LITERATURE REVIEW
Study of interethnic differences in gene frequency and its relation to drugs and their action is termed as *Pharmacanthropology*. Well-established differences exist between Caucasians and Orientals including the responsiveness to ethanol (involving both alcohol and aldehyde dehydrogenases), and also the frequency of slow and fast acetylators (N-arylamines determined by N-acetyltransferase). More recently cytochrome P450 mediated oxidative metabolism of a number of drugs has also been shown to differ between the ethnic groups\(^4\).

Traditionally, twin studies have been used to search for genetic influence on the disposition of drugs. In a second step, family studies have been used to define the mode of genetic transmission. Such studies follow the selection of subjects at basal or near basal conditions and do not usually take environmental influences into account. Studies in small groups from different ethnic populations have suggested geographic variations in the frequency of this phenotypes\(^12\).

**Pharmacogenetics**

Pharmacogenetics is the study of the influence of genetic factors on drug response and metabolism. If the knowledge of Pharmacogenetics is applied during drug dosing or drug selection, one can avoid adverse reactions or therapeutic failure and thus enhance therapeutic efficiency\(^11\).
The birth of pharmacogenetics was brought about following a number of landmark discoveries at the end of the 19th century. As a consequence of the rapidly developing field of organic chemistry, it became apparent that most drugs were eliminated from the body with a chemical composition which differed from that which was administered. It suggested that some 'internal factor' had transformed or metabolised the parent compound. Following the pioneering work of Gregor Mendel and the establishment of the rules of heredity in 1886, a number of independent reports suggested that the rules of heredity also governed the biotransformation of drugs and detoxification of foreign compounds. Central to this theme was the observations of Archibold Garrod, firstly on the physiology of human urinary pigments and secondly in the study of alkaptonuria.

It was 68 years ago (1932) that the first report of an inherited difference in response to a chemical (inability to taste phenylthiourea), demonstrated with Mendelian inheritance, was published. Although the molecular basis of this phenotype has never been studied, this report is regarded by most as the first example of a pharmacogenetic study. In 1957, Arno Motulsky proposed that 'inheritance might explain many individual difference in the efficacy of drugs and in the occurrence of adverse drug reactions'. In 1959, Friedrich Vogel first coined the term 'Pharmacogenetics' and defined it as the study of the role of genetics in drug response.
The vast majority of drugs are degraded via small number of metabolic pathways, mainly by microsomal enzymes localized in the liver and, to a minor extent, in the small intestine and kidney (Figure-1)\(^2\).

Nearly all lipophilic small molecules, including most drugs, which enter the body, must be metabolised to more polar products before they can be excreted. This
metabolic process, which is primarily catalysed by hepatic enzymes, consists of a sequence of enzymatic steps. This normally involves oxidation of the drug (phase-I metabolism) by the cytochrome P450-dependent monooxygenases, followed by conjugation involving sulphation, glucuronidation or acetylation (phase-II metabolism). A number of enzymes including the glutathione S-transferases, N-acetyl transferases and UDP-glucuronosyl transferases are involved in catalysing the phase-II reactions\(^2\). The recent and very rapid progress in the field of drug metabolism has allowed the identification of the major phase-I and phase-II enzymes responsible for the metabolic conversions\(^1\).

All enzymes involved in the metabolism of drugs are regulated by genes and gene products. Because of evolutionary and environmental factors, there is a remarkable degree of genetic variability built into the population. Thus, the genetic factor represents an important source for inter-individual variation in drug metabolism. Mutations in the gene for a drug-metabolizing enzyme result in enzyme defect with higher, lower, or no activity or may lead to a total absence of the enzyme. Therefore, it is not unusual to find a 10-50 fold difference in the rate of drug metabolism among patients\(^7\).
GENETIC POLYMORPHISM OF CYP2D6

Genetic polymorphism is defined as the inheritance of a trait controlled by a single genetic locus with two alleles, in which the least common allele has a frequency of about 1% or greater. Genetic polymorphism of drug-metabolizing enzymes gives rise to distinct subgroups in the population that differs in their ability to perform certain drug biotransformation reactions. Polymorphism are generated by mutations in the gene for drug-metabolizing enzymes, which cause decreased, increased or absent enzyme expression or activity by multiple molecular mechanism. One of the most extensively studied genetic polymorphisms known to influence drug metabolism and response is the debrisoquine type (CYP2D6) oxidation polymorphism. The discovery of CYP2D6 polymorphism created new interest in the role of pharmacogenetics in clinical pharmacology.

Genetic polymorphism has been linked to three classes of phenotypes based on the extent of drug metabolism. Extensive Metabolism (EM) of a drug is characteristic of the normal population; Poor Metabolism (PM) is associated with accumulation of specific drug substrates and is typically an autosomal recessive trait requiring mutation and/or deletion of both alleles for phenotypic expression; and ultra extensive metabolism (UEM) results in increased drug metabolism and is an autosomal dominant trait arising from gene amplification.
CYP

'CYP' is the abbreviation for cytochrome P-450, a subgroup of related enzymes or isoenzymes located in the endoplasmic reticulum and expressed mainly in the liver. It also presents in other organs, such as the intestine and the brain\textsuperscript{18}.

The cytochromes are conjugated proteins containing a ferroporffirin pigment. They were originally known as myohematins, a name coined by C.A. McMunn in 1886 to describe pigments obtained from animal tissues and with characteristic visible absorption spectra. McMunn's discovery did not receive the recognition it deserved until 1925, when Keilin confirmed their existence by spectroscopic means. Keilin made an extensive study of the myochematins and changed their name to cytochromes, after finding that they are distributed not only in animals but also in plants, yeast, and bacteria\textsuperscript{19}.

Keilin identified three cytochromes on the basis of their absorption spectra and named them cytochromes a, b, and c. Since then more cytochromes have been identified (about 30), and their designations are made according to which of the original 3 they resemble; thus a1, a2, a3, c1, c2, c3, etc\textsuperscript{19}. Cytochrome P450 enzyme are characterized by a maximum absorption wavelength of 450nm in the reduced state in the presence of carbon monoxide\textsuperscript{20}.
In mammals, most xenobiotics are metabolized via hepatic phase-1 metabolism by means of CYP monooxygenases. They catalyze the metabolism of a wide variety of exogenous chemicals including drugs, carcinogens, toxins and endogenous compounds such as steroids, fatty acids and prostaglandins. Thirty or more different forms of these haem thiolate proteins have been characterized in humans.

The P450 superfamily is composed of families and subfamilies of enzyme that are defined solely on the basis of their amino acid sequence similarities. With few exceptions, a P450 protein sequence from one family exhibits up to 40% resemblance to a P450 from another family. P450s within a single subfamily always share greater than 55% sequence similarity.

**Invention of CYP2D6 polymorphism:**

Between 1975 and 1977 two groups independently discovered the genetic deficiency of debrisoquine and sparteine metabolism. The discovery of the genetic polymorphism in the metabolism of the two prototype drugs was not the result of a planned strategy but rather of the incidental observation. A dramatic event in a pharmacokinetic study prompted the initial search for a specific metabolic defect: the investigator who was participating in a study on debrisoquine a sympatholytic antihypertensive drug, had a much more pronounced hypotensive response than his colleagues, collapsing from a sub therapeutic dose. This was found to be due to impaired 4-hydroxylation of debrisoquine.
Similarly in 1975, during the course of pharmacokinetic study of a slow release preparation of sparteine, two subjects developed side effects such as diplopia, blurred vision, dizziness and headache. When analyzing the plasma levels of sparteine in them the reason for the development of side effects become evident. Compared to all the other subjects studied, their plasma levels of sparteine were 3 to 4 times higher, although the same dose had been given to every subject.

Family and population studies uncovered a genetic polymorphism and later work established that the two independently discovered defects in drug oxidation co-segregated in Caucasians (PM for sparteine exhibit impaired debrisoquine metabolism and vice versa) and the term sparteine/debrisoquine polymorphism was coined. However there are apparent exemptions to this rule. For instance, in a study in Ghana, the ability of Ghanaians to oxidise sparteine was independent of their capacity for debrisoquine oxidation.

Nomenclature:
Guidelines on nomenclature for individual cytochrome P450 isoform have been internationally agreed upon and are regularly updated. Genes encoding the P450 enzyme are designated as CYP. Because of the diversity of the cytochrome family, a nomenclature system based on sequence identity has been developed to assist in unifying scientific efforts in this area and to provide a basis for nomenclature of
newly recognized members of this gene superfamily. For example, \textit{CYP2D6} is isoform 6 of subfamily D included in the CYP 2 family\textsuperscript{11}.

In the past, \textit{CYP2D6} allele have been named arbitrarily using a single letter after the gene name,\textsuperscript{24} but with increasing numbers of alleles being detected, this system is now inadequate. The general recommendation is that the gene and allele are separated by an asterisk. Specific alleles are named by Arabic numerals or a combination of Arabic numerals followed by a capitalized Latin letter. There are no space between gene, asterisk and allele and the entire gene-allele symbol is italicized (e.g. \textit{CYP2D6*1A})\textsuperscript{30,31}.

Since a number of \textit{CYP2D6} alleles share common key mutations but differ with respect to other base changes, they should be given the same Arabic number (denoting their allele group) and distinguish by capitalized Latin letters (denoting the allele sub groups). For example both \textit{CYP2D6*4A} and \textit{CYP2D6*4B} has same mutation but are differ by a single silent base substitution\textsuperscript{30}.

Extra copies of an allele (duplicated or amplified) may exist in tandem; for example, the \textit{CYP2D6*2N} allele contains \(N\) copies of \textit{CYP2D6*2}. Here \(N\) may be any number between 1 to 13. For two copies, the entire arrangement of alleles should be referred to as \textit{CYP2D6*2X2}. When duplication is not with the same sub-group, they are separated with a coma (e.g. \textit{CYP2D6*10B,10C})\textsuperscript{30}. 
A non-italicized form of the allele is used to name the protein with asterisk omitted and replaced by a single spacing e.g.: CYP2D6*1. Both alleles italicized and separated by slash to name the genotype designation \((CYP2D6*1/CYP2D6*4A)\)\(^{30,31}\).

This nomenclature system is used for other P450 alleles also like \(CYP2A6*1, CYP2C9*2, CYP2C19*2\) etc. A current list of \(CYP2D6\) alleles are given in Table-1 with the trivial name and the new nomenclature system. Descriptions of the alleles as well as the nomenclature and relevant references are continuously updated at the new web page (http://www.imm.ki.se/CYPalleles/).
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<td>M9</td>
<td>E410K</td>
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<td>M11</td>
<td>V7M; Q151E; R296C; S486T</td>
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<td>V136M; R296C; V338M; S486T</td>
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<td>R296C; E410K; S486T</td>
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<td>Mutations</td>
<td>Enzymes</td>
<td>Phenotype</td>
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<td>V11M; R296C; S486T</td>
<td>Decrease (d)</td>
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<td>CYP2D6Ch2</td>
<td>P34S; S486T</td>
<td>Decrease (b)</td>
<td></td>
</tr>
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<td>Frameshift</td>
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<tr>
<td>CYP2D6*39</td>
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<td></td>
<td>S486T</td>
<td>None</td>
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</table>

b, bufuralol; d, debrisoquine; dx, dextromethorphan; s, sparteine

§ = Source: Homepage of the human cytochrome P450 (CYP) allele nomenclature committee.
Editors: Ingelman-Sundberg M, Daly AK and Nebert DW.
Web manager: Dr. Mikael Oscarson
(Reproduced with the permission from the publishers, Annexure-2)
Molecular genetics:

**Figure-2 : Arrangement of CYP2D6 gene in chromosome 22**

![Diagram of CYP2D6 gene arrangement](image)

The CYP2D6 gene resides in the CYP2D6-8 clusters on chromosome 22 in association with the CYP2D7P and CYP2D8P pseudogenes. Defective alleles can be the result of gene deletion, gene conversions with related pseudogenes and single base mutations causing frameshift, missense, nonsense or splice-site mutations. The homozygous presence of such alleles leads to a total absence of active enzyme and an impaired ability to metabolise probe drugs specific for the drug-metabolizing enzyme. These subjects are classified as PM.

In addition to defective CYP genes, there are also alleles that cause diminished or altered drug metabolisms. This results in an enzyme product that exhibits an impaired folding capacity and therefore the expressions of functional enzyme is severely diminished. Among extensive metabolisers, heterozygotes (one functional gene) have higher medium metabolic efficacy than those who are homozygous for the wild-type allele (two functional genes), but with pronounced overlap.
Figure 3: Some major molecular mechanisms that can result in altered human drug metabolism\textsuperscript{16}.

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Another type of metabolism known as ultra rapid metabolism and is caused by occurrence of duplicated, multiduplicated or amplified \textit{CYP2D6} genes (Figure-3). At present, alleles with two, three, four, five and 13 gene copies in tandem have been reported\textsuperscript{16}. In a Swedish family, a father, a daughter and a son were shown to have 12 copies of a functional \textit{CYP2D6L} gene with one normal gene and showed extremely high \textit{CYP2D6} activity\textsuperscript{40}. 
The number of individuals carrying multiple CYP2D6 gene copies is highest in Ethiopia and Saudi Arabia, where up to one third of the population displays this phenotype (Figure-4)\textsuperscript{16}.

Although clear criteria have not been formed to structurally assess whether a compound will be metabolized by CYP2D6 or not, it is observed that most of the substrates and inhibitors of this enzyme have a basic nitrogen and are
oxidized at a site within 0.5-0.7nm of this basic nitrogen. It may also have a flat lipophilic region and functional groups which have capacity for electrostatic interactions or the ability to form hydrogen bonds\textsuperscript{41,42}.

Using homology modeling to predict the active site structure of CYP2D6, a specific aspartic acid residue (Asp 301) that lies within the active site of the protein and mediates interaction between protein and substrate has been identified. Analysis of a series of mutant CYP2D6 proteins, where residue 301 was altered by site-directed mutagenesis has confirmed that the retention of a negative charge at position 301 in CYP2D6 is an important determinant of the catalytic activity of the enzyme\textsuperscript{6}.

The enzyme even shows stereoselectivity. In extensive metabolisers, inactive R-metoprolol is metabolized faster than the active S-enantiomer whereas this metabolism is not stereoselective in poor metabolisers\textsuperscript{43}. Isoform selectivity of CYP2D6 is observed in mianserin metabolism also\textsuperscript{44}.

**P450 polymorphisms: evolution and adaptation**

The enzyme deficiency manifests only during drug exposure\textsuperscript{36}. It would therefore appear that CYP2D6 is non-essential. If it is true, then why do we have this gene?

It was proposed that CYP2D6 is a remnant of evolution that was once required for metabolism of plant toxins. It is well established that, plants are continuously
evolving biosynthetic pathways in order to synthesis secondary metabolites for their reproductive cycles and to defend themselves from animal and insect predators. As animals began to consume plants, the plants responded by evolving new genes to synthesis toxic metabolites. In order to protect themselves from these new plant toxins, animals adapted and evolved new drug-metabolising genes to cope with the ever-changing plants. In this respect, it is remarkable but not surprising that many currently used drugs derived from natural plant metabolites are metabolised by the P450 super family of enzyme. In the human genome, the number of human P450 genes has been estimated to range between 60 and 100, and about 20 different P450s have been shown to be involved in the metabolism of foreign compounds. Figure-5 illustrates the evolution of the human CYPI-CYP4 gene families in relation to the changes that have occurred in the environment\textsuperscript{16}.

Many of the substrates or inhibitors of CYP2D6 are derived from plant alkaloids (sparteine, codeine, quinidine etc.). However, when plants no longer existed as a major food source, a particular P450 is no longer needed and its gene could be eliminated by lack of selective pressure for its maintenance\textsuperscript{36}.
Figure 5: Phylogenetic tree of the human CYPI-CYP4 gene families in relation to evolutionary distance$^{16}$

(Reproduced with the permission from the publishers, Annexure-3)
It appears that in the past, in contrast to rodent development, three genes in the locus have been eliminated, two completely inactivated (CYP2D7P and CYP2D8P) and one (CYP2D6) partially inactivated. The fact that more than 40% of CYP2D6 alleles are defective indicates that the gene is in the process of vanishing from the gene pool and will eventually become a pseudogene like CYP2D8P or CYP2D7P. The latter two genes were undoubtedly also needed by early humans. It is likely that other P450s are also now nonessential to humans and show variability in human liver specimens.

However, the underlying questions of what is the origin of these polymorphic alleles and to what extent the interethnic differences in the distribution of these alleles are dependent on the environment remain to be conclusively answered.

In Ethiopia, however, a different evolution of this locus has taken place compared with that in Caucasians. Alleles containing multiple gene copies have been formed, indicating that the population recently has been exposed to a selection pressure. The highest selection pressure for this was of dietary origin, with plant toxins probably playing a major role. However, in Ethiopia, dietary adaptation by means of induction has not been possible because CYP2D6 is a non-inducible enzyme. Thus, the adaptation has instead occurred through recombination events creating alleles with multiple gene copies. Duplication can invade only if it provides a direct advantage to the organism.
Inhibition and induction of CYP2D6

Although individual metabolic capacity is determined mainly by genetic background, several internal and environmental factors might influence the activity of most of the CYP enzymes: for example, age, gender, certain diseases with hepatic involvement, smoking, nutrition and alcohol\(^4\).

Quinidine is the most potent inhibitor (ki=0.03) of \(CYP2D6\)\(^42\). Quinine, which is a diastereoisomer of quinidine, is several hundreds times less potent inhibitor than quinidine. However, quinidine is not a substrate of \(CYP2D6\)\(^3\). Single oral dose of 200mg-quinidine sulphate is adequate to convert most extensive metaboliser to the poor metaboliser\(^46\). Fluoxetine\(^47-49\), paroxetine\(^49\) and propofenon\(^50\) are also potent inhibitors of CYP2D6 with inhibition constant in the low nanomolar range. A list of inhibitors of \(CYP2D6\) is given in Table -2.
Table 2: Inhibitors of *CYP2D6*:

<table>
<thead>
<tr>
<th>Ajmalicine</th>
<th>Ajmaline</th>
<th>Amitriptyline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amesergide</td>
<td>Aprindine</td>
<td>Budipine</td>
</tr>
<tr>
<td>Bufuralol</td>
<td>Chloroquine</td>
<td>Chlorpromazine</td>
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<tr>
<td>Cimetidine</td>
<td>Cisthiothixene</td>
<td>Citalopram</td>
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<tr>
<td>Clomipramine</td>
<td>Clozapine</td>
<td>Desmethyliimipramine</td>
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<tr>
<td>Diphenhydramine</td>
<td>Flecainide</td>
<td>Fluoxamine</td>
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<td>Fluoxetine</td>
<td>Fluphenazine</td>
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<td>Levomepromazine</td>
<td>Methadone</td>
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<tr>
<td>Meclobemide</td>
<td>Olanzapine</td>
<td>Oxprenolol</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>Perazine</td>
<td>Perphenazine</td>
</tr>
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<td>Propranolol</td>
<td>Quinidine</td>
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</tr>
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<td>Reboxetine</td>
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<td>Sertraline</td>
<td>Terbinafine</td>
<td>Terfenadine</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>Ticlopidine</td>
<td>Venlafaxine</td>
</tr>
<tr>
<td>Yohimbine</td>
<td></td>
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Several studies have shown that the effect of liver disease on *CYP2D6* enzyme is not significant\(^{51-53}\). The activity of *CYP2D6* was investigated in a group of patients with mild or moderate liver disease and a group of healthy control subjects using debrisoquine. The disposition of debrisoquine and 4-hydroxy debrisoquine were comparable between the control subjects and the patients with liver disease\(^{51}\). In another study, the impairment in dextromethorphan oxidation by liver disease was much less than that caused by the genetic deficiency. As a result, the prevalence of
the poor metaboliser phenotype remained the same range in patients with cirrhosis (1.8%) and with moderately severe liver disease (2.0%) as in controls (3.9%).

Debrisoquine hydroxylation study during acute viral hepatitis showed that viral hepatitis has only a marginal effect on the activity of CYP2D6. The study suggests that, substrates of this enzyme may be given in normal therapeutic doses to patients with viral hepatitis.

Unlike many members of the CYP enzyme family, the CYP2D6 enzyme is not affected by classic enzyme inducers such as phenobarbitone. Rifampicin treatment has given only a 30% increase in clearance of sparteine, but metabolic ratio was not significantly changed. About 33% reduction in the metabolic ratio of debrisoquine has been observed with female EM using contraceptives.

Heavy cigarette smoking is shown to increase CYP2D6 activity. Straka et al. reported a significant association between metabolic ratio (MR) and smoking status in White subjects. However, the same study could not found an association between MR and smoking status in Hmong population. Other population studies also observed that smoking is not significantly altering the CYP2D6 activity.

It has been postulated that drug metabolising enzyme activity may be influenced by hormonal fluctuations. The clearance of R-propranolol, desipramine and
clomipramine\textsuperscript{62} have been found to be greater in men than women. However, these studies have been criticized for not normalizing substrate doses for bodyweight, or for not controlling confounding variables such as age\textsuperscript{59}.

A recent study using dextromethorphan observed that, there is no significant difference in the CYP2D6 activity of male and female extensive metabolisers\textsuperscript{63}. CYP2D6 activity does not change with the age\textsuperscript{64}. Several population studies also have been shown that age and sex difference is not influencing the CYP2D6 activity\textsuperscript{57, 58, 65, 66}. Therefore, until a larger, well-designed investigation is performed, the influence of age and sex on CYP2D6 activity cannot be established.

In one study the metabolic ratio of debrisoquine was assessed in different phases of menstrual cycle. A 25\% decrease in metabolic ratio during the luteal phase compared with the preovulatory phase was demonstrated\textsuperscript{67}. However, the study using dextromethorphan shows that, there is no difference in CYP2D6 activity in extensive metabolisers between the mid-follicular and mid-luteal phases of menstrual cycle activity\textsuperscript{63}. The study conclude that, if an effect exists at ovulation, it is most likely transient and would not be expected to significantly affect pharmacokinetic or clinical trial results.

In contrast, there is evidence that pregnancy has a profound influence on CYP2D6 activity. The oral clearance of metoprolol was 4 -5 fold more in pregnancy
compared with the clearance after 3–6 months after delivery among the same subject\textsuperscript{68}.

Marked increase in the metabolism of dextromethorphan also have been reported during pregnancy\textsuperscript{69}. During pregnancy, the dextromethorphan/dextrorphan metabolic ratio was significantly reduced (P=0.0015) among homozygous and heterozygous extensive metabolisers. In contrast, poor metabolisers showed an increased metabolic ratio during pregnancy. Both studies indicate an increase in \textit{CYP2D6} activity during pregnancy, which may be caused by an induction of the \textit{CYP2D6} enzyme. Ovariectomy also induces this enzyme activity but only to a minor extent\textsuperscript{70}.

Compared to other CYP enzymes, \textit{CYP2D6} activity is not significantly induced by any of the environmental or dietary factors. Moreover, as discussed before most of the classic inducing drugs also did not increase \textit{CYP2D6} activity. Hence \textit{CYP2D6} is generally considered as a non-inducible enzyme\textsuperscript{16}. 
Assessment of individual CYP2D6 activity

The activity of CYP2D6 enzyme can be assessed by means of phenotyping or genotyping\(^1\).

**Phenotyping**

Phenotyping requires intake of a probe drug; the metabolism of which is known to be solely dependent on CYP2D6 enzyme. The excretion of parent compound and/or metabolite in urine allows to calculate the metabolic ratio, which is a measure of individual CYP2D6 activity\(^{11,28}\). Discovery of a specific phenotyping probe, development of a reliable analytical assay, and external validation are the three major steps in developing a phenotyping procedure for a drug-metabolizing enzyme\(^{10}\).

In a typical phenotyping experiment, individuals are administered an oral dose of the probe drug usually at a subtherapeutic level, and urine is collected over a period of 8-12 hours. Total yield of parent compound and metabolites are determined and the parent compound/metabolite ratio, termed metabolic ratio (MR) is plotted as frequency distribution histogram. A polymorphism is indicated by bimodal frequency distribution curve with the antimode between the two populations. Antimode which separate the extensive metabolisers from poor metabolisers serve as a baseline to distinguish these two groups\(^{36}\). A probit plot\(^{71}\) or normal test variable (NTV) plot\(^{72}\) also can be used to express the bimodal distribution.
Different probe drugs are used for \textit{CYP2D6} phenotyping. Earlier phenotyping studies have been performed with debrisoquine and sparteine. Later dextromethorphan\textsuperscript{73}, metoprolol\textsuperscript{74} and codeine\textsuperscript{75} were also used for phenotyping \textit{CYP2D6} activity.

The antimodes of this bimodal distribution in Caucasians are about 20, 0.3 and 12.6 for sparteine\textsuperscript{11}, dextromethorphan\textsuperscript{11, 73} and debrisoquine\textsuperscript{11, 73}/metoprolol\textsuperscript{74} respectively. The metabolic ratio is a function of factors such as renal drug clearance as well as enzyme activity. Environmental factors may modify these variables, which may give rise to differences in the antimode of the MR between ethnic groups\textsuperscript{58}.

**Dextromethorphan**

Debrisoquine was initially administered to subjects or patients to determine metabolic phenotype. But it is not available in all countries, and it sometimes causes severe adverse effects (eg. orthostatic hypotension in poor metabolisers). Dextromethorphan is used increasingly as a substitute for debrisoquine, to assess the oxidative genetic polymorphism\textsuperscript{63}.

Dextromethorphan is the nonopioid dextrorotatory stereoisomer of the levorotatory narcotic methorphan (n-methyl-methoxymorphinan) pharmacophore group. Because of its potent cough suppressant activity, dextromethorphan was introduced in the
early 1950s and was widely accepted thereafter. Due to its lack of addictive and analgesic properties, the drug became available without prescription as a safe antitussive over-the-counter drug even for pediatric use. The potential importance of dextromethorphan metabolism in pharmacogenetic investigations became apparent when 12 subjects from a study of dextromethorphan pharmacokinetics became available for debrisoquine phenotyping. This phenotyping study showed that the dispositions of dextromethorphan and debrisoquine are closely related and hereditary factors also influence the dextromethorphan metabolism. To rule out the influence of CYP2C19 in the metabolism of dextromethorphan, the metabolic ratio of dextromethorphan also compared with the mephenytoin hydroxylation. However, there was no indication for co-inheritance between mephenytoin and dextromethorphan metabolism in man.73

Finally the hypothesis that, dextromethorphan might represent an alternative candidate for assigning the debrisoquine drug hydroxylation phenotype in normal subjects, was proved by a population study conducted by Schimid et al.73 The study conducted among 268 Swiss healthy volunteers proved that dextromethorphan is a suitable substitute for debrisoquine in population phenotypic studies. The observations further confirmed by pedigree analysis of 14 family studies.

A urinary metabolic ratio of 0.3 was used to delineate extensive metaboliser and poor metaboliser phenotypes. Once the metabolism of dextromethorphan was
established to genetically cosegregate with that of debrisoquine, it has been used widely to determine the metabolic phenotype of individuals.  

Figure 6: Metabolism of dextromethorphan.  

Dextromethorphan elimination depends mainly on extensive hepatic oxidative metabolism. The drug is mostly O-demethylated to dextorphan by the CYP2D6 enzyme and to a much lesser extent, N-demethylated to 3-methoxymorphinan by
the CYP3A enzyme. Both metabolites are subsequently converted to 3-
hydroxymorphinan by the CYP3A and CYP2D6 enzymes, respectively\(^6^9\) (Figure-6). These metabolites are then partially conjugated with sulphate and glucuronic acid. O-methylation, the rate limiting step in dextromethorphan metabolism and elimination, is subject to CYP2D6 polymorphism\(^5^2\).

After oral administration, more than 85% of the dose is excreted in urine within 24 hour as free and conjugated metabolites and as a very small proportion, unmetabolised dextromethorphan\(^5^2\). Most dextromethorphan CYP2D6 phenotyping studies use 30mg dose. This moderate dose may provide an accurate reflection of actual CYP2D6 activity. To find out the effect of dextromethorphan dosage on phenotyping, a dose dependency study was conducted\(^7^7\). A statistical difference was found for the molar ratio between the higher doses of dextromethorphan (0.8 mg/kg and 1.2 mg/kg) compared with the moderate doses (0.05 mg/kg to 0.3 mg/kg and 30mg dose). The study suggested that the use of moderate doses of dextromethorphan for phenotyping can avoid the dose dependency.

In most of the studies reported in literature, dextromethorphan metabolic phenotype was determined in urine samples that were treated with β-glucuronidase/sulfates before extraction and analysis. Blood\(^2^1\) and salivary\(^7^6,^7^8\) analysis also have been used for phenotyping studies. The determination of dextromethorphan phenotype by
analysis of salivary samples may be of value in patients for whom urinary samples cannot be collected, such as inpatients with end-stage renal disease. 

Although salivary analysis can identify subjects with the poor metaboliser phenotype in normal volunteers, it cannot replace urine collection for dextromethorphan metabolic phenotyping in subjects with normal renal function. This is because, salivary analysis requires a larger dose of dextromethorphan hydrobromide (50mg), which could cause more side effects. Moreover, technically, the salivary assay is more time-consuming and difficult than the urinary assay. Also, some people dislike being asked to collect saliva samples. All of these factors may preclude the widespread use of this method. The shorter time required for collection of samples (3 hours for saliva versus 8 hours for urine) might be important in some clinical setting. The serum analysis for dextromethorphan phenotyping has not been used widely. This may be due to the difficulty in getting consent from the volunteers and the technical difficulty for the detection of low concentration of the compounds in the serum.

Dextromethorphan can be used by pregnant and breast-feeding mothers. It also meets some of the essential prerequisites for a phenotype test drug, viz. specificity, general availability, and innocuousness. Several methods including gas chromatography (GC), high performance liquid chromatography (HPLC), radioimmunoassay (RIA), and enzyme immunoassays (ELISA) have been
developed for the analysis for dextromethorphan. The availability of several analytic methods in combination with the safety and general availability of dextromethorphan renders this drug attractive for worldwide pharmacogenetic investigations. However metabolism of this drug proceeds simultaneously via other enzyme such as CYP3A4 and results should there be interpreted with some caution.

**Pittsburgh cocktail**

The current problems with most phenotyping procedures for drug-metabolizing enzymes are:

- Poor specificity and/or insufficient validation of many probe drugs and phenotyping assays.
- Invasiveness, several point sampling and necessity of elaborate technical equipment.
- High sensitivity of procedures to noncompliance on behalf of the subjects
- Unknown clinical relevance of phenotyping.

For reasons of practicability, only simple, reproducible and non-invasive procedures can be routinely used in clinical and epidemiological studies. If more than one enzyme is to be characterized, a cocktail approach appears to be the less costly procedure. It implies the simultaneous administration of two or more model drugs phenotyping different enzymes. Thus, it allows the prediction of multiple enzyme activities in a single session. This approach is of clinical importance since many
medications are eliminated via more than one metabolic pathway (e.g. dapsone, dexamethasone) or are a substrate for one enzyme and an inhibitor/or inducer of another (e.g. quinidine, omeprazole). Furthermore, an increasing number of patients are treated with multi-drug regimens, which involve more than one enzyme in their metabolism. Presently, the ‘Pittsburgh cocktail’ is best validated and includes caffeine, mephenytoin, debrisoquine, chlorzoxazone and dapsone for CYP1A2, CYP2C19, CYP2D6, CYP2E1 and CYP3A phenotyping respectively.

Metabolic interactions among administered probes and the potential for analytical interference among parent drugs and metabolites represent major limitations of the cocktail approach and this could be overcome by careful selection of both model drugs, administered doses and sampling procedures.

Genotyping

Genotyping involves identification of defined genetic mutation that give rise to the specific drug metabolism phenotype. These mutations include genetic alterations that leads to overexpression (gene amplification), absence of an active protein product (null allele), or production of a mutant protein with diminished catalytic capacity (inactivating allele).

DNA isolated from peripheral lymphocytes can be used for genotyping. Two commonly used methods in genotyping are PCR-RFLP method and allele-specific
PCR. In the former technique, specific region of the gene of interest is amplified by PCP followed by digestion of the amplified DNA product with restriction endonucleases. The size of the digestion products is easily evaluated by agarose gel electrophoresis with ethidium bromide staining and UV transillumination.

In allele specific PCR amplification, oligonucleotides specific for hybridizing with the common or variant alleles are used for parallel amplification reactions. Analysis for the presence or absence of the appropriate amplified product is accomplished by agarose gel electrophoresis.

The number of known defective alleles is growing and a total of more than 30 different defective CYP2D6 and 55 CYP2D6 variations have been identified (Table-1). Some of the well characterized null alleles, containing single amino acid changes, are associated with altered phenotype. Many CYP2D6 alleles, however, do not yet have a clearly defined phenotype. Inheritance of these rare CYP2D6 alleles does not at present clearly defines a susceptible group and their usefulness in predicting therapeutic response therefore remains uncertain. However, it appears that depending on the ethnic group, genotyping for only 5-6 most common defective alleles will predict the CYP2D6 phenotype with about 95-99% certainty. For example, the most common CYP2D6 variant alleles in the Caucasian, Chinese/Japanese and Black African/Afro-American population are CYP2D6*4, *10 and *17 respectively.
**Phenotyping vs. Genotyping**

Phenotype is the visual expression of genotype\(^2\). The genotyping methods require small amount of blood or tissue. It is not affected by underlying diseases or drugs taken by the patient and provide results within 48-72 hours, allowing for rapid intervention\(^\text{11}\). Phenotyping has several drawbacks. It is hampered by complicated protocol of testing, risks of adverse drug reactions, problem with incorrect phenotype assignment due to co-administration of drugs and confounding effect of disease\(^\text{11}\). This approach may be hampered in patients who concomitantly receive drugs that are metabolized by *CYP2D6* and/or inhibit this enzyme. As a consequence metabolite formation of the probe drug may be reduced despite a normal enzyme activity and the metabolic ratio in urine would indicate a poor metaboliser. Such apparent transformation of an EM-phenotype to a PM-phenotype is termed as phenocopying\(^2,93\).

However, phenotyping is the only approach to evaluate enzyme function. If post-translational variation contributes to the individual *CYP2D6* activity then phenotyping will be the only way to identify such phenomena\(^\text{28}\). In other words, phenotyping is a measure of true individual metabolizing capacity at the time of study. Genotyping analysis, in contrast, gives an unequivocal genetically-based prediction of individual drug metabolism, but does not allow for the effects of liver function, exposure to environmental chemicals, alcohol consumption or a range of other factors that may influence enzyme activity. For genotyping analysis to be a
truly accurate predictor of individual drug-metabolizing ability, it is necessary to identify all the variant alleles within the population to be studied and to understand the phenotypic consequences of inheriting each of them. Otherwise certain allelic variants may be erroneously classified as wild-type, making it more difficult to rationalize idiosyncratic responses to drug treatment. Moreover, phenotyping is useful in revealing drug-drug interactions or defect in overall process of drug metabolism.

Ethnic aspects.

Different populations are characterized by their racial backgrounds and their exposures to different environments. Therefore it is not surprising to find interethnic differences in drug metabolism. Four major ethnic regions have been defined viz., Caucasian, Asian, Black and Australian Aborigines. Criteria such as geography (distance reduces an exchange of genes), anthropology (similarity of physical appearance), languages (relations between 4736 human languages) and genetic analysis [blood groups, mitochondrial DNA (mt-DNA), gene polymorphisms] have been employed to establish population groups and determine how distant or close they are to each other. Once a closed relatedness between two populations has been established according to these criteria, the question arises whether the genes encoding drug-metabolizing enzymes have evolved in a similar way to result in similar phenotype profiles.
Apart from genetic factors, the influence of environmental factors like food, natural medicines or environmental xenobiotics may also play a role in this difference. The activity of many hepatic enzymes can be inhibited or induced by a variety of substances. One way to distinguish between genetic and environmental factors is to undertake 'parallel' pharmacokinetic studies. It is well known that for a number of drugs, the daily dose prescribed in Japan is lower than in the US and Europe. Discrepancies between Europe and the US were also observed. The previous observations show that, for neuroleptics, the US dose was often higher than in Europe.

Racial and ethnic studies of drug metabolism have shown substantial inter-population differences in the polymorphic distribution of CYP2D6 activity and corresponding genetic materials. The prevalence of PM and UEM in different ethnic groups is shown in Tables 3 and 4. This polymorphism has been extensively studied in Caucasians and Orientals with results consistently showing a prevalence of PMs of 5-10% in Caucasians (Europeans and white North Americans) and 1% in Orientals (Chinese, Japanese and Koreans). In these populations, there is a high correlation of metabolic ratios with different probe drugs for CYP2D6. The studies, which compared Oriental population with Caucasians, showed an interethnic difference in the metabolism of CYP2D6 substrates.
However, studies in African populations have yielded inconsistent results with prevalence of PMs ranging from 0-19%\(^7\). There seems to be a regional variation among African population. The wide variation in the CYP2D6 phenotype in black Africans suggest that the black populations are not genetically homogeneous as is often assumed\(^62\). Moreover, in some African populations, there is a lack of metabolic co-segregation of different CYP2D6 probe drugs\(^7,98\).

Another major interethnic difference is a shift in the metabolic ratio distribution to higher values in Chinese populations as compared with Whites. Although the PM frequency is significantly lower, the mean CYP2D6 activity is also lower in Orientals than in Caucasians. Many Orientals consequently have a reduced ability to metabolize antidepressant and neuroleptic drugs that are substrates for CYP2D6\(^6\).

The molecular basis of this difference has been attributed to the relatively low frequency (<0.5%) of the CYP2D6*3 and CYP2D6*4 alleles in Oriental populations, which are the most abundant gene-inactivating allelic variants in Caucasians. In contrast, the most abundant CYP2D6 allele in Orientals, comprising 51% of Chinese alleles, is CYP2D6*10B, which contain a Pro→Ser change at codone-34. This substitution influence the protein stability and results in a form of CYP2D6 with reduced catalytic activity\(^99\). CYP2D6*10B is not present in Caucasian population. A further allele, CYP2D6*10A has been described in the Japanese population, which is closely related to CYP2D6*10B\(^100\).
The ultra extensive metabolisers has been reported with prevalence of 1.5-29% in different ethnic groups. The frequency of the \textit{CYP2D6} gene duplication was found to be 2-3% among most European populations and a proportion of 12% in Turkish subjects. The carriers of gene duplication in Saudi Arabia\textsuperscript{101} and Ethiopia\textsuperscript{102} are 21% and 29% respectively (Figure-4). The mechanism behind this high proportion of UEM awaits further elucidation.

In India, an earlier unpublished result cited by Idle and Smith\textsuperscript{103} reported a 2% poor metabolisers of debrisoquine in 147 Indians residing in Bombay (presently Mumbai, Western India). Another study in a Sinhalese population in Sri Lanka (immigrants from the Indian mainland) showed no poor metabolisers with respect to debrisoquine hydroxylation\textsuperscript{104}. A much more recent study with dextromethorphan showed a frequency of 3% PM in a North Indian population\textsuperscript{8}. A similar study also has been reported with Hyderabad city population from South India and the PM frequency observed was 3.2 \%.\textsuperscript{9} Though the incidence of PM was similar between North Indian and South Indian population, the mean metabolic ratio of South Indian EM (0.02) population was much lower than the North Indian EM (0.088) subjects. However, the genetic basis of this marked difference is not known.

DNA marker studies reported that Indian and European populations have common Caucasoid ancestor and are genetically distinct from those of Oriental population\textsuperscript{105}. However, the studies of \textit{CYP2D6} activity in India shows that the Indian population
is a separate group with the enzyme activity in-between the Caucasian and Oriental subjects. The study of CYP2C19 polymorphism in North Indian subjects (11%) also indicated that cytochrome P-450 activity in Indian population is different from other ethnic groups. However, very less information is available about the genetic analysis of CYP2D6 gene in Indian population. Since UEM cannot be determined by only phenotyping, the prevalence of UEM in Indian population is not available.

It is difficult to predict population-specific patterns in drug-metabolising enzyme genotypes even for closely related populations. Therefore, data from one population should only be projected with caution to other populations. Also, since most investigations focus on subjects of defined ethnic origins, pharmacogenetic information concerning populations subject to ethnic admixture is generally not available.
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db=debrisoquine, sp=sparteine, dm=dextromethorphan, mp=metoprolol, cd=codeine.
Table 4: Frequencies of CYP2D6 poor metabolisers and ultra extensive metabolisers in different ethnic groups (genotyping).

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PM = Poor metabolisers  
M x N = Ultra extensive metabolisers (Gene duplication)
Clinical Significance

Polymorphic drug oxidation

If sparteine and debrisoquine being the only drugs affected by CYP2D6, the discovery of this polymorphism in drug oxidation would have been of rather theoretical interest because both these drugs cannot be regarded as essential drugs. However, further studies identified a variety of structurally different compounds which are metabolized by the CYP2D6 enzyme. A current list of CYP2D6 substrates is given in Table -5.

There are enormous individuality in drug response. Side effects with the prescribed drug therapy is also very common. Although most of the side effects are usually not life threatening, it can be unpleasant and debilitating to the patient².

Although CYP2D6 is only a relatively minor form in human liver (1.5% of total cytochrome-P450 isoforms), it metabolizes upto one quarter of all prescribed drugs (Figures 7and 8). This may be because many of the drugs metabolized by CYP2D6 are targeted to the central nervous system². The initial observation which led to discovery of the CYP2D6 polymorphism was that of exaggerated response following intake of regular doses. Thus it was the general opinion that patients with a genetic defect in this enzyme have a high risk of side effects following administration of a polymorphically oxidized drug.
Figure 7: Relative abundance of P450 isoenzymes in human liver\(^2\).

*(Reproduced with the permission from the publishers, Annexure-1)*

![Pie chart showing the relative abundance of P450 isoenzymes in human liver.]

- CYP1A2: 12.7%
- CYP2D6: 1.5%
- CYP3A: 28.8%
- CYP2A6: 4.0%
- CYP2E1: 7.0%
- CYP2C: 18.2%
- Others: 27.6%
- CYP2B6: 0.2%

Figure 8: Relative contribution of P450 isoenzymes to drug metabolism\(^2\).

*(Reproduced with the permission from the publishers, Annexure-1)*

![Pie chart showing the relative contribution of P450 isoenzymes to drug metabolism.]

- CYP1A2: 19%
- CYP2D6: 24%
- CYP2C: 19%
- CYP3A: 51%
- CYP2E1: 1%
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**Antihypertensives**

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</tr>
<tr>
<td>Debrisoquine</td>
<td>Guanoxan</td>
<td>Indoramine</td>
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<tr>
<td>Losartan</td>
<td>Metoprolol</td>
<td>Nimodipine</td>
</tr>
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<td>Nitrendipine</td>
<td>Oxyphenolol</td>
<td>Propranolol</td>
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<td>Timolol</td>
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**Antiarrhythmics**

<table>
<thead>
<tr>
<th>Amiodarone</th>
<th>Aprindine</th>
<th>Encainide</th>
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<tbody>
<tr>
<td>Flecaainide</td>
<td>Mexiletine</td>
<td>Procainamide</td>
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<tr>
<td>N-propylajmaline</td>
<td>Propafenone</td>
<td>Sparteine</td>
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**Antidepressants**

<table>
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<tr>
<th>Amiflavine</th>
<th>Amitriptyline</th>
<th>Brofaromine</th>
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<tr>
<td>Citalopram</td>
<td>Clomipramine</td>
<td>Desmethylcitalopram</td>
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<td>Desipramine</td>
<td>Fluvoxamine</td>
<td>Fluoxetine</td>
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<td>Imipramine</td>
<td>Maprotiline</td>
<td>Minaprine</td>
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<td>Moclobemide</td>
<td>Nefazodone</td>
<td>Nortriptyline</td>
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<tr>
<td>Paroxetine</td>
<td>Tomoxetine</td>
<td>Tranylcypromine</td>
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<td>Trimipramine</td>
<td>Venlafaxine</td>
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**Neuroleptics**

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<tr>
<th>Clozapine</th>
<th>Haloperidol</th>
<th>Levomepromazine</th>
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<td>Olanzapine</td>
<td>Perphenazine</td>
<td>Pimozide</td>
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<tr>
<td>Risperidone</td>
<td>Sertindole</td>
<td>Thioridazine</td>
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**Opiates**

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<th>Dihydrocodeine</th>
<th>Dextromethorphan</th>
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<td>Ethylmorphine</td>
<td>Hydrocodone</td>
<td>Norcodeine</td>
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<td>oxycodone</td>
<td>Tramadol</td>
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**Chemotherapeutic agents**

<table>
<thead>
<tr>
<th>Clotrimazole</th>
<th>Doxorubicin</th>
<th>Ketoconazole</th>
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<tr>
<td>Mefloquine</td>
<td>Pyrimethamine</td>
<td>Rifampicin</td>
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<tr>
<td>Ritonavir</td>
<td>Roxithromycin</td>
<td>Sulfasalazine</td>
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**Antihistamine**

<table>
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<tr>
<th>Azelastine</th>
<th>Cinnarizine</th>
<th>Loratadine</th>
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<td>Promethazine</td>
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**Miscellaneous**

<table>
<thead>
<tr>
<th>Apigenine</th>
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<th>Chloral Hydrate</th>
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<td>Cyclobenzaprine</td>
<td>Dexfenfluramine</td>
<td>Dibucaine</td>
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<td>Dihydroergotamine</td>
<td>Dolansetron</td>
<td>Ethinyloestradiol</td>
</tr>
<tr>
<td>Fenoterol</td>
<td>Formoterol</td>
<td>4-hydroxyamphetamine</td>
</tr>
<tr>
<td>Laudampsome</td>
<td>MDMA (ecstasy)</td>
<td>Methoxamine HCl</td>
</tr>
<tr>
<td>Methoxyamphetamine</td>
<td>Methoxyphenamine</td>
<td>Methoxypsoralen</td>
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<td>Metoclopramide</td>
<td>MPTP</td>
<td>Nicergoline</td>
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<td>Ondansetron</td>
<td>Perhexilene</td>
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<td>Quercitin</td>
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</tr>
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<td>Tacrine</td>
<td>Tamoxifen</td>
<td>Tolterodine</td>
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<tr>
<td>Tropisetron</td>
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There are in fact a variety of drugs, which cause a higher incidence of side effects following administration of a polymorphically oxidised effect in poor metaboliser when compared to the remainder of the population. However, it is readily understandable that the assumption of a uniform consequence of polymorphic oxidation does not reflect reality since not only the lack of CYP2D6 in poor metabolisers but also unusual high expression as a result of gene amplification may influence drug response. In this case, regular doses would result in subtherapeutic plasma concentrations and extreme doses would be required to achieve and maintain effective concentrations.\textsuperscript{28} Hence, the PM trait is characterized clinically by an impressive deficiency in forming the relevant metabolite(s) of affected substrate, which can result in either drug toxicity or inefficacy. The reverse in case of UEM\textsuperscript{11}.

Brosen and Gram\textsuperscript{154} suggested that clinical significance of polymorphism can be evaluated by asking the following questions: Does the kinetics of active principle of a drug depend significantly on a specific enzyme? Is the resulting pharmacokinetic variability has any clinical importance? Can the variation in response be assessed by direct clinical or paraclinical measurement? On the basis of these criteria, significance exists for those drugs for which plasma concentration measurement are considered useful and for which the elimination of the drug and/or its active metabolite is mainly determined by CYP2D6 enzyme.
Thus, the extent to which lack of CYP2D6 expression is of clinical significance is largely determined by the width of the therapeutic window of the drug in question, i.e. the range of concentrations between the minimum dose required to achieve the desired therapeutic effect and that at which toxicity occurs. For example, the therapeutic window is relatively wide for β-blockers such as metoprolol and timolol but is much narrower for the antiarrhythmic flecainide and propafenone.

The clinical relevance of polymorphism at the CYP2D6 gene locus is also determined by the extent to which CYP2D6 is the major route of metabolism of the compound of interest. For example, perhexiline is almost uniquely metabolised by CYP2D6 and as a consequence, the therapeutic usefulness of the drug in poor metabolisers is severely compromised by the associated side-effects. In contrast, propranolol can be metabolised by other P450 enzymes and is also excreted at relatively high concentrations as unchanged drug; variation in CYP2D6 expression is therefore relatively unimportant.

The polymorphism of CYP2D6 is clinically more significant for tricyclic antidepressants, certain neuroleptics, antiarrhythmics, antihypertensives, β-blockers and morphine derivatives. For tricyclic antidepressants, both the PM and UEM phenotypes of CYP2D6 are at risk of adverse reactions. PM individuals given standard doses of these drugs will develop toxic plasma concentrations, potentially leading to unpleasant side effects including dry mouth, hypotension, sedation and
tremor or in some cases life threatening cardiotoxicity. For example, it has been reported that identical dosing regimen treatment of imipramine in EM and PM patients showed the absolute concentrations of both the parent drug (imipramine) as well as its desmethyl metabolite (desipramine) are greater in PM individuals, resulting in reduced ratio of parent drug to metabolite in them. Here the N-methylation of imipramine to its pharmacologically active desmethyl metabolite desipramine is catalyzed primarily by CYP2D6, and CYP1A2, where as the 2-hydroxylation of desipramine to its pharmacologically inactive metabolites is catalyzed by CYP2D6.

Administration of CYP2D6 substrates to UEM individual may result in therapeutic failure because plasma concentrations of active drug at standard doses will be far too low. The clinical presentation of UEM and PM patients are at times similar, leading to confusion in understanding the basis of adverse drug reaction. Because of lack of individualization of drug dosage, patients may be subjected to recurrent depressive episodes and may not respond to treatment.

Patients requiring treatment with antidepressant or antipsychotic substrates of CYP2D6 may begin the normal treatment regimen. Because of the long half-life of these drugs, toxic drug concentrations may take 5-7 weeks to develop. Therefore, it is suggested that the patients should be phenotyped before starting the treatment with drugs, which are metabolized mainly by CYP2D6 enzyme. A recent US study
showed that, in patients prescribed with psychiatric drugs that are CYP2D6 substrates, adverse drug reactions were observed in every patients with inherited mutations inactivating the CYP2D6 gene\textsuperscript{159}.

A lack of CYP2D6 enzyme would be expected to result in reduced drug response where prodrugs requiring activation by CYP2D6 are used. For example, the analgesic effect of tramadol is severely reduced in PMs\textsuperscript{160}. Similarly, following administration of the prodrug codeine, the morphine could not be detected in the plasma of CYP2D6 PMs\textsuperscript{161-163}. On the contrary, severe abdominal pain, a typical adverse effect of morphine, was observed in all UEM treated with codeine\textsuperscript{16}. Moreover, the oro-caecal transit time prolongation of codeine was observed only with EM subjects\textsuperscript{164}.

Several drugs that are substrates for CYP2D6 can also be metabolised by other P450 isoenzymes, often leading to the generation of alternative metabolites. These secondary routes of metabolism may become increasingly important in CYP2D6 PMs and may necessitate alteration of the prescribed dose and/or monitoring for the formation of novel or undesirable metabolites\textsuperscript{6}. 
Drug Interactions

The response of a patient to drug therapy and its side effects are determined by many mechanisms. Some of these are directly related to metabolism or arise as a consequence of drug/drug interactions when several drugs are prescribed concomitantly. Adverse drug reactions due to drug/drug interactions are clearly avoidable². Due to the high polymorphic character of CYP2D6, this enzyme is also the site of a number of drug interactions in vivo, which are of clinical significance. Substrates with a high affinity for the enzyme bind strongly to it and inhibit the metabolism of other compounds which have lower affinity. Consequently drug interaction occur in extensive as well as poor metabolisers⁹. By using this knowledge, pharmacokinetic interactions can be anticipated as follows:

If drug A affect P450 enzyme X
And if P450 enzyme X metabolises drugs B, C and D,
Then drug A may affect the metabolism of drug B, C and D.

This type of knowledge is also being used to decide about drug development, because the inhibition of P450 enzyme is generally result in drug interactions¹⁶⁵.

The interaction of two substrates for CYP2D6 can result in a number of clinical responses. The first pass metabolism of the substrate may be inhibited or the rate of elimination may be prolonged such that higher plasma concentration and associated pharmacodynamic responses may occur²¹, ⁹³, ¹⁶⁶-¹⁷⁰.
Inhibition of metabolism by CYP2D6 can also lead to a lack of therapeutic response when the pharmacological action is dependent on the active metabolite. Since CYP2D6 is not normally inducible by enzyme inducing drugs, drug interactions due to enzyme induction are very unlikely to occur.

The understanding of drug-drug interactions is important because of the widespread practice of polypharmacy. Primary care physicians use polypharmacy because they treat patients suffering from more than one distinct illness. Psychiatrists frequently resort to polypharmacy in part because they deal with patients who have more complicated presentations (i.e., those with 'comorbid' syndromes) or those who have an incomplete response to monodrug therapy.

Today, studying the effects of drugs on cytochrome P450 enzymes is a part of rational drug development. One goal of such development is to minimise the risk of drug-drug interactions: either pharmacodynamic or pharmacokinetic. These two types of interactions often have different clinical presentations. In pharmacodynamic drug interactions, the effect of one drug on its site of action magnifies or diminishes the response to the effect of the second drug on its site of action. The result can be a fundamental and sometimes dramatic change in the nature of the patient's response. In a pharmacokinetic interaction, one drug affects the pharmacokinetics of another drug (i.e., its absorption, distribution, metabolism,
or elimination). The result is generally comparable to what would occur if the dose of the second drug was changed. Thus, pharmacokinetic-interactions in contrast to pharmacodynamic-interactions are more likely to present as a quantitative (i.e., enhanced or reduced) rather than a qualitative change in the nature of the patient’s response. Unless the physician is aware of this possibility, he may not be considered this interaction\textsuperscript{165}.

Advances in our knowledge of cytochrome P450 enzymes have revolutionized our ability to understand, anticipate, and minimize the consequences of pharmacokinetic drug interactions. Previously, physicians had to memorize all known interactions, which had little apparent reason. There was also a vague understanding that some drugs could induce or inhibit the metabolism of other drugs. However, these ‘inducers’ or ‘inhibitors’ did not affect all drugs but, instead, only a few. Now it is possible to develop two sets of complementary knowledge: 1) specific drugs that induce or inhibit specific P450 enzymes and 2) specific drugs that are metabolized by specific P450 enzymes\textsuperscript{165}.
Pathophysiological aspects of CYP2D6 polymorphism

Interindividual variation in the expression of CYP2D6 may be one of the factors that determines individual disease susceptibility. Involvement of CYP2D6 and its variant alleles in the pathogenesis of certain diseases (either by activating xenobiotics or by involvement in neurotransmitter metabolism) is an interesting and yet unsettled area of research.

CYP2D6 polymorphism has been linked to susceptibility to various diseases including certain cancers, early onset of Parkinson’s disease, systemic lupus erythematosus, pituitary adenomas, Balkon nephropathy and ankylosing spondylitis³, ³５. ¹⁷¹-¹⁷⁴.

The CYP2D6 gene is responsible for the metabolism of known human carcinogens, including nitrosamines and, possibly, nicotine (Figure-9). In addition it is suggested that there may be endogenous substrates for CYP2D6, including tryptamine, a well-known neuroactive amine¹⁷³. The possibility that this enzyme may be involved in hormone homeostasis or other metabolic process also cannot be excluded⁶.

Metabolic activation of a procarcinogen may proceed via CYP2D6 which implies that a patient of extensive metaboliser phenotype forms higher amounts of the active compounds and therefore at a higher risk to develop cancer²⁸, ¹⁷⁵.
Figure 9: Initiating event in the most known environmental carcinogens\textsuperscript{175}. (Reproduced with the permission from the publishers, Annexure-1).

However, the influence of \textit{CYP2D6} allelic variance in different types of cancer is a controversy. When some studies suggested a role for \textit{CYP2D6} in the development of cancer, several studies could not support this\textsuperscript{175, 176}.

This difficulty for a conclusion is particularly well illustrated by the results of studies investigating the frequency of \textit{CYP2D6} PMs in lung cancer. The \textit{CYP2D6}
PM phenotype was first reported to be protective in lung cancer. However, further studies identified inheritance of the CYP2D6 PM phenotype as a statistically significant risk factor while others reported only a very weak association. Such marked variation in the PM frequencies in both cases and controls in the different studies makes the conclusion a difficult one\textsuperscript{177}. The variation could be attributable to a range of factors, including the methods used to assign CYP2D6 phenotype and genotype. Selection of the control population may also be an important issue, where the absence of age and sex matching in control populations could be the confounding factor in some of the published studies. However, there is no evidence that this is an important variable in the case of CYP2D6. Even then, if the control and disease populations are not age matched, the possibility of some control developing the disease at a later date cannot be ruled out\textsuperscript{6}.

Although smoking is the primary cause of lung cancer, not all smokers develop the disease. Other factors that determine individual disease susceptibility include the number of cigarettes smoked, the type of tobacco used, and exposure to other synergistic risk factors, such as alcohol. These factors can vary widely in populations of different ethnic origin and may therefore influence the relative importance of polymorphism in CYP2D6. Many CYP2D6 are present at different frequencies in different populations and it is therefore important to know the ethnic origin of all participants in studies\textsuperscript{6}. 
CYP2D6 PM frequencies have also been investigated in a variety of other cancer types, including breast cancer \(^{178-80}\), bladder cancer \(^{181-83}\), pituitary adenomas \(^{184}\) and malignant brain tumor \(^{173}\). In comparison to the lung cancer data, however, relatively few studies have reported any statistically significant associations between CYP2D6 activity and susceptibility to these diseases \(^{6,177}\).

A variety of studies investigated a possible link of Parkinsonism to CYP2D6 expression \(^{185-188}\). Other studies however, failed to show any relation of CYP2D6 activity and Parkinsonism \(^{189,190}\). These trials have been performed in different ethnic groups; since P450 gene structures show interethnic group differences, comparison of these experiments and extrapolation for one ethnic group to another appears to be rather questionable \(^{28}\).

Thus determination of these genetic polymorphism may be of clinical value in predicting adverse or inadequate response to certain therapeutic agents and in predicting increased risk of environmental or occupational exposure-linked disease. The genotyping/phenotyping will lead to increased therapeutic efficacy, improved patient outcome and thus more cost-effective medication \(^{11,191,192}\).
Molecular genetics in clinical laboratory

Because of pronounced individual differences in drug disposition, optimal therapy requires drug administration according to each patient’s needs. Drug metabolising enzymes are mainly responsible for the interpatient differences in drug pharmacokinetics. Since in most cases there is no information available on the individual ability to metabolize a given drug, empirical standard doses are administered which are presumed to represent an average optimal dose in the population. Thus, interindividual diversity may only be taken into account, retrospectively, via feed back from the results of treatment.

While therapeutic efficacy and incidence of adverse events are the clinical data helpful in individualizing drug dosing in patients, therapeutic drug monitoring is a direct method to determine individual pharmacokinetic characteristics. However therapeutic drug monitoring is done only for few medications with a narrow therapeutic range. Therefore, additional information that would reliably predict individual drug disposition is highly desirable. Two approaches are available for this purpose. The first one is to obtain quantitative information on the effect of covariants on drug metabolism e.g. genotyping. The second approach is the direct measurement of the activities of drug-metabolising enzymes, i.e. phenotyping.
The application of genetic testing in the health service remains a difficult and emotive issue. Pharmacogenetics does identify an area where genetic screening as applied to drug use, may provide significant benefits to the patient and could also be of enormous economic importance. This leads to the somewhat futuristic view of tailoring drug use to the unique genetic makeup of each individual patient, where a general practitioner would generate a pharmacogenetic profile of each patient and prescribe drugs accordingly\textsuperscript{2}. In place of simple descriptive information provided by therapeutic drug monitoring, molecular genetics could produce information about why a patient may require a different dose, drug or treatment regimen before a therapy is instituted\textsuperscript{192, 193}. It might also substantially reduce the need for hospitalization because of adverse drug reactions and its associated costs\textsuperscript{11}.

Pharmacogenetic testing is currently used in only a limited number of teaching hospitals and specialist academic centers. It is well established in Scandinavian countries. The most widely accepted application of pharmacogenetic testing is the use of \textit{CYP2D6} genotyping to aid individual dose selection for drugs used to treat psychiatric illness. Several independent testing laboratories provide DNA based testing service for a range of pharmacogenetic polymorphisms to pharmaceutical industry and medical practice\textsuperscript{199}. However, in India, this system has not been developed.
The advantage of combining genotyping/phenotyping with therapeutic drug monitoring is that genotyping can predict the PM or UEM drug metabolism phenotypes, and this information can be used for dosage adjustment or selection of an alternative drug, which is not a substrate of CYP2D6. The cost/healthcare effectiveness of these paradigms has not been extensively studied. Although there would be considerable cost associated with screening all individuals before dosing with CYP2D6 substrates of narrow therapeutic index, this cost may be offset by a reduction in costs associated with toxic episodes or therapeutic failure and subsequent intervention. Polypharmacy and over the counter drug purchase is very common in developing countries like India and Sri Lanka. Since CYP2D6 is responsible for the metabolism of most of the commonly used drugs, this may result in severe drug interactions especially in the poor metabolisers. Routine phenotyping or genotyping may not be economical in developing countries. However, monitoring of CYP2D6 enzyme activity is important for the patients who report adverse reactions with normal dose of the drugs. This may help the physicians in individualization of the therapy especially for long term drugs like anti-depressants and anti-hypertensive.
**Implication for drug development**

The knowledge gained about these polymorphism studies should be incorporated into drug development at an early stage to determine whether or not the drug is metabolized by \textit{CYP2D6} and hence subject to genetic polymorphism. Since phase-1 clinical trials are carried out at a rather later time during drug development, usually five to seven years after the initial discovery, a strategy, which allows for an earlier recognition of this phenomenon would be desirable\textsuperscript{93}. Dosing regimens are normally established during the phase-1 evaluation of drugs and are based on studies of relatively small number of subjects. However, with respect to oxidation phenotype, this subjects may not be representative of the general population\textsuperscript{3}.

If it were possible to predict that the metabolism of a drug cosegregates with a known polymorphism at the preclinical stage, the decision on whether or not to pursue development of the drug would be facilitated\textsuperscript{93}. Several in vitro approaches have been developed which allow a prediction to be made during preclinical testing if the metabolism of a new drug is subject to genetic polymorphism\textsuperscript{194, 195}. Inhibitory monoclonal antibodies are available which determine cytochrome P450 substrate and product specificity\textsuperscript{196, 197}. It is obviously also prudent to exclude potentially susceptible individuals from phase-1 dose escalation trials. This can prevent PM healthy subjects or patients being exposed to additional risk of toxicity during phase-1 and 2 development\textsuperscript{3}.
There is currently great interest in the pharmaceutical industries in pharmacogenetics and an increasing number of companies are genotyping their clinical trial populations. Moreover, the knowledge of genetic variability in drug response is becoming an increasingly important component of the drug registration process\textsuperscript{2}.

**India:**

Indian population is polygenic and is said to be the melting pot of various racial mosaic. The population of India is derived from 6 main ethnic groups:

1. Negroids
2. Proto-Australoids or Austrics
3. Mongoloids
4. Mediterranean or Dravidian
5. Western Brachycephals and
6. Nordic Aryans

**Negroids**, the brachycephalic (broad headed) from Africa were the oldest people to have come to India. In the mainland, these people are now found only in patches among the hill tribes of South India. But they survive in Andaman Island, where they have retained their language. The **Austrics** of India represent a medium height,
dark complexion with long heads and rather flat noses but otherwise of regular features.

Austric tribes spread over the whole of India and then pass to Burma, Malaya and the island of south East Asia. Mongoloids of various types are confined to the north-eastern fringes of India, in Assam, Nagaland, Mizo, Garo and Jainti Hills. Generally, they are people of yellow complexion, oblique eyes, high cheekbones, sparse hair and medium height

The nontribal population of India consists mainly of Caucasoid Aryans in North India and Caucasoid Dravidians in South India. The term Dravidian is derived from the pre-Hellenic Lycians of Asia Minor who called themselves Trmmili, which the Greeks wrote as Temilai. Temilai became Dramiza and became Dravidian. Dravidians antedated the Aryan culture in India by almost a thousand years. It is generally believed that the architects of Indus Valley Civilizations of the 4th millennium BC were Dravidians and that at a time anterior to the Aryans, they were spread over the whole of India. With the coming of the Aryans into North India, the Dravidians appear to have been pushed into the south, where they have remained confirmed. Tamil Nadu, with the other southern states Andhra Pradesh, Karnataka and Kerala, today form the repositories of the Dravidian Culture

Kerala is a small state, tucked away in the south west corner of India. It represents only 1.18 percent of total area of India but 3.43% of the total population of the
Karnataka is the eighth largest state in India both in area and population. Karnataka is situated on the western edge of the Deccan plateau and has for its neighbors Maharashtra and Goa on the north, Andhra Pradesh on the east and Tamil Nadu and Kerala on the south. On the west it opens to Arabian Sea.

Tamil Nadu is situated on the south eastern side of the Indian peninsula. It is bounded on the east by Bay of Bengal, in the south by the Indian Ocean, in the west by the States of Kerala and Karnataka and in the north by Karnataka and Andhra Pradesh. This state represents the nucleus of Dravidian culture in India.

Andhra Pradesh (AP) is the fifth largest state in India, both in area and population. AP forms the major link between the north and the south of India. Andhra Pradesh consists of three distinct regions. (i) Coastal region generally called Andhra, (ii) the interior region known as Rayalaseema and (iii) Telengana region, consisting of the capital Hyderabad and adjoining districts. The earliest mention of the Andhras is said to be in Aitereya Brahmana (2000 BC). It indicates that Andhras originally an Aryan race, living in North India, migrated to the south of Vindhyas and later mixed with non-Aryan stocks.