Chapter 2

REVIEW OF LITERATURE
"If it were not for the great variability among individuals medicine might as well be a science and not an art."

(Sir William Osler, 1892)

The variability of a drug response can lead to therapeutic failure or adverse effects of drugs in individuals or subpopulations of patients. Physicians have to optimize a dosage regimen for an individual patient by a trial-and-error method. This blind approach can lead to adverse drug reactions in some patients and in turn affects drug efficacy. Pharmacogenomics can help in assessing drug response by determining an individual’s risk of developing an adverse drug reaction, predicting pharmacological variability and thereby preventing adverse drug reactions. A combination of genetic, environmental and disease state factors affect drug metabolism, with relative contribution of each depending on the specific drug. There are number of factors associated that determine drug metabolism.

2.1 Factors Affecting Drug Metabolism:

The observed prominent variability in individual response to pharmacotherapy depends on factors like age, sex, weight, liver and renal function, co-medication, heterogeneity in the disease and lifestyle variables like nutritional state, alcohol consumption and smoking. Furthermore, inherited variants in DMEs, transporters, receptors and molecules of signal transduction cascades may have a major impact on drug response.

2.1.1 Age and sex factors:

Age:

Functional cytochrome P450 isoforms and to lesser degree phase II DMEs develop early in the fetal development, but the levels even at births are lower than those found postnatally. Both phase I and phase II enzymes begins to mature gradually. Thus newborn and infants metabolize drugs efficiently but at slower rate than adults. Few generalizations are possible regarding clinical importance of age related changes in drug metabolism in an individual patient. This is particularly true for elderly patients who because of multiple diseases may be taking a large number of drugs, many of which produce drug-drug interactions. In addition, increased sensitivity of target organs and impairment of physiological control mechanisms further complicate the use of drug in elderly population.
Sex:
Many examples indicate differences in drug responsiveness/metabolizing activity in men and women for certain drugs like CYP3A. But such differences are relatively minor and unimportant than other factors\textsuperscript{16}.

2.1.2 Environmental factors:
The activity of most DMEs may be modulated by exposure to certain exogenous compounds like concomitantly administered drug (drug-drug interaction), dietary micronutrients, etc. Two forms of modulation are\textsuperscript{43} –

\rightarrow Induction or up regulation of drug metabolism – This occurs due to enhanced gene transcription following prolonged exposure to an inducing agent. This results in increase in rate of metabolism, enhanced oral first pass metabolism and decrease in bioavailability resulting in decreased plasma concentration of drug. By contrast, for drugs that are metabolized to an active or reactive metabolite, induction may be associated with increased drug effects or toxicity respectively, e.g. Cigarette smoke produces marked induction of CYP1A subfamily of enzymes.

\rightarrow Inhibition or down regulation of drug metabolism – A consequence of inhibiting DMEs is an increase in the plasma concentration of parent drug and a reduction in that of metabolite, exaggerated and prolonged pharmacological effects, and an increased likelihood of drug induced toxicity. This occurs due to competition in two or more substrates for same active site of the same enzyme determined by relative concentration of substrates and their affinities for enzyme. This results in increase in plasma concentration of drug, reduction in metabolism that may further result in drug induced toxicity, e.g. CYP450 isoform required in metabolism of many drugs.

2.1.3 Disease factors:
Liver is major organ involved in drug metabolism. Diseases like hepatitis, alcoholic liver disease, biliary cirrhosis and hepatocarcinomas hamper drug-metabolizing ability of liver. Severity of liver damage is directly proportional to decrease in rate of drug metabolism. Phase I enzymes that is CYP-450 are affected more than phase II enzymes. Other diseases like severe cardiac failure and shock result in decreased perfusion of liver and impaired metabolism.

2.1.4 Ethnicity:
In order to use genomic knowledge to develop drug and to improve health, we need to know the effect of ethnic differences in different populations. The term ethnicity is a
multidimensional classification that encompasses shared origins, social background, culture, and environment. Ethnicity is an important demographic variable contributing to interindividual variability in drug metabolism and response. It is generally recognized that the effects of ethnicity on drug metabolism and response are determined by both genetic and environmental factors to a varying extent, depending on the ethnic groups and probe drugs studied. A significant inter ethnic differences in polymorphisms of gene encoding, DMEs, transporter and disease associated protein exists. Genetic differences are greater within socially defined racial groups. Additionally, it has been found that genetic diversity decreases in non-coding regions whereas diversity of coding non-synonymous SNPs is lower in regions containing a known protein sequence motif in individuals of European origin. Drug treatment may be personalized for greater effect if there is a better understanding of the molecular basis underlying ethnic differences in drug metabolism, transport, and response will contribute to improved individualization of drug therapy. For example Warfarin therapy shows a wide variation among patients of different ethnicities. This variation could be due to polymorphism in gene encoding vitamin K epoxide reductase complex 1. Accordingly Chinese patients require lower dosage of heparin and Warfarin than White patients. This is due to there are biological differences between the two racial groups. Thus the understanding of the genetic variation in different ethnicity is useful and pharmacogenetics is a major tool for accessing genetic variability.

2.1.5 Genetic variation:
Drug response is considered to be a gene-by-environment phenotype. That is, an individual's response to a drug depends on the complex interplay between environmental factors and genetic factors. Variation in drug response therefore may be explained by variation in environmental and genetic factors, alone or in combination. A major difference between genetic and environmental variation is that an inherited mutation or trait is present throughout life and has to be tested for only once in a lifetime, whereas environmental effects are continually changing. Various studies have been carried out to find out what proportion of drug-response variability is likely to be genetically determined.

Advances in molecular biology have identified allelic variants of DMEs with different catalytic activities from that of wild type. The differences involved variety of molecular mechanisms leading to complete lack of catalytic activity, reduction of catalytic activity or enhanced activity in case of gene duplication. These molecular mechanisms result in subpopulations with varied drug metabolizing ability that is genetic polymorphism. A
number of genetic polymorphisms are present in several DME genes that lead to altered drug metabolizing abilities.

2.2 Genetic Variability Influencing Drug Response:
The basic principle of pharmacology is that the given drug must influence one or more constituents of target cells in order to produce pharmacological response. The drug thus needs to reach the proximity of the target cells. Once the drug is administered to the patient, it is first absorbed by the body, and then distributed to the site of its action and then it interacts with the target cell. Following regulatory factors are commonly involved in drug response

2.2.1 Drug Metabolizing Enzymes:
There are over thirty families of DMEs in human beings and all these have genetic variants. Variants may result into functional changes in encoded proteins and consequently affect the metabolism of drug in human body. Drug elimination is an irreversible loss of drug from the body and occurs by two processes: metabolism and excretion. Most drugs leave the body in the urine, either unchanged or as polar metabolites. However, the kidney does not eliminate lipophilic substances efficiently. Metabolism usually converts drugs to metabolites that are more water soluble and thus more easily excreted. It can also convert prodrugs into therapeutically active compounds, and it may even result in the formation of toxic metabolites.

Drug metabolism occurs predominantly in the liver, mainly by enzyme cytochrome P450 system. Pharmacologists classify pathways of drug metabolism as either phase I reactions (i.e., oxidation, reduction, and hydrolysis) or phase II, conjugation reactions (e.g., acetylation, glucuronidation, sulfation, and methylation). Both types of reaction most often convert relatively lipid-soluble drugs into relatively more water-soluble metabolites (Fig. 1a and 1b).
Figure 1a: The Effect of Drug Metabolism on Excretion

Lipophilic (or fat soluble) drugs are metabolized to form relatively more hydrophilic (or water soluble) metabolites than the parent drug, and these metabolites are thus more easily excreted. (Adopted from Weinshilboum R, 2003)

Figure 1b: Drug Metabolism: Phase I and phase II reactions

<table>
<thead>
<tr>
<th>Drug Metabolism</th>
<th>Phase I (CYP gene family enzymes)</th>
<th>Phase II Examples: NAT2, UGT GST, TPMT</th>
</tr>
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<tbody>
<tr>
<td>Drug</td>
<td>Derivative</td>
<td>Conjugate</td>
</tr>
<tr>
<td>Oxidation</td>
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<td>Hydroxylation</td>
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<td>Dealkylation</td>
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<tr>
<td>Deamination</td>
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<tr>
<td>Hydrolysis</td>
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</table>
Phase I reactions consist of oxidation, reduction or hydrolysis and the products are often more chemically reactive. Examples of phase I enzymes are CYP2C9, CYP2C19, CYP2D6. Phase II reactions involve conjugation, which normally results in inactive compounds, which are excreted. Examples of phase II enzymes are TPMT, GST, UGT, and NAT.

![Figure 2.](image)

Figure 2. Essentially all of the major human enzymes classified as phase I reactions (left) and phase II reactions (right). The percentage of phase I and phase II metabolism of drugs that each enzyme contributes is estimated by the relative size of each section of the corresponding chart. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP, cytochrome P450; DPD, dihydropyrimidine dehydrogenase; NQO1, NADPH:quinone oxidoreductase or DT diaphorase; COMT, catechol O-methyltransferase; GST, glutathione S'-transferase; HMT, histamine methyltransferase; NAT, N-acetyltransferase; STs, sulfotransferases; TPMT, thiopurine methyltransferase; UGTs, uridine 59-triphosphate glucuronosyltransferases. (Adapted from Evans WE 1999)

### 2.2.2 Drug transporters:

Membrane transport proteins are important and involved in absorption of drugs into intestinal tract, brain and several other tissues. These proteins are also involved in distribution and excretion of drugs. Amongst various ATP-binding proteins, ATP binding cassette family is one of the most studied.

P-glycoprotein member of this family is encoded by ABCB1 (MDR1) gene. This protein acts as an efflux pump which exports various substrates, like bilirubin, anticancer agents, immunosuppressive agents, human immuno-deficiency virus (HIV) type I protease inhibitor etc outside the cell. Association studies between ABCB1 gene variants and treatment outcome in HIV infected patients receiving combined antiretroviral therapy have been carried out. ABCB1 3435C → T SNP has shown association with significant differences in plasma concentration of nelfinavir and efavirenz. Patients with TT genotype
showed greater and more rapid recovery of CD4 cell count than patients with CC or CT genotype. SNP in exon 26 (3435C → T) shows considerable ethnic differences in the frequencies of allelic variants. TT genotype accounts for 0% - 6% of black Africans and African Americans, 20% - 47% of Asians and 24% - 36% of white population.

### 2.2.3 Drug receptors:

Drug interacts with membrane receptors to exert pharmacological effect. Genetic variations in such drug targets may alter a drug response. Gene polymorphism in various targets such as β2 adrenoreceptor and response to β2 agonist, angiotensin converting enzyme and renoprotective effects of ACE inhibitor, apolipoprotein E and response to HMG co-reductase inhibitors and various others have been studied.

β2 adrenoreceptor plays an important role in regulating cardiac, vascular, pulmonary and metabolic functions. β2 adrenoreceptor coded by ADRB2 gene is extensively investigated for genetic polymorphisms and their clinical importance. SNP resulting in Argenin (Arg) to Glycine (Gly) amino acid change at codon 16 and Glutamine (Gln) to Glutamic acid (Glu) change at codon 27 are comparatively common. Patients homozygous for Arg16 showed nearly complete desensitization from continuous infusion of isoproterenol with decreasing venodilatation. Patients homozygous for Gly16 had no significant change in venodilatation, regardless of their codon 27 sequence. Patients homozygous for Glu27 showed maximal venodilatation in response to isoproterenol than homozygous Gln27 genotype, regardless of their codon 16 sequence.

### 2.2.4 Drug targets

Gene products that are direct targets for drugs have an important role in pharmacogenetics. Whereas highly penetrant variants with profound functional consequences in some genes may cause disease phenotypes that confer negative selective pressure, more subtle variations in the same genes can be maintained in the population without causing disease, but nonetheless causing variation in drug response. For example, complete inactivation via rare point mutations in methylenetetrahydrofolate reductase (MTHFR) causes severe mental retardation, cardiovascular disease, and a shortened lifespan. MTHFR reduces 5,10-CH$_2$ to 5-CH$_3$-tetrahydrofolate, and thereby interacts with folate-dependent one-carbon synthesis reactions, including homocysteine/methionine metabolism and pyrimidine/purine synthesis. This pathway is the target of several antifolate drugs. $MTHFR$ 677C→T SNP causes an amino acid substitution that is
maintained in the population at a high frequency (variant allele). This variant is associated with modestly lower \textit{MTHFR} activity (about 30\% less than the 677C allele) and modest but significantly elevated plasma homocysteine concentrations (about 25\% higher)\textsuperscript{50}. This polymorphism does not alter drug pharmacokinetics, but does appear to modulate pharmacodynamics by predisposing to gastrointestinal toxicity to the antifolate drug MTX in stem cell transplant recipients.

MTX is a substrate for transporters and anabolizing enzymes that affect its intracellular pharmacokinetics and that are subject to common polymorphisms. Several of the direct targets (dihydrofolate reductase, purine transformylases, and thymidylate synthase [\textit{TS}]) are also subject to common polymorphisms. A polymorphic indels in \textit{TS} (two vs. three repeats of a 28-base pair repeat in the enhancer) affects the amount of enzyme expression in both normal and tumor cells. The polymorphism is quite common, with alleles equally split between the lower-expression two-repeat and the higher-expression three-repeat alleles. The \textit{TS} polymorphism can affect both toxicity and efficacy of anticancer agents (e.g., fluorouracil and MTX) that target \textit{TS}\textsuperscript{50}.

Thus, the genetic contribution to variability in the pharmacokinetics and pharmacodynamics of MTX cannot be understood without assessing genotypes at a number of different loci.

\subsection*{2.2.5 Polymorphism-modifying diseases and drug responses}

Some genes may be involved in an underlying disease being treated, but do not directly interact with the drug. For example \textit{MTHFR} polymorphism is linked to homocysteinemia, which in turn affects thrombosis risk\textsuperscript{61}. The risk of a drug-induced thrombosis is dependent not only on the use of prothrombotic drugs, but on environmental and genetic predisposition to thrombosis, which may be affected by germline polymorphisms in \textit{MTHFR}, factor V, and prothrombin\textsuperscript{62}. These polymorphisms do not directly act on the pharmacokinetics or pharmacodynamics of prothrombotic drugs, such as glucocorticoids, estrogens, and asparaginase, but may modify the risk of the phenotypic event (thrombosis) in the presence of the drug.

Cancer pharmacogenetics has an uncommon aspect in that tumors exhibit somatically-acquired mutations in addition to the underlying germline variation of the host. Thus, the efficacy of some anticancer drugs depends on the genetics of both the host and the tumor. For example, the \textit{TS} enhancer repeat polymorphism affects not only host toxicity, but also tumor susceptibility to \textit{TS} inhibitors\textsuperscript{63}. 

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2.2.6 Polymorphisms affecting pharmacokinetics:
The major cause of therapeutic and adverse drug response is the germline variability in enzymes and transporters genes that encode determinants of the pharmacokinetics of a drug in turn affecting plasma concentrations of the drug. Multiple enzymes and transporters may be involved in the pharmacokinetics of a single drug. Several polymorphisms in DMEs were discovered as monogenic phenotypic trait variations, and thus may be referenced using their phenotypic designations (e.g., slow vs. fast acetylation, extensive vs. poor metabolizers of debrisoquine or sparteine). E.g. CYP2C19 codes for a cytochrome P450, that displays penetrant pharmacogenetic variability, with just a few SNPs accounting for the majority of the deficient, poor metabolizer phenotype. Several proton pump inhibitors, including omeprazole and lansoprazole, are inactivated by CYP2C19. Thus, the deficient patients have higher exposure to active parent drug, a greater pharmacodynamic effect (higher gastric pH), and a higher probability of ulcer cure than heterozygotes or homozygous wild-type individuals.

Thus by understanding the genetic variation in the genes coding above regulatory proteins and its effect on kinetics and dynamics of numerous drugs; it is possible to determine individual’s risk for drug inefficacy or toxicity.

2.3 Pharmacogenetics of drug metabolism:
2.3.1 Phase I DMEs-Cytochrome P450 enzymes:
CYP450 super family of heme-proteins metabolizes number of chemically diverse, endogenous and exogenous compounds including drugs, environmental chemicals and other xenobiotics. They function as terminal oxidase in a multicomponent Electron Transport Chain that introduces a single atom of molecular oxygen into the substrate with the other atom being incorporated into water. CYP450 catalyzes reactions involving N-dealkylation, O-dealkylation, aromatic hydroxylation, N-oxidation, S-oxidation, deamination, desulfuration and dehalogenation.

Approximately, 1000 CYP450 enzymes are currently known. Out of these around 50 are functionally active in human beings. These are categorized into 17 families and many sub-families based on the amino acid sequence similarities of the predicted proteins. The mammalian CYP families can be functionally subdivided into two major classes: those that involve the biosynthesis of steroids, fatty acids, and bile acids and those that primarily metabolize xenobiotics. In humans, 12 CYPs (CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) are known to be important for metabolism of xenobiotics. The most active CYPs for drug metabolism are those in the CYP2C, CYP2D, and CYP3A
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subfamilies. CYP3A4 is the most abundantly expressed and involved in the metabolism of about 50% of clinically used drugs. The CYP1A, CYP1B, CYP2A, CYP2B, and CYP2E subfamilies are not significantly involved in the metabolism of therapeutic drugs, but they do catalyze the metabolic activation of many protoxins and procarcinogens to their ultimate reactive metabolites. Each individual CYP isoform has a characteristic substrate specificity based on structural features of the substrate. Two or more CYP isoforms and other drug metabolizing enzymes are often involved in drug’s overall metabolism, leading to formation of many primary and secondary metabolites.

There are large differences in levels of expression of each CYP between individuals as assessed both by clinical pharmacologic studies and by analysis of expression in human liver samples. This large interindividual variability in CYP expression is due to the presence of genetic polymorphisms and differences in gene regulation. The major genetic polymorphisms affecting drug metabolizing enzymes activity of potential clinical relevance are those related to drug oxidation by CYP2C19, 2C9 and 2D6. Few examples are given below:

- **CYP2D6:**
  This is the most extensively studied and characterized example of pharmacogenetic variation in drug metabolizing enzyme. The drugs that are metabolized by this enzyme are tricyclic antidepressants, debrisoquine, codeine, clozapine and metoprolol. The allelic variants of CYP2D6 have different drug metabolism phenotypes – poor metabolizers (PM), extensive metabolizers (EM), ultra extensive metabolizers (UEM). The variants range from SNPs that alter the amino acid sequence of the encoded protein to SNPs that altered RNA splicing or deletion of the CYP2D6. The frequently studied alleles are CYP2D6 *3, *4, *5 and *10 responsible for reduced or null activity of the enzyme. In the Caucasians frequencies of allele CYP2D6 *4 is 19.5% and in Japanese it is 0% while the frequency of allele *10 in Caucasians is only 2% and that in Japanese it is 38.6%. The frequency of *5 allele in Caucasians and Japanese is 4.1% and 6.2% respectively. In Indian mainland these studies were carried out in Tamil Nadu, Kerala and North India. In the Tamilian population the frequency of *3 is 0% that of * 4 is 6.6%, *10 is 20.3% and *5 is 0.9%. 
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➔ CYP3A4:
CYP-450 3A subfamily is predominant isoform in human liver and it contains three known members 3A4, 3A5 and 3A7. CYP3A4 is abundantly present in human liver and small intestine. The drugs metabolized include nifedipine, cyclosporine, erythromycin, midazolam, alprazolam and triazolam. High interindividual variation is observed in CYP3A4 gene. There are four identified allelic variants. The first frequently observed is CYP3A4*1B. Its frequency was low in white and Hispanic subjects (3.6% - 11%), absent in Chinese and Japanese subjects and much higher in black subjects (53% - 69%). Second allelic variant is CYP3A4*2. This allele has low frequency in white subjects (2.7%) and was not observed in black or Chinese groups.

➔ CYP2C19:
This enzyme plays a major role in the metabolism of some clinically important drugs such as omeprazole, diazepam and proguanil. Gene polymorphism of CYP2C19 occurs with the frequency of 18% to 23% in Japanese subjects, 2% to 5% in white subjects, 11% to 20% in Chinese and 12% in Koreans. The most frequently identified alleles are CYP2C19*2 and *3. The resultant phenotype is PM12. Details about this enzyme are given in next section of this chapter.

➔ CYP2C9:
This gene is important in metabolism of drugs like phenytoin, warfarin, tolbutamide, glipizide, losartan and acenocoumarol. There are 34 variant alleles consequent to SNP. Three alleles CYP2C9*1, CYP2C9 *2, CYP2C9 *3 are frequently identified in all ethnic populations. It has been reported that *2 and *3 have significantly reduced enzyme activity which results in poor metabolism of drug leading to drug toxicity. Details about this enzyme are given in next section of this chapter.

2.3.2 Phase II DMEs-
➔ Glutathione S-transferases (GSTs):
GSTs catalyze conjugation reactions in the phase II of drug metabolism. The anticancer agents are substrates for this enzyme. Glutathione is conjugated with several medications and their oxidative damaging metabolites, but due to conjugation they are generally inactivated. GSTs are also termed as ‘a triple threat in detoxification’ as they protect cells in either of the way- a) enzymatically conjugating electrophilic reactive xenobiotics; b) by binding reactive substrates and preventing damage to important cellular components; c) by
suicide inactivation of enzyme and substrate. GST genes are highly polymorphic and amongst all GSTM1 and GSTT1. Studies have shown that 25% of most populations have complete deletion of GSTM1 and GSTT1. Studies have been carried out to investigate distribution of individuals lacking these enzymes due to homozygous gene deletions in South India 30.4% lacked GSTM1 gene while 16.8% lacked GSTT1 & 4.6% lacked both. The frequency of absence GSTM1 is much higher in the Caucasians (53.5%) and Japanese (51.3%). Frequency of GSTT1 null genotypes is lower in South Indians than Japanese (54%) and Afro-American (24.1%).

→ Thiopurine methyltransferase (TPMT):
TPMT catalyzes methylation of drug in phase II of the drug metabolism. The drugs related are azathioprine, mercaptourine and thiouguanine are thiopurine agents commonly used for range of medical indications like leukemia, rheumatic diseases, inflammatory bowel disease. These thiopurines are inactive prodrugs that require metabolism to thioguanine nucleotides (TGN). The TGN incorporates into DNA to exert cytotoxicity. TPMT inactivates these agents. TPMT activity is highly variable and 8 variant alleles have been identified. Three alleles *2, *3A, *3C account for about 95% of intermediate or low enzyme activity. *3A is most common variant (3.2% to 5.7%) on the white population. In Asians the predominant one is *3C. The patients with homozygous mutant compound heterozygous genotype are at high risk of developing severe haematopoietic toxicity if treated with conventional doses of thiopurines.

→ Uridine diphosphate glucuronosyl transferases (UGTs):
Glucuronicas the conjugation reaction wherein UGTs catalyze the transfer of glucuronicacid to aromatic and aliphatic alcohols, carboxylic acids, amines and free sulphahydril groups to form O-, N-, S- glucuronides respectively. These reactions are responsible for elimination of a diverse range of xenobiotics and endogenous compounds. There are 16 functional human UGT genes out of which 6 genes have shown genetic polymorphism: UGT1A1, 1A6, 1A7, 2B4, 2B7, 2B15. UGT1A1 is responsible for the glucuronidation of bilirudin. There are three forms of inheritable unconjugated hyperbilirubinemia:Crigler-Najjar syndrome type I and II and Gilbert syndrome. Studies have shown that Gilbert syndrome arises from polymorphism in UGT1A1 promoter region containing TA repeat element. The frequency of promoters with decreased activity was found to be highest in African population while lowest in Asian population. Studies have been done to show association between genetic polymorphisms of UGT1A7 gene
and irinotecan toxicity in cancer patients. One such study in Japan has shown that amongst 26 patients with severe toxicity allele frequency for UGT1A7*1 was 61.5% while in 92 patients without severe toxicity was 63.6%, suggesting that determination of UGT1A7 genotypes would not be useful for predicting severe toxicity of irinotecan.

Table 1: Drug Metabolizing Enzymes and Gene Polymorphism effects

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Drug</th>
<th>Consequences of polymorphisms</th>
</tr>
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<tbody>
<tr>
<td><strong>Phase I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Warfarin, phenytoin, non steroidal anti-inflammatory drugs</td>
<td>Anti-coagulant effect.</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Omeprazole, mephenytoin, propanolol, proguanil, phenytoin</td>
<td>Peptic ulcer response (toxicity).</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Beta-blockers, antidepressants, antipsychotics.</td>
<td>Narcotic side effects, efficacy and dependence, beta blocker effect.</td>
</tr>
<tr>
<td>CYP3A4/3A5/3A7</td>
<td>Macrolides, cyclosporin, Ca channel blockers, dapsone, quinidine.</td>
<td>Not yet elucidated; polymorphic 3A5 expression linked to 3A5 promoter polymorphism</td>
</tr>
<tr>
<td>DPD</td>
<td>Fluorouracil</td>
<td>5-fluorouracil neurotoxicity</td>
</tr>
<tr>
<td><strong>Phase II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAT</td>
<td>Sulfonamides, isoniazid, amonafide, hydralazine, dapsone, caffeine</td>
<td>Hypersensitivity to sulfonamides, amonafide toxicity, isoniazid neurotoxicity</td>
</tr>
<tr>
<td>GSTs</td>
<td>Several anticancer agents</td>
<td>Decreased response in breast cancer; more toxicity and worse response in AML</td>
</tr>
<tr>
<td>TPMT</td>
<td>Thioguanine, mercaptopurine, azathioprine</td>
<td>Thiopurine toxicity and efficacy, risk of second cancers.</td>
</tr>
<tr>
<td>UGT 1A1</td>
<td>Irinotecan, Bilirubin</td>
<td>Enhanced drug effect, Gilbert syndrome</td>
</tr>
<tr>
<td>Transporters MDR-1</td>
<td>Natural product anticancer drugs, HIV protease inhibitors: digoxin.</td>
<td>Decrease CD4 response in HIV infected patients, decreased digoxin AUC.</td>
</tr>
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</table>
2.4 CYP 2C19:

CYP2C19, one of the members of CYP super family enzymes accounts for about 2% of oxidative drug metabolism in humans and is involved in metabolism of a number of clinically important drugs. The importance of this polymorphism has been recently shown in case of differences in omeprazole concentrations and gastric acid suppression in poor and EM of CYP2C19.

2.4.1 CYP2C19 gene polymorphism

CYP2C19 protein is 490 amino acids long encoded by CYP2C19 gene which has 9 exons and is located on chromosome 10q 24. The enzyme is present in human liver microsomes and is second in quantity to CYP3A subfamily. CYP2C19 is polymorphically expressed with 25 variant alleles (http://www.imm.ki.se/CYPalleles/cyp2c19.htm accessed in July 2009). CYP2C19*1 is a wild type allele while CYP2C19*2 (rs4244285) and CYP2C19*3 (rs4986893) are most frequently occurring mutant alleles. The wild type allele that is *1 is the most frequently found allele. CYP2C19*2 is the most frequently found variant allele. This allele consists of a single base pair transition – G → A, in exon 5, producing an aberrant splice site. This aberrant splice site mRNA lacks first 40 base pairs of exon 5 thus resulting in premature stop codon and a truncated 234 amino acid protein lacking haem binding protein region. Mutation in this exon leads to loss of restriction site for enzyme Smal. CYP2C19*3 is the second most frequently found variant allele. It is also associated with poor metabolism phenotype. It consists of single base pair mutation - G → A at position 636 of exon 4. This creates premature stop codon and lose of restriction site for enzyme BamHI. CYP2C19*4 and *5 is a rare mutant allele having single base pair transition A → G in initiation codon and an amino acid mutation Arg433 Ile in haem binding region respectively.

The two principle alleles CYP2C19*2 and *3 have been reported with PM phenotype in Caucasians and Asian populations. Individuals with homozygous (*1/*1) or heterozygous (*1/*2, *1/*3) wild-type CYP2C19*1 genotype have efficient enzyme to metabolize CYP2C19 substrates and are EMs. CYP2C19*4 mutant allele is responsible for approximately 3% of PMs in Caucasian population. In the Hong Kong Chinese population heterozygous *4 (CYP2C19 *2/ *4) accounted for 2.38% of the defective alleles in all PMs. The frequency of the CYP2C19*5 alleles is low in Chinese (approximately 0.25% in the Bai ethnic group) and Caucasians (< 0.9%). CYP2C19 *2.
CYP2C19 *3, and CYP2C19 *5 have been shown to account for >99% of the defective CYP2C19 alleles in the Oriental population.

Ethnicity is an important determinant of CYP2C19 activity. Studies have reported a marked inter-ethnic variation in the distribution of CYP2C19 polymorphic alleles. The prevalence of PMs varies from 18 - 23% in Japanese subjects, 3 - 5% in European white subjects, 11 - 20% in Chinese, 12% in Koreans and as high as 70% in the residents of Vanuatu island in Melanesia. In case of EMs the activity of CYP2C19 is significantly reduced in Asians compared with white subjects reflecting most probably the higher frequency of heterozygous subjects in these populations. In North Indian population the frequency of the CYP2C19*2 allele was reported to be 29.7% whereas CYP2C19*3 was absent. While in South Indian population (Tamil, Telgu, Kannada, Malayalam) the frequency of the CYP2C19*2 allele was reported to be 35% whereas CYP2C19*3 was 1%. Frequency of CYP2C19 alleles in North and South Indian populations has been reported. However, variant allele of CYP2C19 gene polymorphism in Maharashtrian population has not been studied.

2.4.2 Clinical significance of CYP2C19 polymorphism

CYP2C19 polymorphism is relevant to oxidative metabolism of a number of clinically important drugs such as diazepam, certain barbiturates, tricyclic antidepressants, omeprazole and proguanil. As a result pronounced genetically determined differences in the disposition of these drugs may affect their efficacy and toxicity. CYP2C19 polymorphism also may contribute to a metabolic predisposition to certain diseases including non-aggressive bladder cancer, lung cancer (squamous cell carcinoma), scleroderma or systemic sclerosis and the eosinophilia-myalgia syndrome. Thus defining the frequency of this polymorphism in different populations has considerable epidemiologic importance. The genetic polymorphism of CYP2C19 has been shown to have the most striking interethnic variation of a CYP so far. Several polymorphisms in the CYP2C19 gene have been identified and shown to cause phenotypic variability.

CYP2C19 gene polymorphisms have been shown to have clinical consequences resulting in toxicity of some drugs in the affected individual, and may alter efficacy of the other drugs. It has been shown that a higher concentration of omeprazole in PMs results in great gastric acid suppression as compared with EMs. Cure rates for Helicobacter pylori in patients receiving omeprazole and amoxicillin were found to be 28% in homozygous EMs.
(CYP2C19*1/*1), 60% in heterozygous EMs (CYP2C19*1/*2 and *1/*3), and 100% in PMs (CYP2C19*2/*2 and *2/*3), indicating the importance of dose adjustment in the cases of EMs.

There are several other proton pump inhibitors including pantoprazole, rabeprazole (E3810) and lansoprazole whose metabolism has also been shown to be dependent on CYP2C19. For example, pantoprazole has a 6-fold increase in its plasma AUC, and a 5-fold shorter half-life in EMs of mephenytoin than in PMs. Pantoprazole lacks the 5-methyl group on the pyridine ring of omeprazole which is hydroxylated by CYP2C19. However, the demethylation of the 4-position of the pyridine ring is affected in CYP2C19 PMs. Lansoprazole is structurally related to omeprazole and its 5-hydroxylation is mediated by CYP2C19. The oral clearance of lansoprazole was about 6.5 times lower in PMs of mephenytoin than EMs, and the AUC was also greater. These data indicate that metabolism of lansoprazole, like that of omeprazole, is highly dependent on the CYP2C19 genotype.

Diazepam, an anxiolytic drug is demethylated by CYP2C19. Plasma half-lives of diazepam were dramatically longer in individuals genotyped as homozygous for the defective CYP2C19*2 allele (84 hr) than in individuals who were homozygous for the wild-type allele (20hr), or individuals heterozygous for one defective CYP2C19 defective allele (64 hr). The half life of the metabolite desmethyldiazepam was also longer in homozygous CYP2C19 PMs. Asian populations in general have been reported to have slower metabolism of diazepam than Caucasians, which is attributed to the high frequency of the mutant CYP2C19*2 and CYP2C19*3 alleles in Asians.

CYP2C19 also metabolizes the HIV protease inhibitor nelfinavir to its major circulating metabolite. However, the metabolite has an antiviral activity similar to that of nelfinavir itself as a consequence; the polymorphism does not appear to have a clinical effect on drug toleration or antiviral response to nelfinavir.

Proguanil is an inactive pro-drug that requires biotransformation to its therapeutically active metabolite cycloguanil, which is predominantly catalysed by CYP2C19 and to minor extent by CYP3A4. Therefore, decreased activation of proguanil to cycloguanil in CYP2C19 PMs may result in failure of malaria chemoprophylaxis.
2.5 CYP2C9

CYP2C9, a major isozyme of the CYP2C subfamily in human liver, constitutes approximately 20% of the total human liver microsome P-450 content and metabolizes 10% of therapeutically important drugs, some such as warfarin with narrow therapeutic index\textsuperscript{65,109,110}.

2.5.1 CYP2C9 gene polymorphism

The CYP2C9 gene is located at chromosomal region 10q\textsuperscript{111}, spanning approximately 55-kb with nine exons and encodes a protein of 490 amino acid residues\textsuperscript{112}. CYP2C9 is 92% homologous to CYP2C19, the expressed product of its neighboring gene (CYP2C19)\textsuperscript{113}, differing by only 43 of 490 amino acids. However, the two enzymes have completely different substrate specificity.

Currently 34 allelic variants (http://www.cypalleles.ki.se/cyp2c9.htm accessed on July 2009) for CYP2C9 polymorphism have been reported. At present, at least there are six CYP2C9 alleles studied in humans. The most common allele is designated as CYP2C9*1, and is considered the wild-type allele. A cytosine-to-thymine transversion at nucleotide 430 encodes for a cysteine substitution at amino-acid residue 144, producing the CYP2C9*2 variant allele. An adenine-to-cytosine transversion at nucleotide 1075 encodes for a leucine substitution at amino-acid residue 359, producing the CYP2C9*3 variant allele. Recently, a substitution at base pair 1076 has been detected, coding for an Ile359Thr amino-acid change, producing the CYP2C9*4 variant allele. A cytosine-to-guanine polymorphism at base pair 1080, encoding for an Asp360Glu amino-acid substitution (CYP2C9*5), has also recently been identified. Finally, CYP2C9*6 variant allele has been identified; this is a new null polymorphism containing an adenine base pair deletion at nucleotide 818, which results in a premature stop codon and a truncated inactive protein\textsuperscript{114}. Functional significance of CYP2C9*4, *5 and *6 variant alleles is not well established. However, only three alleles namely CYP2C9*1, CYP2C9*2 and CYP2C9*3 are frequently identified and well established in different ethnic populations\textsuperscript{113}. It has been reported that in individuals with CYP2C9*2 and CYP2C9*3 mutant alleles, the enzyme activity is significantly reduced\textsuperscript{115,116}.

Difference in allelic frequencies has been well documented in population with diverse ethnic origins. The allele frequencies of CYP2C9*2 and CYP2C9*3 generally tend to be higher in white populations than Asian populations\textsuperscript{114}. Furthermore in contrast to African-Americans and whites, the CYP2C9*2 allelic variant was extremely rare in East Asians.
including Korean, Japanese and Chinese populations suggesting that any effects of this variant on CYP2C9-mediated drug metabolism are likely to be negligible in East Asians. CYP2C9*2 allele occurs with a varying frequency between 8 -12% in Caucasians, while by contrast it is absent in Orientals and low in Black Africans. Allelic frequency of CYP2C9*3 allele is lower in Orientals and Black Africans, while it varies between 3 - 8% in Caucasians. Asian individuals homozygous for CYP2C9*3 were rare, and less than 2% of the Asians were heterozygous for this allelic variant. Because of both the greater frequency of the CYP2C9*3 allele in white (-8%) than East Asian (-2%) or black (-1%) populations and the effects of gene dosage on CYP2C9 activity, we would anticipate that concentrations of CYP2C9 substrate drugs would, on average, be higher in whites, assuming expressed levels of the protein are comparable. In South Indian population which comprises of southern part of Indian peninsula belonging to Dravidian race, frequency of CYP2C9*2 allele was found to be 4%, while that of CYP2C9*3 allele was 8%. In spite of interethnic differences in CYP2C9 allelic distribution, no data on the genotype distribution of CYP2C9 variant alleles is available for Maharashtrian population.

### 2.5.2 Clinical significance of CYP2C9 polymorphism

CYP2C9 exhibits marked interindividual variability in its expression and catalytic activity due to functionally significant genetic variations. This can result in either drug toxicity (e.g., warfarin-induced bleeding complications) or therapeutic failure in some patients who take standard doses of CYP2C9 substrate drugs. CYP2C9 metabolizes many clinically important drugs including the diabetic agent tolbutamide, the anticonvulsant phenytoin, the S-enantiomer of the anticoagulant warfarin, D1 tetrahydrocannabinol and numerous anti-inflammatory drugs such as ibuprofen, diclofenac, piroxicam, tenoxicam, mefenamic acid, the antihypertensive losartan, and several new drugs including the antidiabetic drug glipizide and the diuretic torasemide. A rare polymorphism was reported in the metabolism of tolbutamide and phenytoin as early as the 1970s. Subsequently, the tolbutamide polymorphism was shown to be due a rare allele, CYP2C9*3, which carries an Ile359Leu mutation. The same defect was identified in two individuals who were homozygous PMs of losartan. One of these was phenotyped for tolbutamide and was shown to be a PM for this drug. Another individual who was homozygous for the CYP2C9*3 allele was found to have diminished clearance of S-warfarin and an exacerbated response to warfarin. In addition, a PM metabolizer of phenytoin, glipizide and tolbutamide was found to be homozygous for the CYP2C9*3 allele.
Clinical problems with toxicity and dosage adjustment of both warfarin and phenytoin have been found in CYP2C9 PMs. CYP2C9 probably accounts for 80-90% of the metabolism of phenytoin and therefore polymorphisms in CYP2C9 have a larger effect on clinical toxicity of the drug.

The effects of the CYP2C9 polymorphisms on metabolism of these anti-inflammatory drugs have not been studied in vivo, the in vitro results suggest the possibility that individuals who are homozygous for CYP2C9*3 could have slower metabolism of these drugs. However, the metabolism of Diclofenac, the substrate of CYP2C9, is not affected by the presence of mutant alleles of CYP2C9. It was reported that there is no correlation between diclofenac-induced hepatotoxicity and mutant genotype of CYP2C9. Since therapeutic indices of these drugs are relatively high, these polymorphisms would be less likely to have clinical consequences.

An earlier study reported a slightly higher incidence of the CYP2C*2 allele in individuals who required a lower dose of warfarin for maintenance for anti-coagulant therapy. The higher incidence of variant CYP2C9 alleles (CYP2C9*2 and CYP2C9*3) was reported in individuals requiring a low dose of warfarin to maintain optimum coagulation than in those who required a high dose. The incidence of serious and life-threatening bleeding episodes was four times higher in the group of patients requiring a low dose of Warfarin. Thus, the available evidence indicates that the individuals homozygous for the Leu 359 allele are likely to have impaired clearances of losartan, phenytoin, tolbutamide, toresemide, S-warfarin and possibly other CYP2C9 substrates.
2.6 Genetic basis to concept of Prakriti

“A good physician knows individual variations and specific treatment accordingly”
- Charaka Samhita. (Sutra 1/62)

2.6.1 The science of Ayurveda

Ayurveda (Ayu: Life; Veda: Knowledge) literally means science or knowledge of life has history of over 4000 years of practice. It has strong philosophical, experiential and logical foundation. It is one of the most ancient living traditions that address health holistically. Ayurveda is practiced widely in India, Sri Lanka and other countries. Indian healthcare consists of medical pluralism and ayurveda still remains dominant compared to modern medicine, particularly for treatment of a variety of chronic disease conditions.

The core concept of health and disease in Ayurveda is built around a strong belief in the uniqueness of individual. The five basic elements (Panchamahabhutas) Earth, Water, Fire, Air and Ether constitute living and nonliving (all life forms and nonliving matter evolve out of these basic five elements). Combination proportions of these five elements determine “Prakriti” or constitution of an individual which is presented /manifested in form of “Tridosha”. Ayurveda uses this threefold classification known as tridosha theory that identifies principles of motion (Vata), metabolism (Pitta) and structure (Kapha) as discrete phenotypic groupings. Vata (V) one of the tridoshas and is constituted/governed by Ether and Air. It has kinetic functions and it is manifested in mental functions such as enthusiasm, concentration etc. and physical functions such as respiration, circulation, voluntary actions etc. In modern physiology, activities of nervous system primarily are assigned to it. Pitta (P) constituted by Fire. Mental functions such as intelligence, clear conception and physical phenomena like digestion; assimilation, heat production, hormones and enzymes, and metabolism are attributed to P. Earth and Water form Kapha (K). K manifests in mental functions such as courage and body functions such as tissue building, body strength, immunity, resistance and anabolic activity. Tridoshas V, P and K constitute regulatory systems respectively controlling input/output, turnover and storage making them universal properties of all living systems.

Ayurvedic concept of physiology, pathology, diagnosis, prognosis, medicine and therapeutics are based on the doctrine of V, P, and K tridoshas and their combination at any given moment. These three doshas exist in an unstable equilibrium and are always susceptible to being disturbed as a result of interaction with the environment. The state of
equilibrium leads to health and disturbance or imbalance of the doshik equilibrium represents diseased state. Ayurvedic therapeutics is based on philosophy of maintaining equilibrium or homeostasis. If there is vitiation of dosha; either more or less of it, it could be fine tuned in the living system. Pioneering study by Joshi RR shows that the concept of tridosha has a sound empirical basis. A quantitative measure of tridosha level (V, P, K) is obtained by applying an algorithmic heuristic approach to the exhaustive list of qualitative features/ factors that are used by Ayurvedic physicians. Tridosha could thus be quantitatively estimated from qualitative characterization using core diagnostic criteria of Ayurveda\textsuperscript{146}. Decision support software like AyuSoft has been developed for optimal applications of Ayurveda knowledge. AyuSoft draws from three original major classics namely Charaka Samhita, Sushruta and Vagbhata together with Madhava Nidana another ancient classic on diagnosis and contains over 5000 signs and symptoms\textsuperscript{147}. This will help in correct assessment of Prakriti of subjects and help to control possible variations. Thus Tridoshas being substances that are quantifiable in nature, they could be considered as qualitative and quantitative traits.

2.6.2 Ayurvedic concept of Prakriti

Prakriti means constitution, temperament or fundamental form. The Prakriti assessment is an important step in Ayurvedic advice, diagnosis and therapeutics and indicates the extent of domination of one or more Dosha. According to Ayurveda, the individual constitution or Prakriti classification is based on differences in physical, physiological and psychological characteristics and is independent of racial, ethnic or geographical considerations (Table 2). The importance of such individual variations in health and disease is an important basic principle and was underlined by Charaka 4000 years ago. Relative proportions of tridoshas can be clustered into 7 broad Prakriti types. These include the 3 ‘main types’ i.e. having 70% or more dominance of a single dosha, Vata, Pitta and Kapha, which we have assessed here. In addition, there are 4 mixed types: 3 where two doshas account for more than 80%, Vata-Pitta, Vata-Kapha, and Pitta-Kapha, and 1 where all three doshas are in balance, i.e. Vata –Pitta –Kapha all within 10% of each other. Of these, the single dosha Prakritis Vata, Pitta and Kapha are considered as extreme classes. They exhibit more easily recognizable phenotypes, and are more predisposed to particular diseases.

Prakriti, is specific for each individual. It is said to be determined at the time of conception and remains unaltered during the lifetime i.e. in modern terms by the
recombination of zygotic DNA from sperm and ovum. Disease proneness and Prakriti do not have simple and direct association; however some generalized assumptions can be made. Sama Prakriti has good resistance and generally not prone to diseases. Other types are labeled as atura (diseased), as they have dominance of one or more doshas and need regular preventive measures. Out of rest six types, Prakriti with one dosha (Ekadoshaja) have better resistance than two Dosha Prakriti (Dwidoshaja). V, P and K Prakriti are susceptible to disease in decreasing order. Prakriti specific treatment including prescription of medications, diet and lifestyle is a distinctive feature of Ayurveda.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Vata Prakriti</th>
<th>Pitta Prakriti</th>
<th>Kapha Prakriti</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physique</td>
<td>Lean</td>
<td>Medium</td>
<td>Well built</td>
</tr>
<tr>
<td>Skin texture</td>
<td>Dry, dark with superficial veins and tendons</td>
<td>Bright skin with moles</td>
<td>Oily, whitish</td>
</tr>
<tr>
<td>Hair</td>
<td>Rough, scanty</td>
<td>Soft, brown, early gray or bald</td>
<td>Thick, black, lustrous</td>
</tr>
<tr>
<td>Nails</td>
<td>Dry, thin, small</td>
<td>Pink, soft</td>
<td>Thick, oily, large</td>
</tr>
<tr>
<td>Appetite</td>
<td>Inconsistent</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Food habits</td>
<td>Small quantity, eats fast</td>
<td>Require large quantity, frequently</td>
<td>Eat less and slow</td>
</tr>
<tr>
<td>Bowels</td>
<td>Dry, hard stools, constipation tendency</td>
<td>Loose motions tendency</td>
<td>Solid, oily stools</td>
</tr>
<tr>
<td>Rate of Digestion</td>
<td>Inconsistent</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Movements</td>
<td>Fast &amp; quick</td>
<td>Moderate</td>
<td>Slow</td>
</tr>
<tr>
<td>Psychological</td>
<td>Mental nature</td>
<td>Inconsistent</td>
<td>Intelligent</td>
</tr>
</tbody>
</table>

* Charak Samhita describes over 100 such characteristics for Prakriti determination. We have given only representative important characteristics in this Table.

2.6.3 AyuGenomics

The human genome project has revealed the complexity in the relationship between genotype and phenotype. Better understanding of the human genome has helped in understanding scientific basis of individual variation. It is possible to examine the Ayurvedic concept of Prakriti from a human genome perspective. Permutations and combinations of V, P, K attributes characters along with other host factors such as tissue status (Dhatuvara), twenty Guna, psychological nature (Manas Prakriti), habitat (Desha), season (Kala), digestive capacity (Agni) etc lead to infinite number indicating every individual has unique constitution. If we consider the attributes of each Dosha, its subtype, the seven dhaatus and their interaction in the internal milieu the permutations and the combinations can well be infinite, seven attributes of V interacting with other dosha attribute and that of the seven dhaatus their permutations are infinite hence it is safe to
conclude that the possibilities are innumerable and we have to consider every individual as unique entity. This is how Ayurveda describes the basis of individual variation.

Humans are classified into three major groups: the Negroid, Mongoloid and Caucasoid, and genetically all are 99.9% the same. The difference in terms of color, physique, behavior, etc. is due to SNP which constitutes just 0.1%. Importance of such individual variations in health and disease is an important basic principle of ayurveda and was underlined by Charaka some time 4000 years ago as follows: ‘Every individual is different from another and hence should be considered as a different entity. As many variations are there in the Universe, all are seen in Human being. If it were not for the great variability among individuals medicine might as well be a science and not an art. While medical practice will continue to remain an art, medicine per se has become a science. For years physicians have noted these differences, but had no way to predict them. Pharmacogenetics is the study of the hereditary basis for differences in response of populations to a drug. (Detail review about the various aspects of science of pharmacogenomics has been already talked in the introduction section). The concept of *Prakriti* has central role in understanding health and disease in Ayurveda, which is very similar to pharmacogenetics that is expected to become basis of designer medicine.

Designer medicine is a pharmaceutical product that will give maximum therapeutic efficacy and high safety to the particular person with particular disorder. The fundamental principles of Ayurvedic system of medicine can be used for creating Designer Medicines. One of the step towards this is identifying the genetic basis to *Prakriti*.

Understanding the possible relationship between *Prakriti* and genome will be important. Functionally, this will involve creation of three organized databases that are capable of intelligently communicating with each other to give a customized prescription. These are human constitution (genotype), disease constitution (phenotype) and drug constitution. A golden triangle consisting of ayurveda, modern medicine and science will converge to form a real discovery engine that can result in newer, safer, cheaper and effective therapies. It will be in the interest of pharmaceutical companies, researchers and ultimately the global community to respect the traditions and build on their knowledge and experiential wisdom.
AyuGenomics: a tool for classifying Human population

Every individual is different. It is important to identify factors that determine the ‘individuality’ of a person: genotypes associated with phenotypes and classification of the human population. Systematic surveys of genetic variation form the basis for determination of population frequencies, genetic linkage studies and association studies relating genotype with phenotypes are of interest to identify some of these factors. Genotypic or phenotypic classification of human populations is important in various epidemiological contexts: for better understanding disease, drug response and such like. Current classification of human populations is broadly based on ethnicity; geographical location, language or self reported ancestry. However, such commonly used ethnic labels are inaccurate representations of genetic clusters and do not reflect underlying genetic make up. The inability to explore such relationship is attributed to the complexity of human demographic history giving rise to neither an obvious natural clustering scheme, nor an obvious appropriate degree of resolution.

The Human Genome Project has revealed the complexity in the relationship between genotype and phenotype. Identifying specific phenotypic features and correlating them with genotypes constitutes the major program of phenomics. A Proposed Human Phenome Project anticipates efforts to create comprehensive phenotypic data sets from different populations to find broad based genomic representation. However there is no consensus on how to define phenotypes and which phenotypic features are to be included in the database. Classifying human populations thus remains a major challenge to biomedical sciences. Delineating phenome composition involves cataloging phenotype features and can determine combinations of features that constitute traits.

Ayurveda, a traditional Indian System of Medicine has been explored for this purpose. Ayurveda classifies the whole human population in three major constitutions as using distinct morphological, metabolic and psychological characteristics and these Prakriti types (V, P and K) may offer phenotypic datasets suitable for analysis of underlying genetic variation. As a proof of concept a study evaluated 76 subjects both for their Prakriti and HLA DRB1 types, and found some significant associations in support of it. The study concluded that Ayurveda based phenomes may also provide a model for the study of multigenic traits. It could even offer a new approach for correlating genotypes with phenotypes for human classification. To prove this hypothesis more scientific exploration is required in terms of differential gene expression analysis using micro arrays.
to identify the genes that are up or down regulated in major Prakriti types. These genes could be further used for DNA polymorphism, SNP analysis and gene expression analysis in large populations of different ethnic groups. Thus, an effective integration of concepts of Ayurveda with contemporary genomics could describe pharmacogenomics as the basis of individual variation and could form a basis of future customized medicine.

**Traditional Medicine to Modern Pharmacogenomics: Ayurveda Prakriti Type Associated with the Metabolic Variability**

Ayurveda considers that specific Prakritis are associated with specific disorders. We believe that Prakriti, disease proneness and gene polymorphism will have some association. A broad based population analysis comparing these associations in different racial and ethnic groups should be especially informative to define more clearly these genetic interactions. Genetic polymorphism results in variants of functional importance, which further results in an inactive enzyme, reduced catalytic activity or perhaps even gene duplication. These polymorphisms precipitate in different phenotypic subpopulations of drug metabolizers. PM retains drugs in the body for a longer time than normal and hence the plasma concentration of the drug is high for longer period. Intermediate metabolizers retain drug in the body for the optimal periods. EM retain drug in the body for less time and their plasma concentrations are high but for shorter periods.

It is interesting to note that the three major constitution types described in Ayurveda have unique putative metabolic activities. K being slow, P being fast, while F is considered to have variable metabolism. The hypothesis is that this may relate to drug metabolism and consequently to genetic polymorphism of DME. In my work I have tried to address the said hypothesis.

**2.6.4 Other studies on traditional medicine**

Other than the above mentioned studies an attempt has also been made to integrate Ayurveda with functional genomics to identify pathways associated with activity of crude and active components of an herb, Ashwagandha, which is used for cancer treatment. Studies on whole genome expression conclude that the Ayurveda based method of phenotypic classification of extreme constitutional types allows uncovering genes that may contribute to system level differences in normal individuals which could lead to differential disease pre-disposition.
Similarly study on pharmacogenomics of medicinal plants has also been undertaken. There are several similarities between Ayurveda and Traditional Chinese Medicine including the holistic and individual classification systems. A Few studies have attempted to address the relationship between Traditional Chinese medicine constitutions and HLA polymorphism.

Thus, the integration of Prakriti concept of Ayurveda with genomics nomenclatured as Ayugenomics is worth exploring to unravel many challenges in genomics, therapeutics and personalized medicine.
2.7 Pharmacogenetics of Methotrexate in Rheumatoid Arthritis

2.7.1 Epidemiology of RA

RA is a chronic multifactorial disease of unknown cause with prevalence rates ranging from 0.3 to 1.5% in the population worldwide. Females are two to three times more likely to be affected than males. Indian population have reported a prevalence of 0.5-0.75%. As the disease progresses, the inflammatory process of the synovium of the joints destroys articular structure irreversibly. Inflammatory disease activity is an important predictor of progression of joint damage and the long-term requirement for joint replacement surgery. In the late stage, the disease causes a significant disability and high medical costs. About half of RA patients eventually become work disabled, and mortality is increased in patients with severe active disease.

2.7.2 Treatment in RA

Effective control of the inflammatory process in RA reduces radiographic progression of joint damage and improves physical function and quality of life. Thus the primary goal of therapy is to achieve rapid effective disease control to prevent the long-term damaging effects on the joint structure and function. There is no cure for RA and multiple pharmacotherapies are often required to control the disease. Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, which provide rapid symptomatic relief but have no effect on the progression of joint damage; and disease modifying anti-rheumatic drugs (DMARDs), which reduce the disease activity and slow the progression of joint damage, thereby preserving function; are the two classes of drugs generally used to treat RA. The efficacy of early and aggressive treatment with DMARDs is widely accepted. DMARDs, such as MTX, sulphasalazine (SSZ) and leflunomide, have documented prevention of the structural damage of the joints. Recently, biologic response modifiers targeting specific cytokines, such as tumor necrosis factor-a (TNF-a) or IL-1, have been introduced, and the results indicated the effective suppression of the RA activity. However, the high cost of some of these agents, especially the biological agents, lack of long-term safety and efficacy data and the variability in the response to biologic response modifiers limit their use; as a result the DMARDs are initial choice in treatment of RA.

However, the outcome of the treatment with DMARDs in RA patients is known to vary among patients. Moreover, the use of these agents is limited by the development of unpredictable toxicities that are sometimes severe. Clinicians treating RA face several
challenges. There are no defined clinical or laboratory criteria that reliably predict the clinical course of disease, its severity or response to treatment. Age at disease onset, socioeconomic factors such as education level and income, female sex, prior DMARD use, disease functional class and activity, and rheumatoid factor positivity are some of the non genetic variables that have been studied as possible predictors of outcomes in RA\textsuperscript{183, 184}. However, none of these has consistently been shown to be a marker of response to treatment in RA.

Previously, it was described that the activities of some drug-metabolizing enzymes for DMARDs, such as NAT\textsuperscript{2} for SSZ\textsuperscript{184} and TPMT for AZA\textsuperscript{185}, are different among individuals. Recent advances in genetics have clarified that these individual differences are based on genetic polymorphisms, and this knowledge has encouraged the application of pharmacogenetics to the treatment of RA\textsuperscript{172}.

### 2.7.3 MTX in RA

MTX is the most widely used DMARD for the treatment of RA because of its cost and experience in its use. It has proven to reduce disease activity and delay or stabilize the development of bone erosions\textsuperscript{186, 187}. However, only ~ 50% of the patients experience good clinical response and 30% discontinue therapy due to side effects\textsuperscript{188, 189}. Although MTX is among the best-tolerated DMARDs, major drawbacks of MTX therapy are the large interpatient variability in clinical response and the unpredictable appearance of a large spectrum of side-effects\textsuperscript{190}. There are no useful and reliable clinical or molecular markers of response to therapy. Serum levels of MTX have been considered of little value in determining drug efficacy, since the drug is eliminated from the circulation within 24 hours of administration, which is much shorter than the standard weekly dosing interval in RA\textsuperscript{191}. In contrast, circulating intracellular levels of MTX polyglutamates in erythrocytes and polymorphonuclear cells have been shown to correlate with clinical efficacy in patients with RA, but require a difficult assay system that prohibits its availability in most clinical facilities\textsuperscript{192}. Levels of various cytokines and other mediators of inflammation, such as tumor necrosis factorα (TNFα), interleukin-10 (IL-10), matrix metalloproteinase 3 (MMP-3), IL-6 and tissue inhibitor of metalloproteinases 1 (TIMP-1), may correlate with the efficacy of MTX, but rapid clinical assays to measure them are not readily available\textsuperscript{193-195}. Serum levels of chemokines such as RANTES and growth related oncogene α (GROα) may predict the effect of MTX on radiographic erosions, but are not easily measurable\textsuperscript{196}. Hence, although sophisticated cytokine and enzyme assays may assess
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response to treatment, they are often expensive and are not available in most clinical settings, thus limiting their utility in the clinical management of individual patients. The significant variability in MTX response, coupled with the presence of effective, but expensive, alternative therapies, has led to approaches to identify predictive markers for drug response (efficacy and toxicity) prior to the initiation of MTX therapy. This may help to decrease the morbidity associated with side effects, reduce the need for laboratory tests to monitor toxicity, and help select patients who are more likely to respond to the drug. But no such clinical/ genetic predictors are available to date.

2.7.4 Cellular pathway of MTX

Despite extensive research, the precise mechanism of action of MTX is still unknown. MTX, a folate analog, is a competitive inhibitor of the enzyme dihydrofolate reductase (DHFR). It enters the cells through an active transport mechanism, which is regulated by members of both the ATP-binding cassette (ABC) and solute carrier transporter families. The entry of MTX into the cell is mediated by reduced folate carrier 1 (RFC-1), while MTX efflux from the cell is mediated by ABCC1, ABCC2, ABCC3, ABCC4 and ABCB1 (Figure 3). Once inside the cell MTX is intracellularly converted to MTX polyglutamates (MTXPGs) by a \( \gamma \)-linked sequential addition of glutamic acid residues to MTX\(^{197} \). This process is in competition with deconjugation by \( \gamma \)-glutamyl hyrolase (GGH). Polyglutamation of MTX enhances the intracellular retention of MTX and promotes the sustained inhibition of de novo purine synthesis along with the buildup of adenosine, a potent anti-inflammatory agent\(^{172, 173} \). MTX directly inhibits several enzymes of the folate pathway, including DHFR, Thymidylate synthase (TS) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (ATIC). Other folate enzymes, such as MTHFR, are not directly inhibited by MTX, but their expression level may contribute to the antifolate effects of MTX through subtle alterations in the folate pools\(^{198} \).
Figure 3: Intracellular Folate-MTX Pathway

Figure 3 illustrates schematic representation of the intracellular folate biosynthetic pathway and related pathways. Enzymes involved in different pathways are denoted in italics. Transporter/Enzyme polymorphisms genotyped in the current study are highlighted in bold italics.
2.7.5 Polymorphisms in MTX transporter

→ RFC1:
RFC-1, also known as SLC19A1 is important for the transport of MTX into the cell. Polymorphisms in RFC1 can either inactivate the enzyme or change the function of transcription factors, leading to loss of RFC-1 gene expression resulting into defective transportation. A G80A polymorphism leading to the substitution of arginine for histidine at codon 27 in the first transmembrane domain (TMD1) of the RFC-1 protein and a 61-bp repeat polymorphism in the RFC-1 promoter associated with increased transcriptional activity of the gene have been recently discovered. The results of G80A SNP in RFC1 influencing the uptake and intracellular levels of MTX is controversial. One study showed no significant differences in MTX uptake rates between leukemic blast cells with the arginine-27 RFC proteins versus those with the histidine-27 RFC proteins. However, in another study, children with acute lymphoblastic leukemia homozygous for the RFC1 G80A variant had higher MTX plasma levels ($P = 0.004$) than other genotype groups. Study on RA patients taking MTX showed that the patients having RFC1 80A/A genotype had higher MTXPG levels compared with those who had the RFC1 80G/G or G/A genotypes ($P = 0.007$).

→ ABCB1:
P-glycoprotein (P-gp), the product of the ABCB1 gene (a member of the ABC family of transporters) is a membrane transporter implicated in the disposition and bioavailability of several drugs including MTX. Recently, SNPs in the ABCB1 gene have been identified and correlated with P-gp expression. C1236T in exon 12 and C3435T in exon 26 are the most common exonic but silent variations. One of them is the C3435T polymorphism of the gene found to correlate significantly with intestinal P-gp expression levels and bioavailability of digoxin. Like RFC-1, there is considerable controversy regarding whether genetic variations in ABCB1 and/or P-gp expression influence MTX efflux from the cell. Although there are no data demonstrating that the ABCB1 C3435T SNP directly affects the cellular transport of MTX, some studies indicate that higher P-gp expression may be a marker of MTX resistance, whereas other studies contradict this finding.
2.7.6 Polymorphisms in MTX glutamation genes

- **Folypolyglutamate synthase (FPGS) and GGH:**

  SNPs in the GGH promoter region that increase GGH expression and affect MTX polyglutamation have been described. A GGH C401T polymorphism in the promoter that affects the intracellular MTXPG levels has been described. A GGH C4521 SNP that leads to decreased activity of GGH and accumulation of intracellular long-chain MTX polyglutamates has also been identified. Another GGH T16C SNP has been described; however, its functional effects are unknown. As for FPGS, although one study showed an association between FPGS mRNA expression in peripheral blood mononuclear cells and poor response to MTX in RA patients, the functional roles of SNPs in this gene are yet to be determined.

2.7.7 Polymorphisms in MTX cellular pathway

- **DHFR:**

  DHFR reduces dihydrofolate (DHF) to tetrahydrofolate (THF). THF is important for the production of biologically active folate cofactors, such as 5-methyl-THF, which is required for the generation of methionine from homocysteine and for the synthesis of polyamines. DHFR is a direct target of MTX. A total of two DHFR polymorphisms, G473A and G352X, are important in DNA alignment.

- **Serine hydroxymethyltransferase (SHMT1):**

  SHMT encodes a vitamin B6-dependant enzyme that catalyzes the reversible conversion of serine and THF to glycine and methylene THF. Two different SHMT isoenzymes are known: one located in cytoplasm localized to the SHMT1 gene on chromosome 17p11.2, and the other is in mitochondrion localized to the SHMT2 gene on chromosome 12q13.2. SHMT1 plays a crucial role in generating one-carbon units for purine, thymidylate and methionine synthesis in cytoplasm. A C1420T polymorphism in SHMT1 gene influencing red blood cell folate levels has been described.

- **Methylenetetrahydrofolate dehydrogenase (MTHFD1):**

  5, 10-methylene THF dehydrogenase. 5, 10-methylene THF cyclohydrolase or 10-formyl THF synthetase (MTHFD1), as the name implies, is a tri-functional enzyme responsible for derivatization (by oxidation) of 10-formyl THF from 5, 10- methylene THF. This enzyme consists of two major domains: an N-terminal domain, containing methylene THF.
dehydrogenase and the methylene THF cyclohydrolase activities and a larger formyl-THF synthetase domain. 5, 10-methylene THF and 10-formyl THF are essential cofactors for thymidylate and de novo purine synthesis. A G to A substitution at position 1958 of MTHFD1 gene, causing an alanine to glycine substitution at codon 653 within the 10-formyl THF synthetase enzyme domain has recently been reported. A role of the MTHFD1 A1958G variant in susceptibility to neural tube defects has been suggested.

ATIC:
ATIC, which converts AICAR to 10-formyl-AICAR, is directly inhibited by MTXPGs. ATIC when inhibited by MTXPGs, causes intracellular accumulation of AICAR. AICAR and its metabolites can then inhibit two enzymes involved in adenosine metabolism, adenosine deaminase and AMP deaminase (AMPD), leading to increased intracellular concentrations of adenosine and adenine nucleotides. Subsequent dephosphorylation of these nucleotides results in increased concentrations of adenosine in the extracellular space. Adenosine is a potent anti-inflammatory agent, and some of the anti-proliferative effects of MTX are thought to be mediated through this mechanism. A C to G substitution at position 347 of ATIC gene has been reported.

MTHFR:
This is the best studied of the genes in the MTX cellular pathway. MTHFR is important in the generation of 5-methyl-THF, which is the methyl donor for the methylation of homocysteine to methionine by methionine synthase (MS). About a dozen SNPs in the MTHFR gene have been described, two nonsynonymous SNPs have been extensively studied. The C677T polymorphism, which was first described in 1995, causes an alanine-to-valine substitution at codon 222 of the MTHFR gene. It results in a thermolabile variant of MTHFR with decreased enzyme activity and subsequent increased plasma homocysteine levels. The homozygous C677T variant, with ~30% of the wild-type activity, has a prevalence of ~8–10% in the general population. Heterozygotes have ~60% activity and form ~40% of the population.

Another polymorphism in the MTHFR gene, A1298C, which leads to a glutamine-to-alanine substitution at codon 222, was described in 1998. Homozygotes and heterozygotes for A1298C have reduced activity of the MTHFR enzyme, although they do not have a thermolabile variant of MTHFR. The homozygous genotype with ~60% of
enzyme activity in lymphocytes has been observed in 10% of the Canadian population (worldwide prevalence unknown). The A1298C polymorphism, by itself, does not influence plasma homocysteine levels. However, individuals heterozygous for the C677T and A1298C polymorphisms have significantly decreased activity of the MTHFR enzyme and elevated plasma homocysteine levels comparable to those in individuals homozygous for the C677T polymorphism.

TS:
TS, a key enzyme in de novo thymidylate synthesis, converts Deoxyuridine-5’-monophosphate (dUMP) to Deoxythymidine-5’-monophosphate (dTMP). TS is inhibited directly by polyglutamated MTX and indirectly by folate cofactor depletion induced by MTX (Figure 3). Inhibition of TS leads to dTMP depletion and increased uracil misincorporation into nucleic acid, which in turn, leads to chromosome damage and cell death. A tandem repeat sequence has been identified in the 5’ untranslated region (5’-UTR) of the TS gene. This sequence is polymorphic, with a variable number of 28-bp repeat elements (5’-UTR 28-bp repeat). The repeat element appears to function as an enhancer, because TS messenger RNA (mRNA) expression and TS enzyme activity are increased with an increasing number of repeat sequences in vitro. Patients homozygous for the triple repeat allele (TSER*3/*3) have higher TS mRNA expression than those homozygous for a double repeat allele (TSER*2/*2). Another polymorphism in the TS gene, consisting of a 6-bp deletion of the sequence TTAAAG at nucleotide 1494 in the 3’-UTR (3’-UTR 6-bp deletion), has been described. Although the function of this polymorphism is not fully known, there is evidence to suggest that the 3’-UTR deletion is associated with decreased TS mRNA stability and expression.

2.7.8 Polymorphisms in adenosine pathway

ITPA, ACPD, MS and MTRR:
Inosine triphosphate pyrophosphatase (ITPA) converts inosine triphosphate (ITP) to inosine monophosphate (IMP) in the purine synthetic pathway while AMPD converts AMP to IMP. S-adenosyl homocysteine (SAH) can generate homocysteine in the de novo purine synthetic pathway. SAH is derived from homocysteine, which is in turn generated by the remethylation of methionine. MS is required for the methylation of homocysteine to methionine in the presence of a cobalamin cofactor. A polymorphism in the MS gene, A2756G, resulting in an aspartic acid to glycine change at codon 919 (D919G), has been described. This SNP may be an activating mutation, since individuals homozygous for the SNP (DD genotype) have high homocysteine levels as compared with individuals with
the wild-type (GG) genotype\textsuperscript{207, 208}. Methionine synthase reductase (MTRR) is an enzyme important for the methylation of the cobalamin cofactor of MS. A A66G SNP in MTRR, leading to substitution of methionine for isoleucine at codon 22, with the MTRR 66GG genotype conferring the risk of elevated homocysteine levels, has been identified\textsuperscript{209, 210}.

2.7.9 MTX pharmacogenetic studies

\textbf{MTX transporter and cellular pathway genes}

A recent retrospective study by Takatori et al examined the effects of RFC1 G80A, ABCB1 C3435T, ATIC C347G and the TS 6 bp deletion polymorphisms on MTX efficacy and toxicity in 124 RA patients. Responders were classified as those having maintenance dose of 6 mg/week or less and non responders are those having maintenance dose of more than 6 mg/week or in whom MTX therapy was changed owing to poor response to MTX. A significantly higher proportion of non responders carried the ABCB1 3435 TT genotype compared with CC genotype by both univariate (OR: 8.91; p=0.001) and multivariate (OR: 8.78; p=0.038) analysis. This genotype had no effect on MTX toxicity. The RFC1 G80A, ATIC and TS genotypes had no effect on MTX response and toxicity\textsuperscript{211}.

A study by Grabar et al observed that the RFC1 A80G and ABCB1 C3435T polymorphisms increased the risk for overall MTX toxicity (P = 0.039, OR = 3.574, 95\% CI = 1.065-11.993 and P = 0.032, OR = 7.801, 95\% CI = 1.194-50.960 respectively), while MTHFR A1298C polymorphism had a protective effect on overall MTX toxicity (P = 0.027, OR = 0.170, 95\% CI = 0.035-0.820)\textsuperscript{212}.

RFC1 G80A SNP has been studied in association with response to MTX in 105 patients with RA. Patients homozygous for the RFC SNP 80A/A had a greater response to MTX compared with patients with the wild-type allele (80G/G), suggesting that this SNP is associated with an increased response to the drug\textsuperscript{208}.

Two DHFR polymorphisms, G473A and G35289A, were examined along with MTHFR C677T and A1298C and RFC1 G80A for their effects on MTX efficacy and toxicity in 205 RA patients. The results showed that at 6 months, MTHFR 1298AA was associated with good improvement relative to 1298C (OR 2.3, 95\% CI [95\% CI] 1.18-4.41), which increased with increased copies of the MTHFR 677CC haplotype (OR:3.0; 95\% CI 1.4-6.4; p=0.021). The RFC1 and DHFR SNPs were not associated with MTX efficacy.
Patients with MTHFR 1298C allele carriers developed more Adverse events (AEs) (OR 2.5, 95% CI 1.32–4.72). The MTHFR C677T, RFC1 G80A and DHFR SNPs did not show significant association with toxicity. In a study by Pawlik et al; RA Patients with the ABCB1 3435CC and 3435CT genotypes had a greater risk of having active RA compared with patients with the 3435TT genotype (odds ratio [OR] 2.89 [95% CI 0.87–9.7], P<0.05). The 3435T allele also seemed to confer a protective effect, with patients homozygous for this allele having a less severe form of RA that was more likely to respond to MTX and prednisone. Drozdzik et al demonstrated that the ABCB1 C3435T gene polymorphism may influence the efficacy of RA therapy with disease-modifying antirheumatic drugs. Sharma et al in their study on Indian RA reported that the ABCB1 C3435T gene polymorphism was observed to be significantly associated with response.

Genetic variations in other members of the ABC family have not been studied so frequently in the context of MTX response in RA; SNPs in these transporters are quite common. Ranganathan et al in their study revealed the effect of these gene polymorphisms on MTX toxicity in RA patients and reported that MTX transporter gene SNP could be important markers of MTX toxicity in RA.

Thus, genetic variations in transporters such as RFC-1 and ABCB1 may affect the response to MTX in RA, although this remains a controversial subject. Genetic variations in other transporters occur frequently and need to be studied further for their effects on drug response.

**Pharmacogenetics of MTX glutamatergic genes**

In one study, two SNPs in GGH C452T and T16C and 2 in FPGS. G114A and A1994G were genotyped in RA and healthy controls to assess the association of these SNPs with treatment response to MTX. The FPGS gene SNPs and their haplotypes and the GGH T16C were not associated with MTX efficacy. Although particular GGH variant T16C and GGH 452C-16T haplotype appear to influence response to MTX at 3 months, such an influence is not sustained at 6 months.
MTX cellular and adenosine pathway pharmacogenetics

- **MTHFR**

A recent Japanese study looked at the effect of the C677T and A1298C SNPs on MTX efficacy and toxicity in 159 patients with RA. Genotype analysis in these patients revealed that patients with the 1298AA genotype require higher doses of MTX (indicating less drug efficacy) than those with CC and AC genotypes (p=0.008, relative risk (RR) 1.84; 95% CI: 1.12-3.01). There was no association between the C677T genotype and MTX dose. The effect of *MTHFR* SNPs on MTX related AEs was studied in 156 RA. Overall MTX-related AEs were more frequent in patients with 677T allele (CT and TT genotypes) than those with 677CC genotype (p=0.003, RR 2.4; 95% CI: 1.29-4.55). Furthermore, the 677T allele was associated with alanine aminotransferase elevations among subgroup of patients with MTX related liver toxicity (p=0.004, RR 2.66; 95% CI: 1.30-5.44). The A1298C SNP had no effect on MTX toxicity. Thus this study suggests that the *MTHFR* C677T SNP may have effects on MTX efficacy.

Haagsma et al found that increases in homocysteine levels during MTX treatment were higher in RA patients with the C677T mutant allele, which may be related to the increased frequency of gastrointestinal adverse affects. Van Ede et al reported that the presence of the C677T mutation (homozygous or heterozygous C677T mutant alleles) was associated with an increased risk of discontinuation of MTX treatment because of adverse events in 236 RA patients, mainly due to increased risk of elevated liver enzyme levels. Urano et al examined the relation between genotypes and haplotypes concerning polymorphisms of the MTHFR gene and MTX efficacy and toxicity in 106 Japanese RA patients for at least 3 months in a retrospective study. Patients with the A1298C mutant allele were receiving significantly lower doses of MTX than patients without it, while a higher rate of overall MTX toxicity was observed in patients with the C677T mutant allele than those without it. Aggarwal et al. studied correlation between methotrexate efficacy & toxicity with *MTHFR* C677T polymorphism in Indian RA patients on folate supplementation. The study concluded that the *MTHFR* C677T polymorphism is not predictive of toxicity or efficacy of MTX treatment in RA patients receiving folate supplementation. Further studies need to be done to look at polymorphisms in other enzymes that may have association with MTX clinical efficacy and toxicity.

In a more cross-sectional study, 93 patients with RA who were taking MTX and 377 healthy subjects were genotyped for the C677T and A1298C SNPs. The frequency of the
1298CC genotype (24.7%) in the rheumatoid study group was greater than expected in the general population (12.8%, p<0.001). This genotype was associated with a significantly low rate of methotrexate related side effects. The odds ratio for side effects in patients with wild type 1298AA genotype vs 1298CC genotype was 5.24 (95% confidence interval, 1.38 to 20). No correlation of disease activity variables or plasma homocysteine with MTHFR A1298C and C677T polymorphisms was observed. The authors concluded that RA may be more common among 1298CC homozygotes in their population and that 1298CC homozygosity may confer protection from MTX-related side effects through a homocysteine-dependent mechanism.

In another study, 193 Caucasians and 30 African-American patients with RA were genotyped for the C677T and A1298C, and 3 additional SNPs in MTHFR coding region and genotypes were correlated with MTX efficacy and toxicity. In addition, another 308 RA patients and 103 controls, which included Caucasians and African-Americans were genotyped to assess racial differences in the allele frequencies of these SNPs. There were no significant differences in the allele frequencies of the individual MTHFR SNPs between patients and controls irrespective of race or ethnicity. However, the allele frequencies of the rs4846051C, 677T and 1298C alleles were significantly different between African-Americans and Caucasians with RA. There was no association between the individual SNPs or haplotypes and response to MTX. Among Caucasians, there was a significant association between the 1298A allele and MTX related AEs (OR: 15.86; 95% CI 1.51-167.01, P=0.021). There was an association between the rs4846051C allele and the haplotype containing this allele and higher toxicity scores among AA with RA. Thus the genetic markers for MTX toxicity were different in the two racial groups: none of the MTHFR variants were associated with MTX efficacy.

**TS/MTHFR**

In a study analyzing the effects of the TS and MTHFR genotypes on MTX efficacy and toxicity, 167 patients with RA, 115 of whom had been treated with MTX, were recruited. Results showed that 45% of patients treated with MTX experienced toxicity: none of the TS or MTHFR polymorphisms influenced this. The weekly MTX dosage and the C-reactive protein (CRP) levels were used to assess efficacy. Patients homozygous for the TSER*3/*3 repeat allele required higher dosages of MTX (>6 mg/week) compared with those homozygous for the TSER*2/*2 repeat allele (P= 0.033). In contrast, patients
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homozygous for the deletion allele \(0\) bp had at least 50% improvement in their CRP levels after treatment with MTX as compared with pretreatment levels \(P = 0.024\). Based on these findings, the authors speculated that by increasing \(TS\) mRNA expression, the \(TSER^*3\) *3 polymorphism lowered MTX efficacy, whereas by decreasing \(TS\) expression, the deletion SNP \(0\) bp made patients more sensitive to MTX. The MTHFR polymorphisms had no effects on MTX toxicity or efficacy in this study. Since MTX is influenced by many gene products, it is not surprising that polygenic analyses have begun to be reported.

- **MTHFR, TS, ATIC and SHMT**

A total of 214 RA patients on MTX were genotyped for \(MTHFR\) C677T, \(TSER^*2\) *2, \(ATIC\) C347G and \(SHMT1\) C1420T polymorphisms to assess their association with MTX related side effects. Study reported a total of 67 patients (31%) had a side effect and specific genotypes were associated with specific toxicities. \(MTHFR\) 677TT (OR 3.3, \(P < 0.01\)) and \(SHMT1\) 1420CC (OR 2.4; \(P < 0.01\)) was associated with headache and lethargy. \(ATIC\) 347GG (OR 3.0; \(P < 0.01\)) was associated with gastrointestinal side effects, while \(TSER^*2\) *2 (OR 5.4, \(P < 0.01\)) and \(SHMT1\) 1420CC (OR 3.2; \(P < 0.01\)) with alopecia. The toxicogenetic index \((MTHFR 677TT + SHMT 1420CC + TSER^*2\) *2 + \(ATIC\) 347GG) ranged from 0 to 3. An increase in each unit of the index resulted in a 1.9-fold increase in the likelihood of side effects \((P = 0.004\) [95% CI 1.1–3.1]). Thus, homozygosity for \(>1\) of these alleles appeared to confer a “toxicity genotype” to MTX.

- **AMPD1, ATIC, ITPA, MS, MTRR and MTHFDI**

In 205 patients with newly diagnosed RA, 5 polymorphisms in 5 genes coding for enzymes in the adenosine pathway \(AMPD1\) 34C>T, \(ATIC\) 347C>G, \(ITPA\) 94C>A, \(MS\) 2756A>G, and \(MTRR\) 66A>G were analyzed. Patients carrying the \(AMPD1\) 34T allele, \(ATIC\) 347CC, or \(ITPA\) 94CC were more likely to have a good clinical response, as defined by a DAS of \(\leq 2.4\) (OR [95% confidence interval] 2.1 [1.0–4.5], 2.5 [1.3–4.7] and 2.7 [1.1–8.1], respectively). The likelihood of a good clinical response was increased if patients possessed all 3 favorable genotypes (OR: 27.8, 95% CI 3.2–250). Regarding toxicity, only \(ATIC\) G allele carriers experienced a greater frequency of adverse events (OR 2.0, 95% CI 1.1–3.7). Thus this study showed a composite of SNPs in three adenosine pathway genes, \(AMPD1\), \(ATIC\) and \(ITPA\) conferred a favorable phenotype of good clinical response to
MTX treatment, while The ATIC C347G SNP was a marker for MTX toxicity. No association was observed between MTX response and polymorphisms in MS and MTRR genes.

Wessels et al. attempted to construct a clinical pharmacogenetic model to predict MTX efficacy in RA. Two hundred five patients with newly diagnosed RA and active disease were treated with MTX. Twenty-four baseline variables possibly influencing disease state and drug response were selected. In addition, 17 polymorphisms in 13 genes related to the MTX mechanism of action, purine and pyrimidine synthesis, were determined. Factors were compared between responders (defined as patients with a DAS <2.4 at 6 months) and nonresponders. In case of differences, a stepwise selection procedure identified the predictors for response. A clinical score was designed by simplifying regression coefficients of the independent variables. Cutoff levels were chosen based on the clinical score, and positive and negative response rates were calculated. An evaluation of the model was performed in a second group of patients. Among all the genetic and clinical variables examined, sex, rheumatoid factor and smoking status, the DAS, and 4 polymorphisms in the AMPD1, ATIC, ITPA, and MTHFD1 genes (AMPD1 34CC, ATIC 347G allele, ITPA 94A, and MTHFD1 1958AA) constituted the prediction model for MTX efficacy. This prediction model was transformed into a scoring system ranging from 0 to 11.5. Scores of <3.5 had a true positive response rate of 95%. Scores of ≥6 had a true negative response rate of 86%. Sixty percent of the patients were categorized as either responders or nonresponders, whereas 32% of the patients were categorized using a non-genetic model. Evaluation of the model in 38 additional patients with RA supported the results. Thus this pharmacogenetic model was successful in predicting MTX efficacy in the studied RA patients.

**TS, ATIC and RFC1**

A study by Dervieux et al examined the combined effects of the C347G SNP in ATIC, TS 5'-UTR 28-bp repeat polymorphism (TSER*2/*2), and the G80A polymorphism in RFC1 (a MTX transporter) in 108 patients with RA treated with MTX for >3 months. The results showed that the presence of at least 1 homozygous variant genotype (RFC1 80AA, ATIC 347GG, or TSER*2/*2) and/or a higher pharmacogenetic index was associated with a greater response to MTX (Visual Analog Scale [VAS] ≥ 2 cm; P = 0.001), as were increased MTPG levels. Patients with at least 1 homozygous variant were 3.7 times more
likely than patients without a homozygous variant to respond to MTX \( (P = 0.01 \ [95\% \ CI \ 1.7–9.1]) \). Thus, homozygosity for \( \geq 1 \) of these alleles appeared to confer a favorable “response genotype” to MTX\(^{25}\).

- **GGH, ATIC, MTHFR, MTR and MTRR**

A total of 48 MTX naïve patients were initiated on MTX and MTX efficacy (measured by DAS28) and toxicity were assessed prospectively. Red blood cell (RBC) MTX and folate PG levels and 9 polymorphisms in eight cellular pathway genes \( (RFC-1 \ G80A, \ GGH \ C \ 401T, \ ATIC \ C347G, \ MTHFR \ C677T \ and \ A1298C, \ MS \ A2756G, \ MTRR \ A66G, \ TSER \ *2/*2 \ and \ SHMT\( I \ C1420T) \) were assessed. Patients with less response to MTX as reflected by a poorer DAS28 score had lower RBC MTXPG levels \( (p<0.05) \) and higher RBC folate polyglutamates levels \( (p<0.05) \). A multivariate logistic regression analysis revealed that a lower likelihood of therapeutic response to MTX was associated with the \( MTHFR \ 677TT \) genotype (visit 4) \( (OR: 22.2, \ 95\% \ CI \ 1.2–42.2) \); conversely, a greater likelihood of therapeutic response was associated with the \( SHMT\( I \ 1420CC \) genotype \( (OR: 7.4, \ 95\% \ CI \ 1.0–56.4) \). Thus, the \( MTHFR \ 677TT \) and \( SHMT\( I \ 1420CC \) genotypes were associated with a poor response, and patients who were carriers of either or both genotypes (22 patients; 36% nonresponders) were more likely \( (OR: 5.6, \ 95\% \ CI \ 1.0–31.2) \) to have a poor response than those who had none of these genotypes (25 patients; 10% responders) \( (P =0.048) \). Other genotypes were not associated with MTX efficacy \( (P > 0.15) \) RBC MTXPG and folate PG levels were not significantly associated with the occurrence of MTX toxicity. Risk genotypes \( GGH \ 401CC, \ ATIC \ 347GG, \ MTHFR \ 1298AC/CC, \ MS \ 2756AA, \ and \ MTRR \ 66GG \) were associated with toxicity, mainly gastrointestinal and neurological. Other genotypes \( (TSER*2/*2, \ SHMT\( I \ 1420CC, \ RFC\( I \ 80AA, \ and \ MTHFR \ 677TT) \) did not contribute significantly to toxicity. These 5 risk genotypes were summed to create a toxicogenetic index for each patient. An increased toxicogenetic index was associated with an increased occurrence of MTX toxicity \( (p<0.001) \). Thus this study suggests that RBC MTX and folate PG levels influence MTX efficacy but not toxicity, which is influenced by certain risk genotypes\(^{208}\).

Several above studies have demonstrated SNPs of the genes regulating enzymes in the intracellular Methotrexate metabolic pathway\(^{226}\). Many of these gene polymorphisms are being linked to drug efficacy and safety but the evidence available is not yet conclusive\(^{212,227}\). Pharmacogenetic testing may help to optimize therapy\(^{228}\). Much data has emerged on MTX from the west and little is known about Indian population. The present study
evaluated the allelic and genotype frequencies of polymorphisms in genes coding for MTX metabolism in Indian (Asian) healthy controls and patients with RA. A total of 12 polymorphisms in 9 genes of MTX metabolism (including transporters) were studied. The study further explored the relationship of these polymorphisms in folate-MTX pathway with MTX response (efficacy and toxicity) in Indian patients suffering from RA.

"If it were not for the great variability among individuals medicine might as well be a science and not an art." The thoughts of Sir William Osler in 1892 reflect the view of medicine over the past 100 years. The role of physicians in making the necessary judgements about the medicines that they prescribe is often referred to as an art, reflecting the lack of objective data available to make decisions that are tailored to individual patients. Just over a hundred years later we are on the verge of being able to identify inherited differences between individuals which can predict each patient’s response to a medicine. This ability will have far reaching benefits in the discovery, development and delivery of medicines. Sir William Osler, if he were alive today, would be re-considering his view of medicine as an art not a science.

The present PhD work is a step ahead in the direction to prove that medicine is science and not an art.