successful pharmacotherapy. Personalised medicine is concerned with providing medical care tailored to the genomic profile, family medical history and other environmental factors related to the patient.

At present, drug therapy is done by trial and error method and 'one size fits all' concept. Personalized medicine involves conducting genetic testing in order to select the right drug, in the right dosage. Factors such as liver and kidney function, concomitant drug intake etc. that could influence the pharmacokinetics and the pharmacodynamics of the drug are also taken into consideration.

Pharmacogenomics of drug metabolising enzymes

Cytochrome P450 enzymes present in the liver and other parts of the body play a major role in the metabolism of drugs. The important drug metabolising enzymes are shown in Table 1.

Table 1. Important polymorphic genes encoding Phase 1 and 2 drug metabolizing enzymes [4, 5]

Gene	Mutant Alleles	Drug Metabolising Enzymes	Substrates (Drugs)
		Phase 1 enzymes	
CYP2C9	*2, *3, *4, *5, *6	CYP2C9	Warfarin, losartan phenytoin, tolbutamide
CYP2C19	*2, *3, *4, *5, *6, *7, *8	CYP2C19	Proguanil, imipramine, ritonavir, nelfinavir, cyclophosphamide
CYP2D6	*1XN, *2XN, *3,*4,*5, *6 *9,*10,*17	CYP2D6	Clonidine, codeine, promethazine, propranolol, clozapine, fluoxetine, haloperidol, amitriptyline
CYP2E1	*2,*1B	CYP2E1	Acetaminophen, halothane, enflurane, fluoxetine, chlorzoxazone
CYP1A2	*1C, *1F, *1K, *3,*4,*6	CYP1A2	Clozapine, fluvoxamine, haloperidol, imipramine, tacrine, verapamil
		Phase 2 enzymes	
NAT2	*5, *6,*7, *10,*14	NAT2	Isoniazid, hydralazine,
GST	P1, M1 null, T1 null	GST	D-penicillamine
TPMT	*2,*3A,*3C	TPMT	Azathioprine, 6-mercaptopurine
UGT1A1	*28	UGT1A1	Irinotecan
DPD	*2A	DPD	Fluorouracil

Red: Absent; Blue: Reduced; Green: Increased activity

The syntheses of these enzymes are encoded by specific genes. Any genetic variability in these genes will result in either deficiency or increased quantity of enzymes which can predispose an individual to drug toxicity or therapeutic failure.

The various molecular mechanisms that can alter drug metabolism are as follows:

1.Deletion of gene.

This results in complete cessation of production of the concerned enzyme. As a result, the metabolism of the drug by that particular enzyme does not take place (e.g., *CYP2C19*2, *3*)

2.Alteration of function of a single gene.

This can result in three possible scenarios.

a. Production of an unstable enzyme – In this case, the normal drug metabolism is compromised (e.g., *CYP2D6*10*).

b. Normal enzyme – Normal metabolism of drug (e.g., *CYP2C9*1, CYP2D6*1*)

c. Altered substrate specificity – Other metabolites may be formed.

3.Duplication or multiduplicated genes

This results in higher enzyme levels leading to an increased metabolism of drugs (e.g., *CYP2D6*1 X N*)

In the body, drug metabolism takes place in two phases – phase 1 and phase I2. In phase 1, the drugs undergo oxidation, reduction or hydrolysis. In phase 2, drugs undergo glucuronidation, sulfatation or acetylation which makes the drug more water soluble and easily excretable. CYP3A4/A5, CYP2D6, CYP2C9, CYP2C19, CYP2E1 are the important phase I enzymes. Among them CYP3A4/A5 accounts for metabolism of more than 50% of currently used drugs. However, no significant polymorphism has been reported in the genes encoding them. CYP2C9, CYP2C19 and CYP2D6 are the important genes which are polymorphically expressed. Important variant alleles of phase 1 and phase 2 enzymes and their drug substrates are given in Table 1.

The frequency of variation in alleles of drug metabolising enzymes can differ in a population depending on their ethnicity. It was found to be different among Caucasians, Orientals, Africans and Indians. Even among Indians, the frequency of variant alleles was found to be different between South Indians, Western Indians and North Indians. A detailed review on this topic is published elsewhere[6].

Pharmacogenomics of Drug Transporter enzymes

Drug transporters located in the cell membranes are the major determinants of pharmacokinetic profile of drugs. Two major superfamilies, namely, ATP-Binding Cascade (ABC) and Solute Carrier (SLC) were found to have important genetic variation in the coding regions[7]. Among them, P-glycoprotein (ABCB1) and Organic Cation Transporters 1 and 2 (OCT1 and OCT2) are functionally well characterized. In ABCB1 P-glycoprotein, a synonymous polymorphism (3435C>T) was extensively studied and found to be associated with various drug responses. Some of the drug substrates for P-glycoprotein are mentioned in Table 2

Table 2. Important drug substrates of P-glycoprotein

Drug Category	Substrates for P-glycoprotein
Anti-cancer agents	Actinomycin D, vincristine, paclitaxel .
Cardiac drugs	Digoxin, quinidine
HIV protease inhibitors	Ritonavir, indinavir
Immunosuppressants	Cyclosporine A, tacrolimus
Antibiotics	Erythromycin,levofloxacin
Lipid lowering agents	Lovastatin, atorvastatin

Pharmacogenomics of Drug Receptors

Drugs produce their effect by binding to receptors, present either in the membrane or inside the cell. The genes encoding these receptors too show polymorphism, which may modify the receptor activity and drug response[8]. For example – asthmatic patients with Gly[16] polymorphism of beta 2 receptor gene are likely to be about 5 fold less responsive to salbutamol[9]. Beta 1 receptor blockers such as metoprolol may have more profound action in individuals having Gly[49] polymorphism of beta 1 receptors[10].

Clinical applications

A number of papers have been published supporting the role of pharmacogenomics in clinical practice. A few examples of works done by the author at JIPMER, Pondicherry and by other workers elsewhere are given below

Anticonvulsant drug: Influence of *CYP2C9* polymorphism on phenytoin dosage requirement and toxicity were studied in the Indian population. It showed that epileptic patients with homomutant genotype of *CYP2C9* may require about 110 mg/day of phenytoin when compared to its normal dose of 300 mg/day. Phenytoin induced neurological toxicity was found to be 4 to 10 times higher if patients have either of *CYP2C9*2* or **3* variant allele.[11]

Oral anticoagulants: Warfarin related bleeding complications were about 3 to 4 times more in patients who were carriers of at least 1 mutant allele (*2 or *3) of CYP2C9. The optimum dosage of warfarin or acenocoumarol can be predicted by genetic testing of *CYP2C9* and *VKORC1*. The genetic factors contribute about 45 to 55% of oral anticoagulant response in the Tamilian population[12].

Antiplatelet drug: Clopidogrel's action was significantly influenced by CYP2C19, CYP3A5, MDR1 and P2Y12 genetic polymorphism. Poor metabolisers having CYP2C19 polymorphic genes fail to respond satisfactorily to clopidogrel even after doubling the dose[13].

Antidiabetic drugs: The beneficial effect of glibenclamide and metformin were partially determined by CYP2C9 and OCT1 polymorphic genes respectively [14, 15].

Anticancer drug: Tamoxifen is used as an adjuvant therapy in carcinoma of breast. It is a prodrug which is metabolised by CYP2D6 enzyme into an active endoxifen metabolite. It was reported that in patients having carcinoma of breast with CYP2D6 variant alleles and treated with tamoxifen, disease recurrence was increased and survival period was reduced[16].

Antipsychotic drugs: Tardive dyskinesia is a known complication of antipsychotic drugs occurring in 10 to 30 % of cases. Most severe form of tardive dyskinesia was reported in individuals with Ser9Gly DRD3 gene polymorphism and CYP1A2*1F polymorphism[17].

International Status

Worldwide the potential value of pharmacogenomics testing is being realised. New and rapid methods for pharmacogenetic testing are also being developed. A technology called SmartAmp claims to provide the result within 45-60 minutes without the need for DNA extraction and PCR amplification[18]. However, there are just a few controlled clinical trials to establish the cost-effectiveness for recommending routine genetic testing. Precision medicine (personalized medicine) received a big boost in 2015 by President Obama's initiative to establish a voluntary national research cohort of at least 1 million USA population. Under this initiative, the participants are required to contribute their core data including genetic profile, metabolites profile, microbiome profile in and on the body, life style data etc. which shall ultimately promote precision medicine. FDA (USA) based on the available pharmacogenomics data has recommended label changes for abacavir, azathioprine, carbamazepine, citalopram, clopidogrel, warfarin and a few other drugs. An article published in Cleveland Clinical Journal of Medicine recommends pharmacogenetic testing for abacavir, carbamazepine, allopurinol (HLA testing), clopidogrel (CYP2C19), tamoxifen (CYP2D6), azathioprine, 6-mercaptoprine (TPMT) and few more drugs[19, 20]. Now, many biotechnology companies have started commercial genetic testing for important drugs. But the issue of reimbursement by insurance companies is still unresolved.

National Status

Most of the genetic studies done in India have focussed on disease susceptibility to cancer, diabetes and other conditions. Relatively fewer studies have been carried out with anti-epileptic, cardiovascular, and anti-cancer drugs which were briefly discussed earlier. In private sector, there are more than a dozen companies offering genetic testing. Among them, Acton Biotech (India) Pvt. Ltd, Ayugen Biosciences, Datar genetics are more focussed on pharmacogenetic tests.

Government funding agencies such as Department of Biotechnology and Indian Council of Medical Research

have identified pharmacogenomics as one of their priority areas and have established Task Forces to promote pharmacogenomics research. The Indian government is planning to link Pharmacovigilance Program of India (PvPI) with pharmacogenomics research. With this, PvPI will include pharmacogenomics as a part of its scientific component to address concerns of vulnerable populations susceptible to adverse drug events induced by a certain drug triggered due to genetic factors.

Proposed Role of CIDRF

CIDRF is planning to start collaborative research work in cancer and cardiovascular diseases. It also plans to start hospital services for genotyping important genes such as CYP2C9, CYP2C19, CYP2D6, P2Y12 and VKROC1. The results of the above genetic testing may be useful for tailoring the dosages of anti-epileptics (phenytoin), antiplatelet drugs (clopidogrel), oral anticoagulant (warfarin and acenocoumarol), antimalarial drugs (proguanil), anti-depressant drugs (fluoxetine, imipramine), antipsychotic drugs (clozapine, haloperidol), anti-hypertensives (beta blockers, losartan), antidiabetic drugs (glibenclamide and metformin), antiretroviral drugs (ritonavir, nelfinavir), NSAIDs and anticancer drugs (cyclophosphamide, tamoxifen). The cost of these genetic tests may vary from Rs. 2000 - 15,000 depending upon the number of genes to be tested. Similar to the blood group testing, these results are valid throughout life. We believe that integration of selected pharmacogenetic testing into clinical practice will benefit patients receiving drugs with low safety margin.

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