Chapter 1

INTRODUCTION
Over the years there have been major improvements in the length and quality of life. While the infectious diseases are on a decline, the non-communicable and chronic diseases like coronary heart disease, cancers, diabetes, rheumatoid arthritis (RA), hypertension and metabolic syndrome are increasing. There have been number of drugs developed for these diseases. Variable response to these drugs by patients is of a major concern in the disease management. Inter-individual variability in drug response can be attributed to genetic polymorphism in genes encoding different drug-metabolizing enzymes (DMEs), drug transporters and enzymes involved in DNA biosynthesis and repair. Mutation in a gene coding for a drug-metabolizing enzyme can cause enzyme variants with high, low or no activity. As a result of inter-individual variation patients develop adverse drug reactions; patients do not respond to drug at the given dose or respond positively to the drug administered in same dose. Unfortunately, prospective identification of those patients who are most likely to benefit from a specific therapy is not routinely possible for many diseases and medications. This is particularly important in the current health care environment, where cost containment and evidence-based initiatives are having a significant influence on patient care. Understanding the molecular basis of drug action and genetic determinants of drug response should enlighten our use of many medications, toward the ultimate goal of giving the right drug at the right dose to the right patient at the right time. It should also lead to mechanism-based approaches to the discovery and development of new medications. There exists a clear evidence of correlation between genetic polymorphisms and efficacy and toxicity of drugs. Genetic polymorphisms explain why a small proportion of the population may be at higher risk of drug inefficacy or toxicity; the study of such polymorphisms has given rise to the field of pharmacogenetics and personalized medicine.

1.1 Pharmacogenetics
Genetics forms the basis of human life. All the individuals have physical and physiological self, determined by the interactions of genes and environment. Pharmacogenetics is the connotation used to study how heritability affects the response to drugs. The term ‘Pharmacogenomics’ represents systematic identification of all human genes, their products, inter-individual and intra-individual variation in expression and function over time. It focuses on candidate genes and may include transcriptome and proteome information that affect drug metabolism, pharmacokinetics, and pharmacodynamics. It helps in predicting patient’s response to the available drugs and to
design new drugs. The two terms, pharmacogenetics and pharmacogenomics, are used interchangeably though mostly pharmacogenetics is focused on pharmacological consequences of a single gene mutation, pharmacogenomics tries to simultaneously consider numerous genes and their mutual interaction. Research in pharmacogenetics has two main parts: Identification of specific gene products and consequently genes associated with various drugs and identification of allelic variants of genes that affect response to drug.

Pharmacogenetics had its beginning in the 1950s when researchers realized that adverse reactions of drug could be a consequence of genetic variations in enzyme activity. Impairment in a phase I reaction: hydrolysis of the muscle relaxant succinylcholine by butyrylcholinesterase (pseudocholinesterase) was inherited and served as an early lead for the development of pharmacogenetics. Approximately 1 in 3500 white subjects is homozygous for a gene encoding an atypical form of butyrylcholinesterase and is relatively unable to hydrolyze succinylcholine, thus prolonging the drug-induced muscle paralysis and consequent apnea. It was observed that a common genetic variation in a phase II pathway of drug metabolism —N-acetylation — could result in striking differences in the half-life and plasma concentrations of drugs metabolized by N-acetyltransferase. Such drugs included the antituberculosis agent isoniazid, the antihypertensive agent hydralazine, and the antiarrhythmic drug procainamide, and this variation had clinical consequences in all cases. The bimodal distribution of plasma isoniazid concentrations in subjects with genetically determined fast or slow rates of acetylation in one of those early studies strikingly illustrates the consequences of inherited variations in this pathway for drug metabolism. These early examples of the potential influence of inheritance on the effect of a drug set the stage for subsequent studies of genetic variation in other pathways of drug biotransformation.

1.2 Genetic polymorphism and drug response:

A polymorphism is a variation in the DNA sequence that is present at an allele frequency of 1% or greater in a population. Two major types of sequence variation have been associated with variation in human phenotype: single nucleotide polymorphism (SNPs) and insertions/deletions (indels). In comparison to base pair substitutions, indels are much less frequent in the genome and are of particularly low frequency in coding regions of genes. SNPs are present in the human genome at approximately 1 SNP every few hundred to a thousand base pairs, depending on the gene region. By the completion of Human genome project in February 2001 study in this field is accelerated dramatically. It was found that over 60,000 SNPs present in the coding region out of 1.4 million SNPs
identified in the human genome\textsuperscript{18}. These SNPs leads to miss coding of the various proteins that plays crucial role in the biological processes. This point mutation if present in the gene coding for various drug-metabolizing enzymes, drug target and drug transporters, leading to many non-therapeutic and unpredictable drug response. These polymorphisms precipitate into four phenotypic subpopulations of drug metabolizers:

\begin{itemize}
  \item Poor metabolizers (PM): retain drug in the body for long time than normal and hence the plasma concentration of the drug is high for longer period.
  \item Intermediate metabolizers (IM): retain drug in the body for optimal period.
  \item Extensive metabolizers (EM): retain drug in the body for less time and the plasma concentration of the drug is high but for shorter period.
  \item Ultra rapid metabolizers (UM): metabolize drug much faster and extensively than all the other subpopulations
\end{itemize}

1.3 Applications of Pharmacogenetics:

Various studies have demonstrated applications of pharmacogenetics in the treatment of various forms of cancers, hypertension, respiratory diseases like asthma, central nervous system disorders.

1.3.1 Cancer:

Drugs such as azathioprine, mercaptopurines and thioguanine have been used extensively to treat childhood acute lymphoblastic leukemia and inflammatory bowel disease. Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that is involved in the metabolism of thiopurines. It has been reported that the TPMT genotype has a substantial impact on the mercaptopurine treatment response\textsuperscript{19}. Studies have shown that patients with homozygous mutant TPMT alleles exhibit very low enzyme activity and develop a severe hematopoietic toxicity after treatment with standard doses of thiopurines\textsuperscript{20-22}. Similarly, the response rate of 5-fluorouracil (5-FU) based treatment of advanced colorectal cancer is significantly linked to C677T polymorphism in the methylenetetrahydrofolate reductase gene\textsuperscript{23}. Irinotecan, a camptothecin derivative indicated in the treatment of colorectal cancer, is a prodrug transformed by carboxylesterases forming SN-38, the active compound inhibiting topoisomerase I. Patients with a low rate of glucuronidation accumulate SN-38 and develop toxic reactions manifested by severe diarrhea\textsuperscript{24}. 

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1.3.2 Hypertension:
Hypersensitivity drug reactions may be potentially life threatening and result in a socioeconomic burden. The variation in two genes encoding angiotensin-converting enzyme and endothelial nitric oxide synthase influence the effects of standard therapies.
In addition, polymorphism in the sodium channel gamma subunit promoter region is significantly associated with blood pressure response to hydrochlorothiazide. Similarly, SNPs in angiotensinogen (T1198C), apolipoprotein B (G10108A) and adrenoreceptor alpha 2A (A1817G) significantly predict the change in left ventricular mass during antihypertensive treatment. Although common variants may influence the blood pressure response to a given class of antihypertensive medication, studies of polymorphisms have generally provided conflicting results. For instance, polymorphism in the alpha 2B adrenergic receptor gene does not show any association with azepexole response.
However, patients with Glycine (Gly) 389 variant and Ser 49 homozygous of the beta-adrenergic receptor require increases in heart failure medication.

1.3.3 Respiratory drugs:
Asthma is a multifactorial disorder occurring in genetically predisposed persons exposed to environmental factors. Different mechanisms can trigger bronchoconstriction. Several treatments known to modify the 5-lipoxygenase pathway are only beneficial for certain patients whose leucotrienes contribute to their disease susceptibility. Asthma patients exhibit an extensive inter-individual variation in the response to beta-agonists acting at beta 2 adrenergic receptors. This could be due to one nonsynonymous polymorphism (I772M) of adenylyl cyclase type 9 (AC 9) genes. This variation results in decreased catalytic activity (M772) and, therefore, alters albuterol responsiveness in the presence of a corticosteroid. Additionally, in an Indian population, response to salbutamol treatment of asthmatic patients depends on polymorphisms of the beta 2 adrenergic receptor.

1.3.4 Antipsychotic drugs and their receptors and transporters:
Considerable drug variability has been observed in the case of mood disorder. Approximately 30 - 40% patients do not completely respond to pharmacological treatment. However, serotonin transporter promoter length polymorphism has been implicated in the pathogenesis of mood disorders as well as in the therapeutic response to serotonergic drugs. In patients with schizophrenia, Taq I polymorphism in the dopamine D2 receptor is associated with greater improvement of symptoms after treatment. Similarly, Gly 9 allele (Ser 9 Gly) of the dopamine D3 receptor and His 452 Tyr polymorphism in the 5-hydroxytryptamine 2A receptor (5-HT2A) are associated with response to clozapine. The side effect (weight gain) induced by antipsychotics seems to be
associated with the –759C allele of the 5-HT2C receptor. Additionally, Gly 9-variant of dopamine D3, the 102C-variant of the 5-HT2A and the Ser 23-variant of the 5-HT2C receptors (in females) seem to increase the susceptibility to tardive dyskinesia.

The disorder epilepsy is a difficult disease to treat because different patients require different ranges of doses and some patients may even experience side effects such as increase in seizures, depression and double vision. In order to control epilepsy, drugs such as phenytoin and carbamazepine have been extensively prescribed throughout the world. At present, evaluation of the allelic variation between individuals relies on the prior identification of candidate genes and their therapeutic effects of antiepileptic drugs.

Recently, variants in the CYP2C9 and SCN1A (encodes a brain protein) genes are found significantly more often in patients treated with the highest doses of both phenytoin and carbamazepine.

1.4 Advantages of Pharmacogenetics:
There are two major advantages of using science of pharmacogenetics for the patient treatment and for drug development in following ways:

1. For patient treatment
   ➔ Optimal drug prescription at the outset of therapy (without going through series of experiment to find the best treatment).
   ➔ Early selection of optimal therapy (reducing medical costs and increasing patients satisfaction and therapy compliance)
   ➔ Identification of genetic causes of patient variability preventing adverse drug events and increasing drug efficacy.

2. For drug development
   ➔ Choose correct patient and control population which will reduce trial size
   ➔ Shorten trial length
   ➔ Reach tighter end point
   ➔ Decrease cost of clinical trials
   ➔ Choose better drug candidate
   ➔ Improve and revive old drugs
   ➔ Enable extensive drug target interaction search

Thus it will help in giving right dose of right drug, for the right indication for the right patient at right time.
1.5 Use of Pharmacogenetics in Clinical Practice:

Despite considerable research activity, pharmacogenetics is rarely utilized in clinical practice\textsuperscript{40, 41}. In order to implicate a polymorphism in clinical care three major types of evidence are necessary to build up: screens of tissues from multiple humans linking the polymorphism to a trait; complementary preclinical functional studies indicating that the polymorphism is plausibly linked with the phenotype; and multiple supportive clinical phenotype/genotype studies. Because of the high probability of type I error in genotype/phenotype association studies, replication of clinical findings will generally be necessary. Although the impact of the polymorphism in TPMT on mercaptopurine dosing in childhood leukemia is a good example of a polymorphism for which all three types of evidence are available, proactive individualized dosing of thiopurines based on genotype has not been widely incorporated into clinical practice\textsuperscript{42}.

Most drug dosing takes place using a population "average" dose of drug. Adjusting dosages for variables such as renal or liver dysfunction is often accepted in drug dosing, even in cases in which the clinical outcome of such adjustments has not been studied. Even though there are many examples of significant effects of polymorphisms on drug disposition, there is much more hesitation from clinicians to adjust doses based on genetic testing than on indirect clinical measures of renal and liver function. Whether this hesitation reflects resistance to abandon the "trial-and-error" approach that has defined most drug dosing, distrust of the genetic tests (which are constantly being refined), or unfamiliarity with the principles of genetics is not clear.

There is enormous potential utility of pharmacogenetics for optimizing drug therapy. Genetic tests have the advantage that they need only be conducted once during an individual's lifetime. Once adequate genotype-phenotype studies have been conducted, molecular diagnostic tests will be developed that detect >95% of the important genetic variants for the majority of polymorphisms. With continued incorporation of pharmacogenetics into clinical trials, the important genes and polymorphisms will be identified, and data will demonstrate whether dosage individualization can improve outcomes and decrease short- and long-term adverse effects. Significant covariates will be identified to allow refinement of dosing in the context of drug interactions and disease influences. Although the challenges are substantial, accounting for the genetic basis of variability in response to medications is likely to become a fundamental component of diagnosing any illness and guiding the choice and dosage of medications\textsuperscript{43}.

The developments in the field of pharmacogenomics and the concept of "Personalized Medicine" are new to the field of modern medical science. However, there is a long-
standing tradition regarding this concept of “personalized medicine” in the Indian Medical System of Ayurveda. According to the principles of Ayurvedic medicine, every person has a unique trait, which is called as “Prakriti”. Prakriti is defined by specific and permanent composition of doshas at conception. It is one of the important factors for management of health and diseases. Each Prakriti has certain physical - psychosomatic characteristics, defined proneness to diseases and specific response to treatment. These concepts can be correlated with principles of immunogenetics and pharmacogenetics.

This Ph.D. work was planned in this background, to study the genetic polymorphism of DME in Maharashtrian population. Most of the current literature is available on CYP2C19 gene polymorphisms in Caucasians, African and Oriental population while far less is known about other ethnic groups such as Indians. CYP2C19 gene polymorphism is reported in North Indian and South Indian populations, however, not much is known about Maharashtrian population. In the same cohort, this research work explored genetic basis for concept of Prakriti in Ayurveda. By considering Rheumatoid Arthritis (RA) as an example of a complex, chronic disease, this research work studied the pharmacogenetics of Methotrexate (MTX) response (efficacy, toxicity) in Indian patients suffering from RA.

1.6 Aim

To study genetic polymorphisms in Indian population and effect of these polymorphisms on drug response (efficacy and toxicity) in diseased population.

1.7 Objectives

1.7.1 To study Phase I DME (CYP2C19, CYP2C9) gene polymorphisms in Maharashtrian population.

- To compare the allele and genotype frequency of Maharashtrian population with other populations.

1.7.2 To study inter-individual variability in drug metabolism and its association with metabolically polymorphic Prakriti if any.

1.7.3 To study pharmacogenetics of MTX response (efficacy, toxicity) in Indian patients suffering from RA.

- SNPs in genes coding for MTX metabolism in Indian RA patients
- The association of gene polymorphism in MTX metabolic pathway with efficacy and toxicity related to MTX therapy in Indian RA patients.
- The effect of gene polymorphism on plasma levels of MTX and 7-OH MTX metabolite at different time points (pharmacokinetics) in RA patients.