CYTOCHROME P450 2D6 POLYMORPHISM IN LEBANON

A Research Project Submitted In Partial Fulfillment of the Requirements for the Degree of Doctor of Pharmacy

By

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Santa Saade
To my family
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Accomplishment of this research study represents the conclusion of a six year-period of laborious effort, commitment and perseverance. Many have supported me through all these years providing me with incessant assistance, guidance and constancy. Without their perpetual presence, all achievements would have been tougher and more demanding.

My family granted me inestimable and valuable encouragement, counsel and devotion. My parents, George and Julia guided me through the way, providing endless support, resolution and love. My father’s wisdom, calmness, ardor and persistence consisted of the major drive urging me to stay on the right track. My mother’s indulgence, thoughtfulness and determination were indispensable in order to bestow me the strength to continue and overcome all barriers. My sister Sylvia’s help and loyalty were essential, as her eagerness to assist me constituted of a major relief anytime an obstacle emerged.

I am also thankful to my friend Ola who distinguished herself with her zeal, continuous cooperation, and reliability.

Deep gratitude is imparted to Dr Mroueh who assisted me through the exhausting lab hours, granted me a massive amount of knowledge and information, with great thoughtfulness and selflessness.
Method: One hundred and fifty six volunteers (75 males and 81 females) were recruited for the study. All volunteers were administered a 30 mg dose of dextromethorphan hydrobromide. Urine samples were collected 8 hours after dextromethorphan administration. Upon retrieval, the urine samples were stored at -80°C until analysis for dextromethorphan levels and its metabolites using a sensitive, simple HPLC assay.

Results: The distribution frequency histogram of CYP2D6 metabolic ratios (MRs) showed a bimodal distribution with a gap between the metabolic ratios of 0.14 and 0.31 corresponding to log MR between -0.85 and -0.51. This gap correlates well with the antimode of MR=0.3 reported by previous studies in Caucasian populations. Sixteen subjects were classified as poor metabolizers accounting for 10.25% of the whole population sample with metabolic ratios ranging from 0.310559 to 25.76923; in contrast, 140 (89.75%) volunteers were found to be extensive metabolizers according to the aforementioned antimode of 0.3 exhibiting metabolic ratios between 0.000439 and 0.139.

Conclusion: The findings of this phenotyping study demonstrate the presence of a high proportion of CYP2D6 poor metabolizers in the Lebanese population and hence the significance of potential clinical implications in these subjects.
1.1 THE HISTORY OF PHARMACOGENETICS

The field of pharmacogenetics was originally introduced in the 1950s when significant adverse effects of drugs were documented in certain patients and not in others and these findings were later understood to result from genetic polymorphisms in drug metabolizing enzymes. However, the concept that a medication can be safe and effective in one individual while ineffective or even harmful in another individual, dates back to the observations of Pythagoras that fava bean ingestion was dangerous for some but not for others.  

Summary of the history:

510 BC: Pythagoras recognized the occurrence of dangerous complications resulting from the ingestion of fava beans in some individuals that were not apparent in others.

1952: Interesting observations were reported regarding significant drug side effects mainly primaquine-induced hemolysis in Blacks and prolonged apnea following succinylcholine administration.  

1956: Carson suggested genetic polymorphism of glucose-6-phosphate dehydrogenase was responsible for primaquine-induced hemolysis.
1957: Motulsky pointed out that genetic variations in drug metabolizing enzymes could play a role in adverse effects caused by certain drugs.\(^5\)

1957: It was recognized that mutation in the pseudocholinesterase enzyme affected the hydrolysis of the neuromuscular blocking agent succinylcholine leading to an extended half-life of the drug and consequently prolonged apnea.\(^6\textsuperscript{-8}\)

1959: Vogel first suggested the term "pharmacogenetics" to define the observed genetically induced interindividual variability in drug response.\(^9\)

1960: Isoniazid adverse effects were explained by the presence of genetic variants in the N-acetyltransferase enzyme.\(^10\)

1962: Kalow published the first monograph about pharmacogenetics.\(^11\)

1968: Vesell and Page reported that monozygotic or identical twins had remarkably comparable plasma half-lives of numerous drugs whereas wide variations were observed in dizygotic or fraternal twins; suggesting the contribution of multiple genes in the metabolism of drugs: multigenic inheritance.\(^12\textsuperscript{-14}\)
1.2 PHARMACOGENETIC INFLUENCES ON THERAPEUTIC RESPONSE

Pharmacogenetic variability affects the therapeutic response of a medication by influencing the pharmacokinetic and the pharmacodynamic factors that promote drug effects (Figure 1).

![Diagram showing the relationship between Pharmacokinetics and Pharmacodynamics]

**Figure 1: Pharmacogenetic variability and the ways it influences therapeutic outcome**

Pharmacodynamic differences can explain the variability in therapeutic response despite similar drug concentrations and these represent the biochemical and physiological consequences of the administration of a certain agent and its affinity to its receptors.
The pharmacokinetic contribution to interindividual variability consists of any genetically induced variation in one of the processes that determine drug and metabolite concentrations in plasma and tissue after drug ingestion and thus influencing the therapeutic outcome. These processes include drug absorption (e.g., active transport systems), distribution (e.g., plasma protein binding), biotransformation (e.g., drug metabolizing enzymes), and excretion by the renal or biliary routes.

Because of the primordial role of proteins in governing the pharmacokinetic and pharmacodynamic processes in the body, they are both subject to genetic influences. In pharmacokinetics, drug-metabolizing enzymes are mostly affected whereas in pharmacodynamics, the structure and conformation of receptors, carrier proteins or ion channels are generally involved. Genetic influence on pharmacodynamic processes often surpasses the impact on pharmacokinetics.\textsuperscript{15} This can be justified by the difference in the degree of complexity between genetic controls of an enzyme compared to receptor structure: A single locus is usually responsible for the control of an enzyme whereas receptor structure is extremely complex, multiple protein units might be involved, several metabolic pathways might contribute to the translation into an expected response, and therefore multiple genes govern the pharmacodynamic processes hence increasing the potential for genetic polymorphisms.
1.3 GENETIC MUTATIONS: THEIR IMPACT ON THE GENOTYPE AND
THE PHENOTYPE

According to Lennard et al., "The majority of inborn errors affecting the response to
drugs are caused by alterations in the functional activity of an enzyme... These inborn
errors are of two types: some are very rare to the extent that only 1 in 10000 to 1 in
100000 people might be affected, while others take the form of classical genetic
polymorphisms in which the abnormal gene has a frequency of more than 1% in the
general population." 16

Genetic variation results generally from two types of allelic variants: point mutations
and polymorphisms. Point mutations are rare allelic variants (<1% in frequency)
frequently associated with changes in encoded amino acids in congenital diseases
such as cystic fibrosis, the congenital long-QT syndromes or the familial epilepsies.

A genetic polymorphism is defined as a monogenic trait which occurs in the
population in at least two phenotypes and genotypes [a phenotype being the visual
expression of a genotype] neither of which has a frequency of less than one percent.
The most common types of polymorphisms are single nucleotide polymorphisms;
known as SNPs. 17 SNPs arise from nucleotide insertions, base deletions and
duplications, base pair substitutions or variations in copy number of single base pairs
(Figure 2). Other types of polymorphisms include dinucleotide or trinucleotide
polymorphisms, and insertion or deletion of larger portions of DNA.
Figure 2: Examples of genetic variation

There are in general three classes of SNPs: the intergenic SNPs, iSNPs; the perigenic SNPs, pSNPs; and the coding SNPs, cSNPs. The number of intergenic SNPs accounts for 2 million approximately and these iSNPs are located between the genes. Perigenic SNPs, counting about 200000 to 500000, are located in non-coding gene regions such as the upstream regulatory regions, and in introns. Both iSNPs and pSNPs result in silent mutations that do not affect the phenotype. If a polymorphism affects the promoter, rate of transcription can be altered.

A polymorphism located at an intron/exon boundary in a gene may produce incomplete or inactive proteins as a result of incorrect mRNA splicing. Less than 500000 SNPs reside in coding regions, mainly exons. If a whole gene deletion occurs, functional enzyme activity is deleted, whereas duplications of the entire gene may result in higher levels of activity. Many coding SNPs have no apparent clinical importance (silent): no change in the amino acid transcription results, due to the
existence of several codons that encode the same amino acid (synonymous substitution); or the amino acid substitution does not affect neither the binding site nor the structure of the protein and thereby has no functional significance. Some coding SNPs have minimal effects on the phenotype since amino acid substitution will produce little functional difference in protein structure or function. Finally, a small fraction of SNPs will have significant impact on protein structure, function or response to endogenous agents or drugs, producing either functionally altered gene product (altered enzyme or receptor) or altered amounts of a normal gene product; generally lower rather than greater amounts are produced.

These polymorphisms are responsible for the interindividual variability in therapeutic response, or represent the variants that underlie a hereditary disorder such as cystic fibrosis, sickle cell anemia...

The population is generally divided into two phenotype categories: poor and extensive metabolizers. An individual with a poor metabolizer phenotype will experience adverse effects after ingestion of a standard dose of a drug because of decreased catalytic activity of the metabolizing enzyme involved and hence accumulation of drug in plasma. If biotransformation into active metabolites is carried out by the polymorphic enzyme with diminished activity, then lower levels of the active metabolite will be achieved resulting in therapeutic failure. On the other hand, the extensive metabolizer phenotype corresponds to the normal or standard metabolic activity of an enzyme and in this category, appropriate therapeutic outcome is achieved with standard drug dosages and minimal or absent toxicity.
The poor metabolizer phenotype is a result of two inactive or low activity allele variants and therefore expresses a homozygous recessive genotype. The extensive metabolizer phenotype is an expression of both heterozygous and homozygous normal allele dominant genotypes that usually cannot be distinguished by phenotyping methods.

Often, a third category is added: the intermediate metabolizer phenotype is a subset of the extensive metabolizer group and results from the expression of a heterozygous genotype coding for one functional allele and a non-functional allele. This subset also cannot be differentiated from other phenotypes by phenotyping techniques.

Finally, an additional category is sometimes used to describe the phenotype resulting from dominant allele multiplication. This category is known as the ultra rapid metabolizers and is usually associated with specific drug metabolizing enzymes mainly the cytochrome CYP2D6. This category of individuals will probably complain of therapeutic failure and may require higher than standard therapeutic doses of a certain medication because of increased enzymatic activity and consequently low therapeutic levels in plasma.
1.4 DRUG METABOLIZING ENZYMES

Two categories of drug metabolizing enzymes (DME) exist in the human body: Phase I and Phase II enzymes. The polymorphic Phase I enzymes of functional importance for the metabolism of drugs are mainly the cytochrome P450 enzymes (CYPs). N-acetyltransferase, pseudocholinesterase and thiopurine methyltransferase (TPMT) are the main conjugative (Phase II) enzymes that display polymorphic variability.

The P450 proteins are an expression of a gene super-family (CYPs) that currently contains more than forty different members in humans. The P450s catalyze reactions functional in the biosynthesis of steroid hormones; the metabolism of xenobiotics to reactive metabolites (free radicals) that interact with cellular macromolecules (DNA, RNA, proteins) or undergo detoxification by reaction with cellular constituents such as glutathione; the synthesis and degradation of prostaglandins and other unsaturated fatty acids; the conversion of vitamins to their active forms...
An evaluation of the mechanism for the metabolism of 315 different drugs revealed that 56% of them were primarily cleared through the action of the cytochrome P450 enzymes. CYP 3A4 alone is responsible for the clearance of 50% of the drugs studied, CYP 2D6 covers generally 25%-30% of all clinically used medications, among them 50 of the 100 best selling drugs in the US, CYP 2C9 and 2C19 metabolize 15% of the drugs and the remaining metabolism is carried out by CYP 1A2, CYP 2A6, and CYP 2E1.

Phenotypic and/or genotypic polymorphisms have been described for the following cytochrome P450s: CYP 1A1, CYP 1A2, CYP 1B1, CYP 2A6, CYP 2C9, CYP 2C19, CYP 2D6, CYP 2E1, CYP 3A4, CYP 3A5, CYP 5A1 and CYP 8A1. Consequently, polymorphic enzymes perform the majority of P450 dependent drug metabolism.

N-acetyltransferases (NAT) are phase II conjugating liver enzymes that catalyze the N-acetylation (usually deactivation) and O-acetylation (usually activation) of arylamine carcinogens and heterocyclic amines. Common drugs metabolized by N-acetyltransferases include isoniazid, sulfonamides, procainamide, amrinone, caffeine, aminoglutethimide and hydralazine. Allelic variation at the NAT2 gene locus accounts for the polymorphism seen with acetylation of drugs.

S-methylation is an important pathway in the metabolism of aromatic and heterocyclic sulphydryl compounds such as captopril, azathioprine, 6-mercaptopurine and D-penicillamine with methyl groups from an endogenous donor, S-adenosylmethionine. Two enzymes are responsible for clearance of these medications:
thiopurine methyltransferase (TPMT) or thiolmethyltransferase (TMT). TPMT is a cytoplasmic enzyme that preferentially catalyzes the metabolism of 6-mercaptopurine and azathioprine whereas TMT is a membrane bound enzyme responsible for the clearance of D-penicillamine and captopril. 21

Pseudocholinesterase is an enzyme found in human plasma. It is responsible for the metabolism of cocaine and the neuromuscular junction blockers succinylcholine and mivacurium.

A summary of the major human drug metabolizing enzyme characteristics, with their main substrate drugs, is presented in Table 1. 22

1.5 ENVIRONMENTAL FACTORS AND INTERETHNIC VARIABILITY

Pharmacogenetics, as already mentioned is the study of the hereditary basis of the differences in therapeutic responses to medications. Ecogenetics is the broader field of interindividual variations in responses to all environmental, chemical and physical agents such as heavy metals, insecticides, compounds formed during combustion, and UV radiation. Environmental genomics, in contrast, try to recognize how genetic variability influences individual responses to environmental stress, taking into consideration the idea that high-risk genotypes are at greater risk of developing exposure-related diseases because they suffer more injury than others do.
<table>
<thead>
<tr>
<th>Drug metabolizing enzymes</th>
<th>Polymorphic</th>
<th>Representative substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Yes</td>
<td>Carcinogenic polycyclic aromatic hydrocarbons</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Yes</td>
<td>Arylamines, nitrosamines, aflatoxin B1, caffeine, paracetamol, theophylline, imipramine, fluvoxamine</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Yes</td>
<td>Coumarin, nicotine</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Yes</td>
<td>Tolbutamide, ibuprofen, mesalamic acid, tetrahydrocannabinol, losartan, diclofenac</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Yes</td>
<td>S-mephenytoin, amitriptyline, imipramine, citalopram, domperidone, metoclopramide, omeprazole, proguanil, hexobarbital, propranolol</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Yes</td>
<td>Debrisoquin, sparteine, metoprolol, propranolol, timolol, encainide, propafenone, flecainide, mexiletine, codeine, ethylmorphine, dextromethorphan, clonazepam, perphenazine, thioridazine, chlorpromazine, remoxipride, zuclopenthixol, desipramine, haloperidol, amitriptyline, imipramine, domperidone, nortriptyline, paroxetine, fluoxetine, desmethylcitalopram, mianserin</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Yes</td>
<td>Ethanol, nitrosamines, paracetamol, chlorzoxazone, halothane</td>
</tr>
<tr>
<td>CYP3A</td>
<td>Yes</td>
<td>Erythromycin, ethinyl estradiol, nifedipine, triazolam, cyclosporine, amitriptyline, imipramine, aflatoxin B1</td>
</tr>
<tr>
<td>NAT</td>
<td>Yes</td>
<td>Procainamide, hydralazine, depson, sulfasalazine, sulfamethoxazole, sulfadiazine, sulfacetamide, aminglutethimide, phenelzine, isoniazid, caffeine, p-aminohippuric acid, nitrazepam, p-aminobenzoic acid</td>
</tr>
<tr>
<td>Pseudocholinesterase</td>
<td>Yes</td>
<td>Cocaine, succinylcholine, mivacurium</td>
</tr>
<tr>
<td>TPMT</td>
<td>Yes</td>
<td>6-mercaptopurine, azathioprine</td>
</tr>
</tbody>
</table>
Survival of the fittest governs the process of natural selection. Life must therefore adapt to environmental pressure in order to survive. A phenocopy is an environmentally induced phenotype that mimics the one usually associated with a specific genotype. In general, environmental factors include diet, pollutants and toxins, drugs, alcohol and tobacco use, and diseases unrelated to the genotype of interest. The existence of a very large variety of polymorphic genes coding for drug metabolizing enzymes, suggests that these genetic mutations were beneficial to man at some point in time. Besides the metabolism of drugs, metabolizing enzymes are also responsible for detoxification of toxic products found in food, mainly plants; and for biotransformation of harmless compounds into toxic metabolites such as the conversion of precarcinogens into carcinogenic agents. This concept demonstrates the logic behind the existence of allelic variants that cause loss of activity to an enzyme, or mutations that result in complete gene deletion and thereby disappearance of the enzyme that it encodes, or otherwise, polymorphisms that induce gene multiplication and therefore significantly increased levels of a single enzyme. It could have been advantageous on a certain occasion during evolution to inhibit metabolism in response to environmental pressure; while in another geographic area, survival would have been challenged if metabolic activities of drug metabolizing enzymes were not enhanced by gene multiplication in response to the abundance of toxic chemicals in dietary customs of the specific area involved.

Environmental pressure and natural selection represent the basis of the geographic differences in drug metabolizing enzyme polymorphisms resulting in the significant interethnic variability in therapeutic outcome observed. Interindividual variability in
drug metabolism between two individuals in the same population is mostly of genetic origin. In contrast, the variations observed between two populations are often due to differences in dietary habits and exposure to variable toxins in the surroundings. Pharmacooanthropology examines the interethnic or interracial differences in therapeutic response. Differences can be extreme. For example, the $\beta_1$ variant of alcohol dehydrogenase predominates in Europe whereas the atypical alcohol dehydrogenase variant, the $\beta_2$ form is more common in East Asia.

Table 2 provides an overview of the polymorphic distributions of the major drug metabolizing enzymes in different populations.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Ethnic Group</th>
<th>Percentage of subjects with monogenic enzyme variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19</td>
<td>Caucasian</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>Oriental</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>Central American Indian</td>
<td>1</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Caucasian</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Oriental</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Central American Indian</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Greenland</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Arab</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>San Bushman</td>
<td>18.8</td>
</tr>
<tr>
<td>NAT</td>
<td>Europe</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>North Pacific</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>South Pacific</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Other Asian populations</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>North and South American Indian</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Africa</td>
<td>46</td>
</tr>
</tbody>
</table>
1.6 CLINICAL IMPLICATIONS OF GENETIC POLYMORPHISMS

1.6.1 Adverse drug reactions

Adverse drug reactions and drug interactions constitute a major drawback to therapeutic management of diseases and an endless concern in drug development. A meta-analysis conducted by Lazarou et al., in 1998 demonstrated that serious adverse drug reactions are considered as the fourth to sixth cause of death in hospitalized patients in the United States. In the US, 2.216 million cases of severe drug reactions occur from appropriate drug therapy in inpatients every year accounting for 6.7% of all hospitalized patients and resulting in increased health care expenses by 100 billion US dollars. In 0.32% of these hospitalized patients, adverse drug reactions provoked death, amounting to approximately 100000 deaths annually.

A significant proportion of adverse drug reactions in addition to therapeutic failure are probably due to the genetically based interindividual variability in drug metabolism as well as the impact of drug interactions on the catalytic activities of the different polymorphic enzymes and the alterations in therapeutic outcome induced. Genotyping or phenotyping individuals for drug metabolizing enzymes represents an attractive idea in order to identify, prior to initiating therapy, patients at risk of developing adverse drug reactions or failing to respond to a certain medication. It allows for optimization of drug therapy with respect to safety by predicting and preventing the occurrence of adverse drug reactions and fatalities and with respect to efficacy by avoiding treatment of individuals who, based on their genetic identity, will experience
therapeutic failure. This would provide substantial medical and financial benefits to health care and society.

1.6.2 Polymorphism of drug metabolizing enzymes: the impact on therapeutic outcome

Genetic polymorphism of drug metabolizing enzymes can affect a multitude of molecular mechanisms that result in altered drug clearance. An overview of the major mechanisms is illustrated in Figure 3.

Figure 3: Major molecular mechanisms that can result in altered human drug metabolism
As mentioned earlier, gene deletions and frameshift, nonsense, missense and splice-site mutations can lead to translation into an inactive enzyme or to the absence of enzyme production completely. Single nucleotide polymorphisms causing substitutions in amino acids can provoke the creation of an unstable enzyme or an enzyme with an altered active site, resulting in a change in substrate specificity. Finally, duplication or multiplication of active genes can give rise to exaggerated levels of m-RNA and excessive levels of active enzyme thus increased catalytic activity of the enzyme involved. Examples of common human cytochrome P450 allelic variants that are associated with such variations in drug metabolism are presented in Figure 3.

Significant clinical implications have arisen in response to genetic polymorphisms in xenobiotic metabolism. Since drug metabolizing enzymes are responsible for detoxification of drugs into less toxic or inactive metabolites or biotransformation of prodrugs into active agents, genetically induced variations in enzyme catalytic activities can affect therapeutic outcome with respect to safety by increasing the incidence of adverse drug reactions in poor metabolizers, and with respect to efficacy by resulting in decreased drug plasma levels in ultra rapid metabolizers or decreased transformation of a prodrug into its active metabolites in poor metabolizers; both conditions leading to therapeutic failure.

An overview of the main allelic variants involved in the major cytochrome P450 polymorphisms is summarized in Table 3 along with their effect on enzyme catalytic activity and the resulting consequences on prototype substrates in poor metabolizer phenotypes.
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Metabolism affected in PMs</th>
<th>Consequence</th>
<th>Reduced prodrug activation in PMs</th>
<th>Major allelic variant</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP 2A6</td>
<td>Nicotine</td>
<td>Elevated levels</td>
<td>CYP 2A6*2</td>
<td>Inactive enzyme</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CYP 2A6*3</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CYP 2A6*4</td>
<td>No enzyme</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CYP 2A6*5</td>
<td>Defective enzyme</td>
<td></td>
</tr>
<tr>
<td>CYP 2C9</td>
<td>S-warfarin</td>
<td>Bleeding</td>
<td>Losartan</td>
<td>CYP 2C9*2</td>
<td>Reduced affinity for P450 reductase</td>
</tr>
<tr>
<td></td>
<td>Phenytion</td>
<td>Ataxia</td>
<td></td>
<td>CYP 2C9*3</td>
<td>Altered substrate specificity</td>
</tr>
<tr>
<td></td>
<td>Tolbutamide, glipizide</td>
<td>Hypoglycemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NSAIDs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP 2C19</td>
<td>Omeprazole</td>
<td>Increased levels and</td>
<td>Proguanil</td>
<td>CYP 2C19*2</td>
<td>Inactive enzyme</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H. pylori eradication rate</td>
<td></td>
<td>CYP 2C19*3</td>
<td>Inactive enzyme</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe sedation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP 2D6</td>
<td>TCAs</td>
<td>Cardiotoxicity</td>
<td>Encainide</td>
<td>CYP 2D6*2n</td>
<td>Increased activity</td>
</tr>
<tr>
<td></td>
<td>SSRIs</td>
<td>Nausea</td>
<td>Codiaine</td>
<td>CYP 2D6*4</td>
<td>Inactive enzyme</td>
</tr>
<tr>
<td></td>
<td>Haloperidol</td>
<td></td>
<td>Ethylmorphine</td>
<td>CYP 2D6*5</td>
<td>No enzyme</td>
</tr>
<tr>
<td></td>
<td>Perphenazine</td>
<td></td>
<td>Tramadol</td>
<td>CYP 2D6*10</td>
<td>Unstable enzyme</td>
</tr>
<tr>
<td></td>
<td>Zuclopenthixol</td>
<td></td>
<td></td>
<td>CYP 2D6*17</td>
<td>Reduced affinity for substrates</td>
</tr>
<tr>
<td></td>
<td>Perhexilone</td>
<td>Neupathogy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antiarrhythmic drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenformin</td>
<td>Arrhythmias</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tolterodine</td>
<td>Lactic acidosis</td>
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</tbody>
</table>
CYP2A6 catalyzes the C-oxidation of nicotine, which constitutes the major pathway in nicotine metabolism. It is also responsible for the 7-hydroxylation of coumarin. Two allelic variants were reported in addition to the wild-type allele: CYP2A6*2, CYP2A6*3 and the wild-type CYP2A6*1. Both variants cause the translation into an inactive enzyme. Wild type CYP2A6 gene duplication has also been suggested. 26,27 A study conducted by Pianezza et al. showed that individuals with at least one defective allele constituted a very small proportion of a group of smokers compared to a control group of non-smokers. In addition, it revealed that smoking individuals with a variant allele smoked a significantly lower number of cigarettes suggesting the implication of a genetic factor for nicotine dependence. 28

CYP2C9 plays a major role in the metabolism of a variety of drugs with narrow therapeutic indices including S-warfarin, tolbutamide, NSAIDs and phenytoin. Two allelic variants of clinical importance have been documented: the CYP2C9*2 and CYP2C9*3, both responsible for diminished enzyme catalytic activity. 26 Patients homozygous for the CYP2C9*3 allele exhibit 90% decreased clearance of S-warfarin compared to homozygous individuals for the wild-type allele. 29 These individuals may require racemic warfarin doses as low as 0.5 mg per day to achieve appropriate therapeutic outcome while keeping a low side effect profile, compared to the 5-8 mg standard daily dose required for patients homozygous for the wild-type CYP2C19 alleles. 30 Poor metabolizers have a much higher risk of experiencing side effects and major bleeding complications. 31
CYP2C19, also called S-mephenytoin hydroxylase, is a member of the cytochrome P450 family of drug metabolizing enzymes. This polymorphic enzyme catalyzes the enantiomer-selective-4'-hydroxylation of S-mephenytoin after which it is named. In addition, CYP2C19 metabolizes diazepam, omeprazole, imipramine, propranolol, and hexobarbital and converts the antimalarial prodrug proguanil into its active metabolite cycloguanil. CYP2C19 polymorphism expresses itself through three phenotypes: the rapid, intermediate and poor metabolizer groups. The deficient metabolism of this drug is inherited as an autosomal recessive trait. The poor metabolizer phenotype results from two non-functional alleles, whereas the extensive metabolizer phenotype consists of heterozygous or homozygous dominant genotypes. The most common variant alleles in poor metabolizers are CYP2C19*2 and CYP2C19*3.

Mephenytoin is marketed as a racemic mixture. Among extensive metabolizers, it exhibits stereoselective disposition because the S-enantiomer is more rapidly eliminated than the R-enantiomer. In contrast, the two enantiomers have similar elimination characteristics among poor metabolizers.

Furthermore, CYP2C19 polymorphism affects the efficacy of omeprazole in the management of peptic ulcer disease and H. pylori infection. Because of impaired omeprazole metabolism and thus accumulation of the drug, poor metabolizers of CYP2C19 exhibit higher plasma levels of omeprazole and higher intragastric pH compared to extensive metabolizers. A Japanese study conducted by Furuta et al. suggested that CYP2C19 genotype could influence the cure rates for H. pylori infection in peptic ulcer patients since they observed dramatically different cure rates with omeprazole and amoxicillin in patients with documented CYP2C19
polymorphisms. They reported a 100% cure rate in poor metabolizers, compared to 60% and 29% in intermediate and extensive metabolizer groups respectively, concluding that the accumulation of omeprazole concentrations in the poor metabolizer phenotype contributed to the greater efficacy in these patients by producing a greater degree of gastric acid suppression. We can therefore postulate that identification of rapid and intermediate metabolizers of CYP2C19 would allow us to achieve better therapeutic outcomes in patients with peptic ulcer disease by selecting appropriate dosing according to individual requirements or by considering more aggressive drug therapy in patients expected to fail on conventional therapies.

Diazepam is another example of substrates affected by CYP2C19 genetic polymorphism. In patients with the poor metabolizer phenotype, the plasma half-life of diazepam is prolonged up to 80 hours approximately compared to 64 and 20 hours in heterozygotes and functional allele homozygotes respectively; resulting in increased plasma diazepam levels and consequently severe sedation. Kumana et al., observed an elevated incidence of the poor metabolizer phenotype and low activity heterozygotes in Orientals that allowed him to explain why physicians in Asia tend to prescribe routinely smaller doses of diazepam for Chinese than for Caucasians.

CYP3A isoenzymes constitute the predominant subfamily of cytochrome P450 enzymes. The genes for CYP3A are expressed primarily in the liver and small intestines. CYP3A4 is responsible for the metabolism of approximately 50% of all currently used medications. Because of its presence in the intestine too, CYP3A4 plays a major role in the first pass metabolism of a multitude of orally administered
drugs. Substrates for the CYP3A isoenzymes include benzodiazepines, calcium channel blockers, HMGCoA reductase inhibitors, chemotherapeutic agents, immunosuppressants, and HIV protease inhibitors. Although there are large interindividual and interracial differences in CYP3A drug metabolism, but the genetic basis behind polymorphic activity of the enzymes is still poorly documented. Several allelic variants have been reported however they do not totally explain the variations observed, especially since the discovery of a new gene, the PXR gene that regulates CYP gene expression and might play a role in the variability in drug metabolism.

The details of CYP2D6 polymorphism will not be covered in this chapter since it represents the core of this review and thus it will be discussed in further details in the following chapter.

The acetylator polymorphism is explained by allelic variation at the NAT2 gene locus. Twenty-seven NAT2 alleles have been reported. NAT2*5B and NAT2*6A account for 72-75% of all the variant NAT2 alleles. They are responsible for changes in the genetic code leading to translation into lower amounts of an N-acetyltransferase enzyme displaying decreased catalytic activity. The population is usually divided into two phenotypes: slow and rapid acetylators. Slow acetylators are generally subject to adverse reactions due to drugs metabolized by N-acetyltransferase such as the occurrence of vitamin B6-induced peripheral neuropathy while taking isoniazid or sulfonamide-induced toxic epidermal necrolysis and Steven-Johnson syndrome, or idiopathic lupus erythematosus while on treatment with procainamide and hydralazine. On the other hand, rapid acetylators may experience failure of therapy
for example when treated with isoniazid for tuberculosis or with hydralazine as antihypertensive agent because the body fails to reach therapeutic plasma concentrations of the agent due to rapid clearance by the metabolizing enzyme. Interestingly, fast acetylators experience more adverse drug reactions due to procainamide because its metabolic N-acetylprocainamide that retains some antiarrythmic activity and has a slower renal elimination rate will accumulate, thus increasing the likelihood of side effects.  

Thiopurine methyltransferase (TPMT) is inherited as a single autosomal dominant gene. The cytotoxic activity of 6-mercaptopurine (6-MP) and azathioprine, a prodrug of 6-MP, is associated with the production of thioguanine nucleotides that are incorporated into the individual's DNA. When TPMT catalytic activity is low because of genetic polymorphism, a larger percentage of 6-MP is converted to thioguanine nucleotides resulting in severe myelosuppression that is considered a major drawback when azathioprine is used for immunosuppressive management of organ transplantation or inflammatory bowel disease, yet it can be beneficial as a cytotoxic agent in the treatment of leukemia.  

In a study of approximately 700 patients with inflammatory bowel disease treated with a fixed dose of azathioprine of 2 mg/kg/d, the authors reported a 5% incidence of bone marrow toxicity including three patients who developed pancytopenia resulting in death due to sepsis in two of them. In addition, a case of fatal myelosuppression was reported following treatment with azathioprine for immunosuppression after cardiac transplantation. Conversely, in the treatment of leukemia, the major concern is prevention of relapse and thus therapeutic failure that can occur as a consequence of
increased catalytic activity of TPMT resulting in lower levels of thioguanine nucleotides, the major factor responsible for the cytotoxic activity of 6-mercaptopurine. It is therefore appealing to be able to select and increase treatment dose according to an individual's needs, keeping in mind that the risks of relapse are minimized while myelosuppression is less likely and is not a chief concern if it occurs since the majority of leukemic patients are usually considered for bone marrow transplantation as long term management of their disease.  

Finally, low thiopurine methyltransferase catalytic activity has been linked with increased risk of hypoprothrombinemia in patients taking medications that yield sulphydryl metabolites such as cephalosporin antibiotics more specifically moxolactam, cefoperazone...  

1.6.3 Clinical consequences of induction or inhibition of drug metabolizing enzymes

Management of chronic diseases requires long-term administration of therapeutic agents and often multiple drug therapy because of co-morbidity. This may result in drug accumulation, enzyme inhibition or induction, and an increased risk of simultaneous exposure to multiple agents. Metabolic drug interactions occur generally at the presystemic (interaction with the cytochromes found in the gastrointestinal tract and responsible for first-pass metabolism) and systemic levels for oral drugs; whereas they occur predominantly at the systemic level when drugs are administered intravenously. Drug-metabolizing enzymes are usually responsible for the detoxification of drugs into inactive nontoxic metabolites; nevertheless, they
sometimes transform them into active or reactive compounds. In general, most drugs are oxidized to several metabolites by different cytochrome P450s. However, when an agent is metabolized extensively by a single CYP, inhibition or induction of the cytochrome involved would have a major impact on therapy with the drug in question. For instance, CYP2D6 extensive metabolizer phenotypes are transiently converted into poor metabolizers by administration of CYP2D6 inhibitors resulting in increased risk of toxicity from accumulation of toxic metabolites or therapeutic failure due to decreased biotransformation of the parent compound into its active metabolites; whereas the drug metabolism in poor metabolizer individuals was not affected. 52

1.6.3.1 Induction of the cytochrome P450s

To study the influence of enzyme inducers on the cytochrome P450 3A4, Pichard et al. screened 60 different molecules and observed their effect on the metabolism of cyclosporine, a substrate of CYP3A4. 53 They noted that rifampin, as well as other inducers of the cytochrome such as phenytoin, carbamazepine, dexamethasone, sulfipyrazone and phenobarbital, markedly increased the metabolism of cyclosporine in hepatocytes.

CYP2C9 is also affected by rifampin, although to a lesser extent than is CYP3A4. 54 Rifampin can cause a three- to eightfold increase in the R/S ratio of mephénytoin in urine and a 40 to 180 percent increase in the eight-hour urinary excretion of the hydroxy metabolite in extensive metabolizer phenotypes as a result of CYP2C9 inhibition without affecting the indices of poor metabolizer individuals who usually produce little if any hydroxymephénytoin. 55
1.6.3.2 Inhibition of the cytochrome P450s

Concomitant administration of enzyme inhibitors as part of a patient’s drug therapy may dramatically affect the metabolism of concurrent drugs thus resulting in significant pharmacokinetic consequences including impairment of drug clearance and accumulation, and prolongation of drug action. Drug interactions are usually transient as a result of reversible inhibition of the cytochrome P450 isoenzymes. However, another group of medications such as macrolide antibiotics, or ethinyl estradiol exert marked effects on the pharmacokinetic behavior of coadministered drugs because of prolonged or irreversible inhibition of the enzymes. 56

The most common type of inhibition is due to the reversible interaction of a drug or a stable metabolite with a CYP enzyme. Reversible inhibition occurs when a drug or stable metabolite attains concentrations near the cytochrome that are sufficient for impairment of enzyme activity. An early step in the cytochrome reaction cycle is usually affected; mostly substrate binding or oxygen coordination to the heme, thus substrate turnover is decreased. Diffusion of the enzyme inhibitor away from the cytochrome restores enzymatic function so that inhibition is transient.

Irreversible cytochrome P450 enzyme inhibition occurs following mechanism-based processes suggesting that at least one CYP reaction cycle is required to achieve inhibition of enzyme activity. 57 There are two basic types of mechanism-based processes that induce irreversible enzyme inhibition: autocatalytic inactivation or suicide processing that occurs when a reactive drug metabolite binds to the
cytochrome and alters its structure irreversibly, resulting in loss of function then in enzyme destruction; and metabolite-intermediate complexation that is characterized by the formation of drug metabolites that bind tightly to the cytochrome heme rendering the enzyme catalytically inert without destroying it. Irreversible inhibitors are more selective to specific cytochrome P450 isoenzymes because only one or few CYPs generate the reactive metabolite that is responsible for the mechanism-based inhibition.

In their study, Pichard et al. confirmed that erythromycin, troleandomycin, ketoconazole, midazolam, and nifedipine are inhibitors of the cytochrome P450 3A4 because they induced clinically significant increases in cyclosporine blood concentrations when given concurrently.

Periti et al. observed an increased incidence of adverse effects when drugs metabolized by CYP3A4 were co-administered with macrolide antibiotics, mainly troleandomycin and erythromycin. These complications consisted of jaundice during concurrent administration of estrogen containing contraceptives, and toxicity associated with theophylline treatment.

Diltiazem is also an inhibitor of the cytochrome P450 3A4 and has been used in conjunction with cyclosporine to impede its elimination. This provides an economic benefit because the dose interval of the expensive immunosuppressant can be increased; nevertheless, replacement of diltiazem by CYP3A4 inhibitors without major pharmacological effects may be of interest.
Back and Tija also observed the influence of different azole antifungals on the CYP3A4 mediated metabolism of cyclosporine. They concluded that ketoconazole markedly inhibited cyclosporine metabolism, whereas itraconazole and fluconazole were 10 times and more than 100 times less potent respectively in inhibiting the cytochrome isoenzyme.

Ketoconazole is also responsible for inhibition of the cytochrome P450 2C9 metabolic activity. It was found to significantly decrease the R/S ratio of mephenytoin in urine.

In addition, Bottiger et al. studied the effect of CYP3A4 inhibition on the metabolism of omeprazole. Hydroxylation of omeprazole is catalyzed by CYP2C19 and sulfoxidation is catalyzed by CYP3A4. The CYP3A4 pathway is a minor pathway in CYP2C19 extensive metabolizers, but a major one in poor metabolizers. A single oral dose of ketoconazole increased the plasma concentration of omeprazole by 99% in poor metabolizers compared to 36% in extensive metabolizers phenotypes.

The effect of multiple drug therapy on CY2D6 and the resulting pharmacokinetic alterations in drug metabolism will be discussed in depth in the next chapter.
1.6.4 Effect of liver disease on drug metabolism

Many tissues express CYP genes, but only the liver exhibits activities of P450 enzymes high enough to play a significant role in drug elimination. It might be expected, therefore, that liver disease, which can potentially reduce hepatic functional mass, could decrease the capacity for drug metabolism. Early studies of drug clearance in patients with liver disease tended to confirm this, but not clearly so because in some conditions, and for some drugs there was no reduction in drug clearance.\textsuperscript{64,65} Further, while pathophysiologic determinants such as hepatic drug delivery (hepatic blood flow, plasma protein binding), hepatic uptake, oxygen supply and bile secretion contribute to altered hepatic drug clearance in cirrhosis, these variables do not explain all the discrepancies between different types of drugs.\textsuperscript{65}

Investigators have observed a decrease in total P450 hepatic levels and related enzyme activities in patients with severe liver disease including hepatitis with liver failure and decompensated cirrhosis.\textsuperscript{66} These alterations in drug metabolizing enzymes levels contribute greatly to impairment of drug clearance and hepatic detoxification and biotransformation. The cytochrome P450 isoenzymes are clearly more susceptible to the effects of liver disease than are P450-reductase or phase II enzymes, such as glucoronyltransferases, sulphatases, N-acetyltransferases and amino acid transferases involved with drug and steroid conjugation reactions.\textsuperscript{67} Drug metabolism catalyzed exclusively by the latter types of enzymes is preserved until the most advanced stages of liver failure. In addition, Debinsky et al. have found increased expression of glucoronyltransferases per unit cell in cirrhotic livers, suggesting that compensatory up-regulation of enzyme expression can occur to offset the reduced number of functional hepatocytes.\textsuperscript{68}
The most profound reported alterations of CYP expression in human liver cirrhosis are decreased levels of *CYP1A2* mRNA. Diminished *CYP1A2* expression in severe liver disease is responsible for significant clinical implications mainly alteration in the metabolism of theophylline, caffeine and antipyrine since these drugs are extensively metabolized by this cytochrome isoenzyme.

The *CYP2C* genes seem to be relatively less affected by liver disease than *CYP1A* and enzyme expression is partly determined by the type and severity of liver disease. George et al. found no change in liver disease where hepatocyte function is compromised such as chronic hepatitis; in contrast, there were significant reductions of P450 2C protein levels in livers from patients in whom cirrhosis resulted from prolonged impairment of bile flow (cholestasis). Adedoyin et al. also noted the major influence of severe liver disease on CYP2C19 enzyme activity by observing significant reductions in the hydroxylation and thus the clearance of the S-mephenytoin enantiomer, a reaction catalyzed by CYP2C19.

CYP3A expression is also arbitrarily affected by liver disease. In some types of cirrhosis such as hepatocellular disease, 3A protein levels were found to be significantly lower than in controls. In contrast, levels of *CYP3A* mRNA and protein in cholestatic cirrhosis were more variable, and mean values were not significantly reduced compared to controls. Furthermore, *in vitro* studies of enzyme activities based on substrates extensively metabolized by CYP3A such as ethylmorphine or aminopyrine have shown the same preservation of enzyme expression and activity in primary biliary cirrhosis (a cholestatic liver disease) with significant reductions in other liver diseases.
Changes in CYPE1 expression are also dependent on the type of liver disease. Similarly to CYP2C and conversely to CYP3A, cytochrome P450 2E1 protein and enzyme activity are decreased in cholestatic types of cirrhosis but remain normal in hepatocellular liver diseases. 69

Activity of cytochrome P450 2A6 appears to be influenced by liver disease, as suggested by changes in coumarin 7-hydroxylation in vivo; 75 however in vitro studies are lacking to confirm this idea.

Finally, metabolism of debrisoquin does not seem to be affected in moderately severe liver disease, indicating that CYP2D6 may be refractory to the influences of liver disease. 73

A summary of the effects of different types of liver disease on individual cytochrome P450 isoenzyme is presented in Table 4.

Table 4: Effects of different types of liver disease on individual P450 proteins

<table>
<thead>
<tr>
<th>Type of cirrhosis</th>
<th>P450 1A2 (%)</th>
<th>P450 2C (%)</th>
<th>P450 2E1 (%)</th>
<th>P450 3A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocellular</td>
<td>29b</td>
<td>57</td>
<td>81</td>
<td>25b</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>18b</td>
<td>34b</td>
<td>49b</td>
<td>41</td>
</tr>
</tbody>
</table>

b Values that were significantly different from control. Modified from George et al. 75
*Hepatology* 1995a; 21: 120-128
1.6.5 Drug metabolizing enzymes and cancer

Drug metabolizing enzymes play key metabolic roles in several aspects of cancer as a consequence of their unusually broad substrate specificities. Approximately three-fourths of drugs like isoniazid and other environmental chemicals are toxic in their non-metabolized parent forms whereas the remaining one-fourth of drug metabolizing enzymes substrates such as acetaminophen or benzene are metabolically potentiated to toxic or mutagenic intermediates.

Phase I drug metabolizing enzymes, mostly the cytochrome P450s are responsible for the activation of the majority of chemical procarcinogens, both exogenous (xenobiotics) and endogenous (e.g., steroid hormones) to genotoxic electrophilic intermediates. Phase II drug metabolizing enzymes such as UDP glucoronyltransferases, glutathione transferases and N-acetyltransferases conjugate the intermediates to water-soluble derivatives completing the detoxification cycle. Reactive metabolites that are not detoxified, may react with DNA to form DNA adducts which, if not repaired, may eventually produce somatic mutations and cancer. Indeed, individuals with genetically determined high-phase I enzymes activity and low phase II activity would presumably produce higher quantities of reactive intermediates and exhibit greater DNA damage. 76 If DNA repair systems can correct carcinogen-induced or endogenous DNA damage that escape detoxification systems, then the consequences of high-risk metabolic genotypes may be less significant. DNA repair enzymes maintain the integrity of the genetic code by minimizing replication errors caused by damaged or rearranged DNA templates and by removing damaged DNA segments.
Cytochrome isoenzymes also play a major role in the biotransformation of anticancer therapeutic agents, enhancing or diminishing the efficacy of the drugs depending on whether the drug or its metabolites are active.

Several CYPs occur as genotypic variants within populations with consequent phenotypic alterations in susceptibilities to carcinogenic compounds and possibly to the therapeutic action of anticancer drugs. The variable expression of the cytochrome P450s between tumor and normal tissue can provide a basis for selective sensitivity to anticancer drugs, thereby localizing drug actions to tumors. The most important cytochrome P450s for the activation of procarcinogens are CYP1A1, CYP1A2, CYP1B1, CYP2D6, CYP2E1, and CYP3A4.

High CYP1A2 metabolic activity has been associated with an increased risk for colon cancer.\textsuperscript{77}

CYP2A6 is responsible for the metabolism of nicotine and might be important for the activation of some carcinogens. However, the enzyme is expressed entirely in the liver and further studies are required in order to show whether the CYP2A6 polymorphism is important for smoking behavior or cancer risk.

Recently, several polymorphic allelic variants of \textit{CYP3A4} have been identified, yet their influence on cancer risk and their importance for procarcinogen activation such as aflatoxin are still unclear.
CYP1B1 protein has been shown to be present in substantial concentrations in human lung. A mutation in the *CYP1B1* gene has been reported in association with lung cancer.  

CYP2E1 is an ethanol-inducible enzyme and is expressed in human lung as well as liver, kidney and brain. CYP2E1 also plays an important role in activating many occupationally relevant carcinogens such as vinyl chloride, benzene, acrylonitrile and butadiene; 79 and high CYP2E1 activity has been associated with benzene poisoning. 80 CYP2E1 polymorphism has been related to upper airway nasopharyngeal carcinoma, 81 a malignancy thought to be linked to nitrosamine exposure as well as oral cancers. 82

A striking relationship between mutations in the *CYP1A1* gene and lung cancer has been observed in the Japanese population, whereas less pronounced correlations have been found among Caucasians. 83-87 Furthermore, individuals with elevated CYP1A1 activity were found to be at higher risk for smoking-induced cancers. 88 Thus, a connection between cancer and a particular polymorphic site in one ethnic group might be of limited value as a genetic marker for cancer in another ethnic group, and extrapolations should be avoided.

The relationship between CYP2D6 polymorphisms and cancer will be discussed in further details in the next chapter.
DT-diaphorase is an important polymorphic enzyme in the activation of chemotherapeutic prodrugs such as antitumor quinines, and for the detoxification of certain potentially carcinogenic xenobiotics. Reduced enzyme activity has been related to the incidence of cancers such as colorectal cancer, basal cell carcinomas, prostatic adenocarcinoma or benign prostatic hyperplasia and myeloid leukemia. The enzyme is not inducible in homozygous patients for the allele responsible for diminished enzyme activity and this can explain the increased risk for benzene toxicity observed among such individuals.

N-acetyltransferases can deactivate by N-acetylation or activate aromatic and heterocyclic amine carcinogens by O-acetylation that usually produces reactive species. Polymorphisms in NAT2 mainly have been associated with an increased risk of bladder and colorectal cancer. Individuals with slow acetylation genotypes or phenotypes appear to be at higher risk for bladder cancer but at lower risk for colon cancer because bladder and colorectal carcinogens reach the target organs via different physiological and metabolic pathways.

The glutathione S-transferases conjugate glutathione to various potentially carcinogenic compounds, which facilitates their elimination from the body. Individuals with a nonfunctional GSTM1 allele seem to have increased risk for bladder and lung cancers. Furthermore, patients with high CYPIA1 activity as well as deficiency for the glutathione S-transferase (GSTM1) gene are at a considerably increased risk for squamous cell carcinoma following tobacco smoke exposure.
Polypharmacy is a fundamental feature of the management of certain diseases or conditions, including asthma, diabetes, epilepsy, psychoses, depression and HIV infection. Because of the large diversity in genetic polymorphisms of drug metabolizing enzymes, it is of utmost importance to recognize the impact of these variations on xenobiotic metabolism.

When an enzyme is responsible for most of the metabolism of a certain drug and thus pharmacokinetics of the drug solely depend on the catalytic activity of this enzyme, then polymorphic changes in that enzyme may translate into important functional metabolic and pharmacokinetic variations that are of clinical relevance. Furthermore, multiple drug therapy increases the likelihood of inhibition or induction of drug metabolizing enzymes activities, thereby inducing more dramatic clinical implications.

Consequently, phenotyping or genotyping may help to individualize drug dosing according to the patient's requirements. They are of greatest value when an enzyme that metabolizes a large variety of commonly used medications displays polymorphic catalytic activity. The polymorphic changes in enzymatic activity are clinically significant when we are considering long term treatment with a drug with narrow therapeutic index; when the enzyme involved constitutes the main pathway for the metabolism of a drug, whether it is responsible for detoxification of the drug or its
biotransformation into active metabolites; when the major clearance mechanism is the metabolism of the agent and other elimination pathways provide a minor contribution to the drug's clearance; when metabolism is the major pharmacokinetic factor affecting drug concentrations in plasma rather than drug absorption, distribution or excretion; when the enzyme is subject to environmental pressure such as concomitant use of drugs that display enzyme induction or inhibition properties; and finally when drug concentration utterly governs therapeutic response or toxicity which implies that pharmacodynamic variability should play a negligible role in this situation.

All the considerations described above should motivate health care professionals for phenotyping or genotyping individuals in order to facilitate drug selection and dosage and to improve therapeutic outcome and safety of medications for appropriate management of diseases when no alternative drugs are available. The clinical usefulness of pharmacogenetic monitoring depends on the balance between the individual patient and the condition being treated, the expense of treating the patient versus the expense of avoidable errors or the severity of insufficient management. In addition, decision between phenotyping versus genotyping should be made carefully regarding test selection and predictive power.

Very few hitherto enzymes fulfill the previously mentioned criteria; hence an enzyme that displays such properties is of great significance to the field of pharmacogenetics.

The polymorphic N-acetyltransferase or acetylation status influences the efficacy and the toxicity of a number of drugs including isoniazid, procainamide, hydralazine and
sulfonamides. Today, however, the use of most of these agents is becoming limited due to the availability of alternative medications. Procainamide prescriptions are decreasing nowadays owing to the existence of a wide variety of antiarrhythmics with similar or different properties and a more advantageous safety profile. The use of sulfasalazine in the management of inflammatory bowel disease is now limited in order to avoid sulfa drug adverse effects and because of the availability of sulfa free aminosalicylic acid derivatives and other forms of immunomodulation. Finally, with the emergence of diverse antituberculosis medications, treatment with isoniazid had decreased; however, its use has been considered recently for the prophylaxis for tuberculosis. 98

Plasma cholinesterase is also an enzyme that displays polymorphic properties and is responsible for the metabolism of cocaine as well as the neuromuscular junction blockers, succinylcholine and mivacurium. Pseudocholinesterase deficiency has been associated with cocaine toxicity nevertheless cocaine use is limited to topical anesthesia, vasoconstriction of mucous membranes and illegal practice among drug addicts where screening for safety by phenotyping or genotyping is not a priority for the user and not of importance nowadays compared to more concerning issues such as HIV or STD transmission. In addition, succinylcholine use in anesthesia has declined spectacularly although mivacurium remains part of the newer clinical practice and cases of apnea have been reported following anesthesia induced by mivacurium. 99 Nonetheless, clinical consequences of prolonged apnea are not of major concern today because of the availability of adequate mechanical ventilation equipment to facilitate its management.
Consequently, there does not seem to be convincing reasons of the usefulness of acetylation or cholinesterase status testing. In addition, the testing is considered inconvenient and expensive; the clinical benefits are restricted to a small number of medications still under common use and therefore do not outweigh the costs of testing.

Cytochrome P450 3A4 is responsible for the metabolism of more than 50% of all drugs. Its catalytic activity varies from 10 to 100 fold across a population therefore predicting metabolic activity of CYP3A4 in certain individuals prior to initiation of therapy could be of major interest. Genotyping for CYP3A4 allele variants is still unfounded since the few polymorphic changes discovered until now are not enough to provide the rationale for genetic testing of CYP3A4. Therefore, only phenotyping is available, using probe drugs such as erythromycin, midazolam and omeprazole. Phenotyping however, is not very practical because of the use of pharmacologically active drugs, as well as the necessity for an unethical washout period to avoid the influences of drug interactions, and cumbersome sample collection in addition to the availability of therapeutic drug monitoring which consists of following the actual drug concentrations in plasma rather than estimating the metabolic capacity of a patient’s drug metabolizing enzymes using another drug and extrapolating the results gathered, then applying them to the treatment drug.

Cytochrome P450 2C19 is responsible for the clearance of several commonly used medications such as omeprazole and other proton-pump inhibitors, diazepam, propranolol, imipramine and amitriptyline. Phenotyping of CYP2C19 with mephenytoin is limited because of concerns about the use of the probe drug,
inadequate results due to low urinary metabolite concentration, and poor stability of the metabolites in urine that are cumbersome and impede proper testing.

Thiopurine methyltransferase represents one of the major enzymes where pharmacogenetic screening would be of utmost value. The occurrence of fatal bone marrow suppression is a major concern when homozygous patients for the defective allele are treated with azathioprine. The benefit from avoiding the fatal outcome of the adverse effect, aside the cost of additional therapy such as antibiotics, colony stimulating factors, blood and blood product transfusions, and hospitalization of an individual, outweighs the cost of screening.

CYP2D6 displays properties that are of particular interest to the field of pharmacogenetics. CYP2D6 is responsible for the metabolic elimination of 25 to 30% of all therapeutic drugs, among them 50 of the 100 best selling drugs in the United States, as mentioned previously.

CYP2D6 polymorphisms often translate into clinical relevance. However, some substrates of CYP2D6 such as beta-blockers have a large therapeutic window with generally mild adverse effects and therefore genetically induced alterations in their metabolic clearance will not cause significant implications when they are administered alone. Yet, if given with enzyme inhibitors, the resulting pharmacokinetic interactions might lead to the occurrence of adverse effects associated with the medications.
In contrast, some neuroleptics and antidepressants have a narrow therapeutic index and accumulate dramatically in poor CYP2D6 metabolizer phenotypes causing adverse effects that may lead to toxicity and death.\textsuperscript{100,101} Also, extensive metabolizer phenotypes often experience therapeutic failure, a major concern in depressive inpatients for example, due to extremely subtherapeutic drug concentrations in plasma as a result of excessive clearance by the highly active cytochrome enzyme.\textsuperscript{102}

Pharmacogenetic screening for CYP2D6 polymorphisms is therefore of extreme importance, yet no comprehensive cohort studies have been published to address the medical and financial value of the screening. Reasons for that include the low prevalence of polymorphisms that require large study populations to demonstrate positive outcome. The excessive funding required hinders the study of currently marketed drugs; conversely, pharmacogenetic screening for CYP2D6 polymorphic alleles may become part of the investigations performed during the development of a new medication.

CYP2D6 phenotyping using probe drugs such as debrisoquin or acetaminophen allows for prediction of metabolic capacity in most cases; however, genotyping is more attractive because it can detect up to 99% of functionally important changes of CYP2D6 gene, it is not affected by co-administered medications, it is specific for the mutation and requires the collection of only one blood sample.\textsuperscript{103-105}

Nevertheless, the financial impact of genotyping is the major factor that can influence the decision to screen individuals for polymorphic CYP2D6 alleles. Chen et al. have
suggested that the cost for genotyping CYP2D6 is about one hundred US dollars for one test. Yet, high throughput systems can diminish the cost to 8-10 US dollars per test. An average of one million patients receive tricyclic antidepressants (TCAs) in the United States, therefore pharmacogenetic screening if these patients will cost 8 million US dollars in minimum and up to 100 million US dollars as a maximum. Furthermore, Chou et al. estimated that the additional cost of treatment due to adverse reactions in poor metabolizers or because of therapeutic failure in ultrarapid metabolizers is between 4000 and 6000 US dollars; 5000 US dollars in average. Assuming that 5-10% of patients on TCAs are poor or ultrarapid metabolizers, additional treatment costs lay in the range of 250 to 500 million US dollars. In other words, although this is only an estimate, yet it represents solid ground for considering pharmacogenetic screening of CYP2D6 polymorphism.

In light of the aforementioned observations, CYP2D6 phenotyping of the Lebanese population appeared to be an interesting initiative in order to estimate the incidence of CYP2D6 polymorphism in this population and to evaluate the need for genotyping as a subsequent step in specific individuals.
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CHAPTER TWO
CYTOCHROME P450 2D6

2.1 CYP2D6 GENOTYPES AND PHENOTYPES

The CYP2D locus is localized on human chromosome 22 and normally consists of the active gene CYP2D6 and the two pseudogenes CYP2D7P and CYP2D8P. Genetic polymorphisms of CYP2D6 with more than 75 allelic variants have been identified since the nomenclature for human CYP2D6 alleles has been suggested by Daly et al. Allelic mutants result from point mutations, single base-pair deletions insertions or additions, gene rearrangements, deletion of the entire gene and gene duplication or multiplication. These mutations cause a reduction, alteration or complete loss of enzymatic catalytic activity, as well as an increase in the metabolic capacity of the enzyme.

Athanassiadis et al. were the first to report a phenotypic polymorphism of CYP2D6. Previously known as debrisoquin 4-hydroxylase, it was discovered during clinical studies on debrisoquin, a sympatholytic antihypertensive agent. Patients possessing a CYP2D6 polymorphism required a lower dose for blood pressure control, and they showed low recovery of metabolized debrisoquin in their urine. Mahgoub et al. later confirmed that this interindividual variability resulted from alterations in the metabolic capacity of CYP2D6 in different individuals and was not a consequence of other pharmacokinetic factors such as absorption or renal elimination.
The debrisoquin hydroxylation capacity was described as the metabolic urinary ratio between the parent drug and its hydroxylated metabolite and was found to follow a bimodal distribution in European and other Caucasian populations; allowing to recognize two main subsets: the extensive and poor metabolizers of debrisoquin.\textsuperscript{7,8}

Poor metabolizers of CYP2D6 are homozygous carriers of two nonfunctional alleles and the phenotype is a consequence of an autosomal recessive trait inheritance.\textsuperscript{4}

Extensive CYP2D6 metabolizers are either heterozygous or homozygous for the normal or wild type $CYP2D6$ allele, $CYP2D6^{*1}$.\textsuperscript{9}

The first evidence of genetic polymorphism for the $CYP2D6$ gene was the observation of homozygous $CYP2D6$ deletion ($CYP2D6^{*5}$ allele) by Southern transfer in individuals demonstrating the poor metabolizer phenotype for debrisoquin hydroxylase.\textsuperscript{10} Subsequent to the identification of the $CYP2D6^{*5}$ allele, studies were conducted in order to correlate the presence of this mutant allele in an individual’s genotype with the poor metabolizer phenotype. However, they were partially successful because the $CYP2D6^{*5}$ allele explained the poor metabolizer phenotype in some individuals but other poor metabolizers had an apparently homozygous genotype for the wild type or normal allele, suggesting that other hitherto unidentified mutations might be responsible for the expression of the genotype into the poor metabolizer phenotype in these individuals.

Several allelic variants have since been identified;\textsuperscript{3,4,5} among which the most common mutant alleles are: $CYP2D6^{*2}$, $CYP2D6^{*3}$, $CYP2D6^{*4}$, $CYP2D6^{*5}$, $CYP2D6^{*9}$, $CYP2D6^{*10}$, and $CYP2D6^{*17}$. 
The \textit{CYP2D6}^{*}3 allele consists of a frameshift mutation resulting from a single adenine deletion in exon 5 leading to the formation of a premature stop codon. \textsuperscript{11} \textit{CYP2D6}^{*}4 mutation results from a G- to A- transition in the last nucleotide of intron 3, producing a splicing defect and a subsequent frameshift in the open-reading frame and premature stop codon. \textsuperscript{11,12} The \textit{CYP2D6}^{*}5 allelic variant is produced as a consequence of the deletion of the entire \textit{CYP2D6} gene. \textsuperscript{11,13} The molecular genetic basis for the poor metabolizer phenotype consists of the occurrence of combinations of any of these defective alleles resulting in complete loss of enzyme metabolic activity.

Reduced \textit{CYP2D6} enzyme capacity has been described in association with allelic variants such as \textit{CYP2D6}^{*}9, \textit{CYP2D6}^{*}10 and \textit{CYP2D6}^{*}17. \textit{CYP2D6}^{*}9 allele results from a three base pair deletion; \textsuperscript{14} \textit{CYP2D6}^{*}10 is created from a proline and serine amino acid substitution and results in altered protein folding and enzyme substrate specificity; \textsuperscript{15,16} and \textit{CYP2D6}^{*}17 occurs in response to three mutations predominantly the substitution of threonine by isoleucine also producing an enzyme with altered substrate specificity. \textsuperscript{17} These mutations are not responsible for the expression of the poor metabolizer phenotype however, they might explain the occurrence of an intermediate metabolizer phenotype that lies on the borderline of the extensive metabolizer group showing reduced enzyme catalytic activity yet not behaving exactly like the poor metabolizer status. \textsuperscript{18}

The ultrarrapid metabolizer phenotype is another identified subset that displays excessively increased enzyme metabolic activity due to an inherited autosomal dominant trait arising from functional gene amplification (\textit{CYP2D6}^{*}2 allele
duplication or multiplication). This allelic variant contains two mutations causing amino acid exchanges: Arg→Cys and Ser→Thr. It is believed to display reduced activity compared to the wild type allele CYP2D6*1 when it occurs in one copy whilst it is responsible for increased metabolism when duplication or multiplication occurs. The gene effect is dramatic and clearance of CYP2D6 substrates is proportional to the number of CYP2D6 gene copies. Two, three and up to thirteen gene copies have been reported in the literature.

2.2 INTERETHNIC VARIABILITY IN CYP2D6 POLYMORPHISM

The metabolic activity of cytochrome P450 2D6 was found in many studies to be bimodally distributed in Europeans and other Caucasian populations. Phenotyping investigations in Caucasians have consistently shown that 5-10% of the population are poor metabolizers with high metabolic ratios for probe drugs such as debrisoquin, whilst in Orientals only 0-1% of populations display poor metabolizing behavior. There is a great variation in prevalence of poor metabolizers in African populations ranging from 0% to 19% presumably an indication of the ethnic heterogeneity on the continent. In general, black Africans (mostly west, central and southern African people) exhibit a 0-2% prevalence of poor metabolizers. The frequencies of the poor metabolizer phenotype in different populations or ethnic groups are presented in Table 5.
<table>
<thead>
<tr>
<th>Ethnic group or country</th>
<th>Number of Subjects</th>
<th>Frequency of poor metabolizers (%)</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td><strong>Amerindians</strong></td>
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<td></td>
</tr>
<tr>
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<td></td>
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<td>8.7</td>
<td>58</td>
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<tr>
<td>USA</td>
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<td>6.1</td>
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Table 5: Poor metabolizer phenotypes among different ethnic groups (cont’d)

<table>
<thead>
<tr>
<th>Ethnic group or country</th>
<th>Number of Subjects</th>
<th>Frequency of poor metabolizers (%)</th>
<th>Reference</th>
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<td><strong>Eskimos</strong></td>
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<td>185</td>
<td>3</td>
<td>60</td>
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<tr>
<td>East Greenland</td>
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<td>3.3</td>
<td>61</td>
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<tr>
<td>West Greenland</td>
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<td>61</td>
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<tr>
<td>Canadian Inuit</td>
<td>90</td>
<td>3.3</td>
<td>62</td>
</tr>
<tr>
<td><strong>Blacks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burundi</td>
<td>100</td>
<td>5</td>
<td>63</td>
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<td>64</td>
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<td>66</td>
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<td>75</td>
</tr>
<tr>
<td>Sinhalese</td>
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<td>0</td>
<td>76</td>
</tr>
</tbody>
</table>

Besides variation in the proportion of poor metabolizers in different ethnic groups and populations, the mean value of metabolic ratios (MRs) in extensive metabolizers also fluctuates between different populations studied. The mean value of metabolic ratios in Oriental extensive metabolizers is higher than that in Caucasians, suggesting a lower average CYP2D6 activity in Orientals. This tendency of Oriental populations to have higher metabolic ratios of probe drugs has been termed the “right shift” in the
frequency distribution and is also characteristic of most African populations phenotyped. Median values of metabolic ratios for debrisoquin for instance, are about 0.6 in Caucasians and 1.0 in Orientals. Furthermore, with the exception of Egyptians who display a median value of 0.6, studies of African populations have reported a median value varying from 0.8 to 1.5, and averaging 1.0.  

Another apparent interethnic difference is the 1000-fold variation (0.01-10.0) in metabolic ratio in Caucasian extensive metabolizers with a group of people with very low metabolic ratios. Among Caucasian extensive metabolizers, this subgroup of ultrarapid metabolizers (debrisoquin MRs<0.2) exists but this phenomenon has not been observed in Orientals and most African populations. Interestingly, Ethiopians and Egyptians have a subgroup of ultrarapid metabolizers.  

The metabolism of the CYP2D6 probe drugs, sparteine, metoprolol, debrisoquin and dextromethorphan cosegregates in Caucasian and Oriental populations. In some African populations, however, poor correlations have been reported and have been attributed to possible poor subject compliance during phenotyping, interfering drugs or dietary components and the existence of CYP2D6 variants with differing catalytic activity towards these compounds. 

The discovery of a number of allelic variants described previously has allowed for setting the molecular genetic basis for the different CYP2D6 phenotypes in Caucasians and Orientals, and to a lesser extent in African populations.  In Caucasians, the genetic basis for the poor metabolizer phenotype has been explained by the presence of a combination of defective alleles mainly the CYP2D6*4,
CYP2D6*5 and CYP2D6*3. The CYP2D6*2 variant has approximately similar activity to the wild-type CYP2D6*1 allele but has a tendency to duplicate or multiply. Subjects with the duplicated or multiplied gene have very high CYP2D6 activity and accounted for 40% of the ultrarapid metabolizers in a Swedish study conducted by Dahl et al. The major allelic variants of CYP2D6 are listed in Table 6 along with their frequency of distribution in a number of different ethnic groups or populations.

<table>
<thead>
<tr>
<th>CYP2D6 activity</th>
<th>Caucasian</th>
<th>Oriental</th>
<th>Saudi Arabian</th>
<th>African American</th>
<th>Zimbabwean</th>
<th>Ethiopian</th>
</tr>
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<tbody>
<tr>
<td>Increased</td>
<td>1.0-2.0</td>
<td>0</td>
<td>10.4</td>
<td>2</td>
<td>1</td>
<td>16</td>
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<td>(CYP2D6*2)n&gt;1</td>
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<td>0</td>
</tr>
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</tr>
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<td>5</td>
<td>5</td>
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<td>26</td>
<td>34</td>
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</tr>
<tr>
<td>Absent</td>
<td>2</td>
<td>0</td>
<td>0.2-0.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>CYP2D6*3</td>
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<td>7.3-8.5</td>
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<tr>
<td>% of PM</td>
<td>5.0-10.0</td>
<td>0-1</td>
<td>1.0-2.0</td>
<td>1.9</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

The CYP2D6 locus in Orientals has a number of differences from that of Caucasians. First, Orientals do not have the CYP2D6*3 and CYP2D6*4 mutant variants, hence the low prevalence of poor metabolizers. The CYP2D6*5 exists at a frequency similar to that in Caucasians and Africans and accounts for the few poor metabolizers in Orientals. A CYP2D6 mutant variant CYP2D6*10 was determined as being the
basis of diminished CYP2D6 activity in Oriental populations with the mutant gene resulting in the production of a low activity and unstable enzyme. In Oriental populations, CYP2D6*10 is the major mutant allele and it exists at a high frequency of 50% compared to a 5% frequency in Caucasians hence explaining the higher median of metabolic ratios and the "right shift" in the frequency distribution in the former populations. Duplication of the CYP2D6*2 gene has been reported in a low frequency of 0.3% in the Japanese population; duplication of the CYP2D6*10 allele has also been observed however in a frequency of 0.6%, a value a great deal lower than the incidence of the single allele.

In African populations, fewer genotyping studies have been done. Genotyping of Zimbabweans, Ethiopians and Tanzanians indicate the absence of the detrimental CYP2D6*3 allele and low prevalence of the CYP2D6*4 hence explaining the low prevalence of poor metabolizers in these populations. The CYP2D6*5 variant occurred at a frequency similar to that found in all other ethnic groups genotyped so far. This implies that this allelic variant predates the evolutionary split of Caucasian, Oriental and African populations. This is in contrast to the CYP2D6*3 and CYP2D6*4 which appear to be Caucasian specific. An exception to the general ethnic pattern for these alleles is found in African-Americans with the CYP2D6*3 allele being present at a frequency of 0.6% and CYP2D6*4 at 7.3% presumably due to intermarriage with Caucasians.

The CYP2D6*10 which explains the "right shift" in metabolic ratios (CYP2D6 low activity) in Chinese was found at a very low frequency of 5% in the Zimbabweans.
and Tanzanians although it was found at a higher frequency (12.5%) in a study of Ghanaians but the number of individuals in this latter study was low (n=21). 78,84-86

Phenotyping and CYP2D6 genotyping led to the discovery of a mutant variant of the CYP2D6 gene in Zimbabweans designated CYP2D6*17. 17 This allelic variant has also been found in the South African Venda and in Tanzanians at an allele frequency of 18-20%, and in Ghanaians with an incidence of 15%. 78,85,86 The CYP2D6*17 was not found in Swedish and Chinese subjects analyzed by Masimirembwa et al. 17 and only one allele out of 1344 was found in another study of Europeans. 88 Since the Zimbabwean population is genetically related to most central and southern African populations, the CYP2D6*17 variant was postulated to be the basis of diminished debrisoquin hydroxylase activity in these populations. 17,84 The presence of the CYP2D6*17 allele is associated with higher metabolic ratios for debrisoquin in phenotyping studies. 17,84,87

Phenotyping results in most African populations have not shown any ultrarapid metabolizers. Genotyping of Zimbabweans, on the other hand showed two subjects having the duplicated CYP2D6*2 but these also carried the detrimental CYP2D6*4 mutation in the locus. 83,84 In a genotyping study done in Ghanaians, none had the duplicated or amplified gene status. 89

Phenotyping and genotyping studies in Ethiopians showed the most interesting admixture of CYP2D6 mutant alleles observed in one population so far. 77 First, the phenotyping studies showed a low prevalence of poor metabolizers which was accounted for by the infrequent occurrence of the CYP2D6*4 variant and absence of
the \( CYP2D6*3 \) variant. Second, most of the subjects phenotyped as extensive metabolizers showed a distribution of debrisoquin metabolic ratios similar to that of Zimbabweans and other African populations, that is, higher mean metabolic ratios compared to Caucasians. Most of the subjects with diminished \( CYP2D6 \) activity were accounted for by the \( CYP2D6*10 \) and the \( CYP2D6*17 \) (9% allele frequency in each case). \(^{77}\) Third, like Egyptians and Caucasian populations, the phenotyping results showed a subgroup of ultrarapid metabolizers (7%) with a debrisoquin MR below 0.2. These subjects had multiple copies (3-5) of the \( CYP2D6*2 \) gene hence explaining the high \( CYP2D6 \) activity with multiplied allele frequency reaching 29%. It must be noted, however, that some subjects carrying the duplicated \( CYP2D6*2 \) and no other known detrimental mutations had relatively high MRs. \(^{77}\) This is suggestive of the possible existence of other hitherto unidentified mutant alleles responsible for the decreased metabolic capacity of the enzyme.

Saudi Arabian, neighbors to Ethiopians, have also been shown to have a high frequency (18%) of duplicated \( CYP2D6*2 \) alleles. \(^{90}\) It is therefore obvious that focus for \( CYP2D6 \) gene duplications has been in Ethiopia and Saudi Arabia with 20-30% of the population having this genotype; an unexpected high frequency that can only be explained by the occurrence in the past of a dietary selective pressure that favored the preservation of the duplicated genes. Furthermore, although a low frequency of gene multiplications has been reported in Asia and Europe, genotyping for \( CYP2D6 \) alleles in Spaniards has yielded a surprisingly elevated frequency of \( CYP2D6*2 \) allele multiplication of approximately 7%. \(^{91}\) This is most likely the result of an Arab migration through Gibraltar about 700 A.D.
2.3 CLINICAL IMPLICATIONS OF CYP2D6 POLYMORPHISM

2.3.1 Impact of CYP2D6 polymorphism on substrate drugs

Cytochrome P450 2D6 is responsible for the clearance of 25-30% of all clinically used medications and has been associated with the metabolism of over 50 of the 100 best selling drugs in the US. Clinical implications of CYP2D6 polymorphism have been investigated and reviewed thoroughly with special attention paid to cardiovascular and neuroactive medications because a large proportion of these drugs are substrates of CYP2D6.

Major substrates of CYP2D6 are those medications with oral absorption greater than 90%, for which CYP2D6 metabolism represents between 60-100% of the clearance of an oral dose into its different metabolites and they include desipramine, venlafaxine, encainide, metoprolol, propafenone and tolterodine; whereas minor substrates are also readily absorbed after oral administration but CYP2D6 metabolism accounts for 10-60% of their excretion. Minor substrates include amitriptyline, imipramine, nortriptyline, mexiletine, propranolol, sparteine, codeine, dihydrocodeine and hydrocodone.

Three decades ago, Idle and Smith reported interesting findings concerning the CYP2D6 enzyme polymorphism, previously known as debrisoquin hydroxylase. They noticed that the antihypertensive agent debrisoquin, released on the British pharmaceutical market but never approved in the United States, caused an unexpectedly and remarkably high incidence of side effects considered at that time as idiosyncratic drug reactions. Smith reasoned that an underlying genetic variation in
the metabolism of the drug might be responsible for this high incidence of undesired outcomes. Consequently, Smith and three of his colleagues ingested the prescribed standard dose of debrisoquin and measured the levels of its metabolites in urine. Besides his becoming hypotensive, Smith also noted that his urinary 4-hydroxy metabolite of debrisoquin was excreted in approximately 20-fold lower concentrations than his three colleagues, despite his intake of the recommended standard dose of debrisoquin.95

Today, poor metabolizers of CYP2D6 account for 5-10% of Caucasian populations whereas they occur in much lower frequencies in Orientals (0-1% of the population). Poor metabolizer individuals usually excrete a large fraction of CYP2D6 substrates unmetylated due to the absent or poor catalytic activity of the enzyme. This results in accumulation of the parent active compound and increases the likelihood of adverse drug reactions.

On the other hand, extensive CYP2D6 metabolizers who are either homozygous for the wild-type allele or heterozygous for one of the defective alleles, metabolize CYP2D6 substrates adequately and reach drug plasma concentrations lying in the desired range with subsequently appropriate response to therapy and favorable outcome. Some of the individuals in this group are classified as “intermediate metabolizers” because they display higher metabolic ratios than those of extensive metabolizers, yet remaining lower than the cut-off point (antimode) separating extensive from poor metabolizers. These individuals usually exhibit mutations of the CYP2D6 gene that encode for an enzyme with reduced activity such as the CYP2D6*10 and CYP2D6*17 alleles found in Oriental and African populations.
Finally, a subset of ultrarapid metabolizers experience excessively enhanced drug metabolism hence they do not reach therapeutic concentrations consequently failing to achieve therapeutic response. Figure 4 illustrates this concept by displaying the functional relevant polymorphisms of CYP2D6 and their clinical consequences.

Figure 4: Functional CYP2D6 polymorphisms and their clinical consequences

Genetic polymorphism of CYP2D6 however, does not result in significant clinical implications in all instances. The clinical relevance of the genetic variability in metabolizing CYP2D6 substrates depends markedly on the therapeutic class of the compound, on the availability of alternate pathways for metabolism, and on the concentration-effect relationship of the desired and unwanted effects.
In other words, this monogenetically determined alteration in drug metabolism becomes clinically significant only in those cases where pharmacokinetic variability is considered important relative to the efficacy and clinical safety of the drug. Thus, treatment with drugs that depend solely on CYP2D6 metabolism for their clearance and display a narrow therapeutic index that prohibits large variations in steady-state blood concentrations is more likely to be affected by CYP2D6 polymorphism. This is applicable for drugs for which therapeutic drug monitoring is considered useful.

Tricyclic antidepressants and to a lesser extent selective serotonin reuptake inhibitors (SSRIs) represent the most thoroughly studied classes of drugs in relation to CYP2D6 polymorphism. In clinical practice, 30% of the patients do not respond to antidepressants, and doses required may vary up to 50-fold. 96 Therapeutic drug monitoring may decrease the proportion of nonresponders to 10-20%, which indicates that there is a relationship between drug concentration and clinical effects.

Differences in metabolism between individuals have long been recognized and are increasingly demonstrated to be of clinical significance. In general, there is a 2-5 times difference between poor and extensive metabolizers in the capacity to metabolize CYP2D6 substrates; hence poor metabolizers achieve the same steady-state serum concentrations as extensive metabolizers at doses which are 50-80% lower than those administered to individuals with the extensive metabolizer phenotype. 96
Genetic variability in the metabolism of the following antidepressants has been reported: desipramine, fluoxetine, fluvoxamine, maprotiline, mianserin, nortriptyline, paroxetine, venlafaxine, amitriptyline, imipramine and clomipramine. \(^{94,97-109}\)

Poor metabolizers typically display slower metabolic rates than extensive metabolizers with marked elevations in drug plasma concentrations and low metabolite recovery in urine. At therapeutic doses, they experience a high incidence of side effects due to the antidepressants compared to the extensive metabolizer group.

Conversely, ultrarapid metabolizers with gene duplications and multiplications have extremely low metabolic ratios of parent versus metabolite concentrations. These patients fail to reach therapeutic serum concentrations of antidepressants when given in the recommended doses for the general population. Case reports have illustrated these observations. Bertilsson et al. reported a patient with a debrisoquin metabolic ratio of 0.07 who had to be treated with 500 mg nortriptyline daily (3-5 times the recommended dose) to achieve desired serum concentrations and appropriate therapeutic outcome, whilst another patient received 300 mg clomipramine daily (2-12 times the recommended dose) for successful outcome. \(^{110}\) On genetic analysis, both patients were found to have a duplicated \textit{CYP2D6}\textsuperscript{*2} allele. \(^{110}\)

All the antidepressants aforementioned are primarily metabolized by the cytochrome P450 2D6 except amitriptyline, imipramine and clomipramine, which are substrates of CYP2D6 and CYP2C19. Some display non-linear pharmacokinetics at therapeutic doses due to metabolism by CYP2D6 that constitutes the high affinity/low capacity
pathway. This pattern is only observed in extensive metabolizer individuals because of enzyme saturation, but not in poor metabolizers who lack this functional isozyme. 99,100,102

Currently, preliminary dosage recommendations based on CYP2D6 genotypes are available for antidepressants (Table 7). 100 This allows for the demonstration of the benefits generated from pharmacogenetic screening prior to initiation of drug therapy in terms of avoiding the occurrence of undesired adverse effects in poor metabolizers or even intermediate metabolizers and preventing therapeutic failure in ultrarapid metabolizers. Prospective studies however are warranted to address the influence of these genotype-based recommendations on therapeutic outcome.

Table 7: Preliminary antidepressant dosage recommendations based on CYP2D6 genotypes

<table>
<thead>
<tr>
<th>Drug</th>
<th>Maintenance/Single Dose</th>
<th>Usual Dose (mg)</th>
<th>Ultrarapid metabolizers</th>
<th>Extensive metabolizers</th>
<th>Intermediate metabolizers</th>
<th>Poor metabolizers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>MD</td>
<td>150</td>
<td>120%</td>
<td>90%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>50</td>
<td>120%</td>
<td>80%</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>Clomipramine</td>
<td>MD</td>
<td>150</td>
<td>120%</td>
<td>90%</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>50</td>
<td>120%</td>
<td>90%</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td>Desipramine</td>
<td>MD</td>
<td>150</td>
<td>130%</td>
<td>30%</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>50</td>
<td>230%</td>
<td>280%</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>SD</td>
<td>20</td>
<td>120%</td>
<td>80%</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>MD</td>
<td>50</td>
<td>120%</td>
<td>90%</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>100</td>
<td>110%</td>
<td>100%</td>
<td>90%</td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td>MD</td>
<td>150</td>
<td>130%</td>
<td>80%</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>50</td>
<td>110%</td>
<td>100%</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td>Maprotiline</td>
<td>MD</td>
<td>150</td>
<td>130%</td>
<td>80%</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>Mianserin</td>
<td>MD</td>
<td>60</td>
<td>110%</td>
<td>90%</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>30</td>
<td>300%</td>
<td>110%</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>MD</td>
<td>150</td>
<td>120%</td>
<td>90%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>50</td>
<td>230%</td>
<td>140%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Paroxetine</td>
<td>MD</td>
<td>20</td>
<td>110%</td>
<td>90%</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>20</td>
<td>130%</td>
<td>70%</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>MD</td>
<td>150</td>
<td>130%</td>
<td>80%</td>
<td>20%</td>
<td></td>
</tr>
</tbody>
</table>
Like tricyclic antidepressants, antipsychotics are known to have a very narrow therapeutic window. Also, it is of general agreement that usually relatively low concentrations are required for a therapeutic effect; higher concentrations only increase the risk of extrapyramidal side effects without further increase in antipsychotic activity.\textsuperscript{111} If not identified, poor CYP2D6 metabolizers may thus be treated with unnecessarily high doses of neuroleptics, resulting in an increased risk of undesired adverse effects which may in turn lead to decreased patient compliance with the treatment.\textsuperscript{112}

In panel studies with haloperidol and zuclopenthixol, poor metabolizers experienced more adverse effects than extensive metabolizers of debrisoquin at assumed subtherapeutic doses leading to the need for administering half the dose to poor metabolizer individuals in these studies. At these halved doses, the poor metabolizers still achieved therapeutic plasma concentrations comparable to the extensive metabolizers taking a double dose.\textsuperscript{113,114}

Brockmoller et al. also demonstrated that pharmacokinetics of haloperidol and its metabolite, reduced haloperidol, as well as the occurrence of extrapyramidal side effects depended significantly of the CYP2D6 genotype.\textsuperscript{115} A large proportion of the patients in the study had haloperidol plasma levels outside the therapeutic range; 34% of patients had subtherapeutic levels whereas 11% of them showed concentrations exceeding the therapeutic limit. Also, patients with reduced metabolic rates were at greater risk of experiencing extrapyramidal side effects.
Other adverse effects associated with antipsychotic and neuroleptics agents include severe cardiotoxicity reported with thioridazine treatment. In poor metabolizers of debrisoquin, thioridazine metabolism into mesoridazine is impaired resulting in the accumulation of the parent compound and in formation of an alternative metabolite, the ring sulfoxide in greater concentrations. The ring sulfoxide seems to be the most cardiotoxic metabolite of thioridazine.

In a retrospective study of the CYP2D6 status in patients who had experienced neuroleptics-induced side effects, an overrepresentation of poor metabolizers was also found.

Similarly to tricyclic antidepressants, subtherapeutic concentrations of the antipsychotic risperidone were reported in two subjects who were subsequently genotyped and found to bear multiplied CYP2D6*2 alleles and therefore pertain to the ultrarapid metabolizer subgroup.

The relatively slow extensive metabolizers or intermediate metabolizers should be considered separately. Orientals and most Africans will on average metabolize CYP2D6 substrates at a slower rate compared to Caucasian populations as a result of the mutations that lead to the “right shift” in the frequency distribution of metabolic ratios for probe drugs. From empirical observations of response to medications and side effects, clinicians in Oriental populations have been prescribing antipsychotics at lower doses than in Caucasians. Reasons for this practice are possible differences in other pharmacokinetic parameters or receptor-drug interactions; however, the difference in metabolism of the drugs remains the basis of lower prescription doses in
the Chinese. As a result of such findings, the Japanese regulatory authorities do not depend on pharmacokinetic studies performed in Caucasians to optimize drug therapy but insist that they be done with Japanese subjects. 

The debrisoquin phenotype influences the kinetics and pharmacologic effects of several antiarrhythmic agents, including encainide, flecainide and propafenone. Propafenone, a sodium-channel blocker used as antiarrhythmic, is metabolized via a CYP2D6-mediated pathway to 5-hydroxypropafenone, which is equipotent to the parent drug with respect to sodium-channel blocking activity. Markedly elevated plasma concentrations of propafenone and decreased concentrations of the hydroxy metabolite are seen in subjects with the poor metabolizer phenotype compared with extensive metabolizers of debrisoquin with a consequently higher incidence of propafenone-related adverse effects such as nausea, vomiting and arrhythmias. In addition, propafenone displays non-linear pharmacokinetics in extensive metabolizers due to saturation of enzymatic activity. When given as a racemate, propafenone is metabolized extensively in extensive metabolizers: more than 95% of the dose is cleared.

Encainide metabolism is also dependent on CYP2D6 activity. O-demethylation resulting in the O-desmethylencaainide and its 3-methoxy derivative is mediated by CYP2D6 and accounts for more than 80-90% of the elimination of intravenous encainide in extensive metabolizers. In contrast, more than 80% of the parent drug is excreted unchanged in the urine of poor metabolizers. However, antiarrhythmic
responses are similar in the two phenotypes, perhaps because antiarythmic effect is mediated by encainide itself in poor metabolizers and by its metabolites in extensive metabolizer phenotypes. The metabolism of oral encainide follows the same kinetics as the intravenous form. 125

Finally, flecainide kinetics were studied by Mikus et al. in order to observe the correlation with CYP2D6 polymorphism. 122 Renal clearance of flecainide was found to be similar in poor and extensive metabolizers thereby decreasing the likelihood of interference of fluctuations in the renal elimination of flecainide in different phenotypes. Despite similar renal clearance, flecainide metabolic clearance was diminished in poor metabolizer individuals; the terminal half-life was therefore longer, and both the serum concentrations achieved and the area under the curve for flecainide were higher. The lower metabolic capacity of poor metabolizers resulted in a larger proportion of the dose excreted as unchanged drug in the urine. Those findings, although significant, did not result in any severe implications in therapeutic outcome.

In a study conducted by Tenneze et al., QRS prolongation did not correlate with CYP2D6 activity after administration of flecainide. 123 However, in patients with renal disease, the half-life of flecainide is further prolonged. Poor metabolizers with impaired renal function will therefore have a restricted capacity to eliminate flecainide both renally and hepatically. The resulting increase in plasma drug concentrations has the potential to cause arrhythmias, which could, in turn, contribute to patient deaths associated with flecainide administration. 127-130
In contrast to tricyclic antidepressants, antipsychotics and antiarythmic agents, \( \beta \)-blocking agents display a wide therapeutic window and thus can be used almost regardless of the patient's phenotype, as large interindividual variations in steady-state blood concentrations are probably of minor clinical significance and certainly do not warrant blood level monitoring.

The influence of CYP2D6 polymorphism on \( \beta \)-blockers can be illustrated by the disposition of metoprolol, a major substrate of CYP2D6. Elimination of metoprolol by CYP2D6 exhibits stereospecific behavior.\textsuperscript{131-133} Following oral administration of the racemic mixture, significantly higher plasma concentrations of the active S-enantiomer compared to the inactive R-enantiomer were observed in extensive metabolizers suggesting preferential O-demethylation of the R-enantiomer, whereas in poor metabolizers the enantioselective disposition was not significant and maximum plasma concentrations of both enantiomers as well as the elimination half-life were more elevated.

Wuttke et al. have also observed that patients experiencing pronounced adverse effects (sustained \( \beta \)-blockade) during treatment with metoprolol had a 5-fold higher likelihood of being poor metabolizers (38%) compared to the general German population (approximately 7%).\textsuperscript{134}

Similarly to metoprolol, stereoselective disposition of propranolol and carvedilol have been reported in extensive metabolizer phenotypes.\textsuperscript{135,136} In contrast, in poor metabolizers of debrisoquin, total propranolol clearance was not reduced in comparison with extensive metabolizers; production of the 4-hydroxy metabolites was
however decreased, demonstrating the role of CYP2C19 in the metabolic elimination of propranolol that compensated for the poor capacity of CYP2D6 by increasing side chain oxidation thus resulting in decreased metabolites through the CYP2D6 mediated ring oxidation of propranolol. In carvedilol poor metabolizers, the R-enantiomer elimination was significantly decreased with no marked effect on the S-enantiomer suggesting that R-carvedilol metabolism depends predominantly on CYP2D6 whereas S-carvedilol is metabolized by both CYP2D6 and other enzymes in a stereoselective fashion.

Although focus has been on the influence of CYP2D6 polymorphism and the poor metabolizer phenotype on the detoxification of drugs, CYP2D6-mediated bioactivation of specific substrates remains a very important issue to be discussed.

Biotransformation by O-demethylation of codeine into its major active metabolite morphine represents the best example to illustrate this idea. The analgesic effect of codeine predominantly depends on its conversion to morphine. Thus deficiency in morphine production in poor metabolizers may result in a failure of codeine-induced analgesia. Compared to placebo, poor metabolizers did not show any analgesic effect, whereas a significant increase in pain tolerance was observed in extensive metabolizers.

In addition to codeine metabolism, conversion of oxycodone and hydrocodone into their active metabolites oxymorphone and hydromorphone respectively, appears to be dependent on CYP2D6 metabolic capacity. The enzyme may not contribute importantly to the overall clearance of these drugs, but it catalyzes the formation of
highly active metabolites. Enzyme-deficient individuals should have much decreased probability of abusing such medications since formation of the primarily active compounds is markedly reduced. In extensive metabolizers, the probability of abuse should be increased, and proportionate to their genotype (homozygous versus heterozygous) and absolute level of enzyme activity. Conversely, poor metabolizers experience greater risk of toxicity because of accumulation of the parent compound and slow metabolic elimination.

When the differential response to endogenous opiates was considered, poor metabolizers were found to be more sensitive to tonic pain in the cold pressure test than extensive metabolizers whereas pain thresholds to phasic pain were similar in both groups. A higher fraction of poor metabolizers withdrew their hands prematurely from ice water. This can be explained by the relation between different kinds of painful stimuli and response to the analgesic morphine: tonic pain substantially responds to morphine treatment while phasic pain only responds marginally. In poor metabolizers who have a poor ability to convert endogenous codeine into morphine, morphine concentrations are markedly decreased thus resulting in decreased pain threshold to tonic stimuli and not affecting phasic pain. In contrast, extensive metabolizers convert substantial amounts of codeine into morphine indicated by the increase in the pain threshold to tonic stimulation and lower perception of the pain.

All the examples described above have allowed us to highlight a major problem of pharmacotherapy induced by genetic polymorphism in drug oxidation. Metabolic capacity is an individual feature of the respective patient and not a therapeutic
disadvantage of a given drug. Side effects in poor metabolizers or therapeutic failure in ultrarapid metabolizers can be avoided by individualizing drug therapy. Instead of considering them as obstacles to appropriate patient care, side effects or therapeutic failure due to genetically determined alterations in drug metabolism can be defined as consequences of inadequate doses for the individual metabolic capacity.

2.3.2 Inhibition of CYP2D6 and its influence on the different phenotypes

Drug interactions have been divided into those that are pharmacodynamic and those that are pharmacokinetic. Pharmacodynamic interactions occur at the level of mechanism of action of drugs. For example, a delirium may result from combining several drugs with anticholinergic activity, or ataxia and incoordination may be exaggerated by combining alcohol and a benzodiazepine. Pharmacokinetic interactions, on the other hand, involve absorption, distribution, metabolism and excretion of drugs.

The cytochrome P450 enzyme system plays a major role in pharmacokinetic interactions affecting the metabolism of drugs. Since the number of xenobiotics vastly exceeds the number of P450 enzymes, it is fortunate that these enzymes are not highly substrate specific. In other words, these enzymes are non selective. At the same time, there is a certain degree of specificity in that a particular enzyme will preferentially metabolize a particular drug. In the absence of such an enzyme, metabolism will not be absent, but it will be considerably less efficient.

Significant drug interactions have been reported with cytochrome P450 2D6, especially that it is responsible for the metabolism of a large fraction of clinically
important medications with narrow therapeutic indices. CYP2D6 polymorphism is genetically determined but drugs might influence the enzymatic activity. CYP2D6 metabolism distinguishes itself from other cytochrome isozymes by its lack of inducibility. However, inhibition of CYP2D6 is a major factor that determines outcome of treatment with CYP2D6 substrates.

Because CYP2D6 is not expressed in poor metabolizers, inhibitors of this isozyme have little or no effect on the metabolism of CYP2D6 substrates in this subgroup. On the other hand, extensive metabolizers receiving a CYP2D6 substrate drug will display the phenomenon of phenocopying when an inhibitor is added; that is, they will behave as poor metabolizers. In other words, the genetic difference in drug metabolism between poor and extensive metabolizers may be lost when a substrate of CYP2D6 is administered with a potent inhibitor, resulting in false phenotyping. Thus, although poor metabolizers may be subject to undesired adverse effects, these usually manifest within several doses after initiation of the drug. Extensive metabolizer individuals conversely may be stabilized with prolonged drug therapy and then display an unusual drug effect when the inhibitor is added.

Some commonly used drugs are potent inhibitors of metabolic pathways mediated by CYP2D6, but not all of them are substrates of this isozyme. Any substrate for this enzyme is potentially capable of inhibiting the metabolism of another substrate. Inhibition of CYP2D6 results in clinically significant complications when the inhibitor is highly potent (k is close to or lower than k_m), if the enzyme catalyses a principal metabolic pathway for the drug, and if the drug has a narrow therapeutic index.
Curiously, the most specific inhibitor of CYP2D6, quinidine, is not a substrate for the enzyme. Concentrations of quinidine that abolish CYP2D6 activity have virtually no effect on other cytochrome P450s. Most investigators agree that metabolic pathways inhibited by low doses of quinidine are likely to be mediated by CYP2D6. Quinidine affinity for CYP2D6 is both specific and stereoselective. Quinine, its diastereoisomer, is several hundred times less potent as an inhibitor than quinidine. 144

Several cases of observed drug interactions have been reported in association with quinidine; and a variety of studies have investigated the influence of quinidine-mediated CYP2D6 inhibition on different drug substrates when coadministered with quinidine. 145

With most β-blockers, oxidative metabolism is required for elimination, and so poor metabolizers have a more intense and sustained β-blockade. Poor metabolizers suffer from excessive β-blockade after receiving usual therapeutic doses of these agents, and although β-blockers have a wide therapeutic index, subjects, mainly extensive metabolizers, given quinidine while receiving stable doses of these agents may require dose adjustment due to alteration in their apparent phenotypes. 146

Quinidine-mediated inhibition of CYP2D6 metabolism of several β-blockers and antiarrhythmic agents has been reported. Examples include metoprolol, propranolol, flecainide, encainide and propafenone. 146-151 Quinidine affected the stereoselective disposition of propranolol by significantly reducing the clearance of (+)-propranolol compared to (-)-propranolol in extensive metabolizers through inhibition of CYP2D6-
mediated 4-hydroxylation. Morike et al. demonstrated that the administration of low-dose quinidine to extensive metabolizer individuals receiving propafenone resulted in increased β-blockade. Increased total propafenone concentrations when concomitantly administered with quinidine were observed previously in patients with arrhythmia but with no changes in QRS interval, an index of sodium channel blockade, or arrhythmia suppression. Those findings demonstrated the contribution of propafenone metabolites to sodium channel blockade. In contrast, propafenone metabolites lack the β-blocking effect. Consistently with this finding, inhibition of propafenone metabolism by quinidine resulted in significantly enhanced β-blockade due to accumulation of the parent compound mainly responsible for this effect.

In addition to quinidine, tricyclic antidepressants, selective serotonin reuptake inhibitors and antipsychotics are major inhibitors of the cytochrome P450 2D6 isozyme.

At present, it is not possible to rank antidepressants in terms of their susceptibility to interactions with drug metabolizing enzymes because of many confounding factors and the differences between in vitro and in vivo data. The Ki values obtained for various antidepressants tested for inhibitory action on sparteine/debrisoquin oxidation in vitro differ quite substantially. As seen in Figure 5, the rank order of potency (that is inversely proportional to the Ki) of selective serotonin reuptake inhibitors determined under identical experimental conditions was paroxetine > fluoxetine > sertraline > citalopram > fluvoxamine. When the choice of substrate differed, the same inhibitor exhibited different inhibition potencies. Serotonin reuptake
inhibitors were less inhibitory when desipramine or imipramine was used as a substrate compared with sparteine and dextromethorphan.

![Figure 5: Relative potencies of different antidepressants as CYP2D6 inhibitors](chart)

Since therapeutic concentrations, elimination half-life and plasma protein binding are among the factors that determine the consequences of CYP2D6 inhibition, caution is needed in interpretation and extrapolation from $K_i$ data alone.

Inhibition of CYP2D6 activity by fluoxetine is the likely mechanism underlying the adverse events reported after combination therapy with drugs that are substrates of this enzyme, such as nortriptyline, desipramine and imipramine.¹⁵⁶
Paroxetine is also a potent inhibitor of CYP2D6 and hence may cause serious pharmacokinetic interactions with drugs metabolized by this isoenzyme notably tricyclic antidepressants (e.g. desipramine), some neuroleptics (e.g. perphenazine) and some antiarrhythmics (e.g. propafenone). \(^{101,155}\)

Imipramine and desipramine are competitive inhibitors of sparteine and debrisoquin in human liver microsomes. \(^{157,158}\) A study of extensive metabolizers showed increased metabolic ratios of both probe drugs during imipramine treatment, yet the phenotypes were not changed. \(^{99}\)

Brosen and Gram have pointed out that neuroleptics (acting in a fashion similar to quinidine) inhibit the metabolism of antidepressant medications, a process mainly mediated by CYP2D6. \(^{10}\)

Llerana et al. studied the effect of neuroleptics and antidepressant agents on debrisoquin hydroxylation phenotypes. \(^{159}\) They noticed that the distribution of the debrisoquin metabolic ratio in extensive metabolizers receiving neuroleptics or antidepressants was shifted toward higher values compared to drug free healthy volunteers. Forty percent of patients treated with neuroleptics including thioridazine, haloperidol, clozapine etc… and 5% of patients on antidepressant treatment including maprotiline, clomipramine, amitriptyline, imipramine and mianserin were classified as poor metabolizers due to elevated metabolic ratios. CYP2D6 genotype analysis however confirmed that these high metabolic ratios were attributable to CYP2D6 inhibition and not to overrepresentation of subjects with poor metabolizer phenotypes.
Compared to tricyclic antidepressants and selective serotonin reuptake inhibitors, moclobemide was a far less potent inhibitor of CYP2D6 \textit{in vitro}.\textsuperscript{160} However, moclobemide was shown to be a rather potent inhibitor \textit{in vivo} causing substantial increases in the sparteine metabolic ratios with an increased excretion of sparteine and decreased excretion of dehydrosarteine.\textsuperscript{161} Therefore, inhibition of CYP2D6 by moclobemide could reach clinical significance if concomitantly administered with substrates having a narrow therapeutic index such as tricyclic antidepressants and neuroleptics.

Although enzyme inhibition causes decreased CYP2D6 metabolic capacity in extensive metabolizers resulting in a higher incidence of adverse effects due to parent drug accumulation; when the enzyme catalyzes a required step for activation of a substrate or prodrug, the opposite scenario occurs: the interaction results in reduced bioactivation of the drug into its active metabolite(s) and leads to therapeutic failure because patients will fail to attain therapeutic plasma concentrations of the active compounds at usual drug dosages.

This has been reported mainly with opiate derivatives such as codeine and oxycodone.\textsuperscript{137,140,162} Concurrent quinidine administration with codeine resulted in a marked decrease in the production of morphine in extensive metabolizers without affecting the output in poor metabolizers.\textsuperscript{137} In a study conducted by Sindrup et al., quinidine effectively blocked the hepatic formation of morphine after codeine administration in extensive metabolizers, but the analgesic effect of codeine was not totally abolished by quinidine pretreatment indicating the impact of conversion of codeine into
morphine in the central nervous system that may not be totally blocked by quinidine because it poorly penetrates the blood brain barrier in humans.  

Quinidine also inhibited the conversion of oxycodone into oxymorphone in extensive metabolizers. However, the absence of oxymorphone after quinidine administration did not affect therapeutic outcome suggesting that oxymorphone does not seem to be important in the pharmacodynamic action of oxycodone, unless oxycodone is metabolized into oxymorphone in the brain.

In conclusion, genetically determined individual responses related to efficacy and side effects of medications can be altered by drug-drug interactions through inhibition of the enzymes responsible for their elimination. It is assumed that there is a relationship between drug concentrations and clinical effects. Concentration-effect studies are often neglected in drug development. However, such information is important for the evaluation of pharmacokinetic and pharmacogenetic data as well as for evaluation of the clinical importance of drug interactions. Major concern is during treatment with substrate drugs with narrow therapeutic index that mainly depend on one isozyme for their metabolism and clearance. Therefore, particular caution is warranted when prescribing antidepressants, neuroleptics and antiarrhythmics with CYP2D6 inhibitors. Decreasing the number of medications prescribed and administering the lowest effective dose can reduce patient risk. Fixed dose schedules should be avoided and health care professionals should focus on the need to establish optimal drug regimens for individual patients.
2.3.3 CYP2D6 polymorphism and disease risk

2.3.3.1 CYP2D6 and cancer

Cytochrome P450 2D6 polymorphism has been linked to the risk for lung cancer. A putative mechanism for a relationship between the debrisoquin noninducible metabolic phenotype and lung cancer has been difficult to define, because CYP2D6 is expressed primarily in the liver. CYP2D6 protein and mRNA expression have been found in the human lung in low concentrations. CYP2D6 catalyzes the activation of 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NKK), a tobacco-derived lung procarcinogen in human liver and lung microsomal preparations but the specific CYPs responsible for this reaction remain uncertain.

The debrisoquin poor metabolizer phenotype has been noticed to be under-represented in smokers diagnosed with lung carcinoma versus controls. A meta-analysis of the published data as of 1992 reported an overall odds ratio of 2.3 of a lung cancer case displaying an intermediate or extensive metabolizer phenotype. A Finnish study pointed out an odds ratio of 6.4 (p=0.05) for lung cancer cases having a genotype that translates to the extensive metabolizer phenotype versus controls. The CYP2D6*9 allele, encoding an active enzyme with lower substrate affinity and higher maximal metabolic rate than the wild-type allele, was observed in a Spanish study to be six-fold more common in lung cancer patients than healthy controls. However, a French group reported no statistically significant relationship between this genotype and lung cancer status, and there are several notable case-control studies and meta-analysis that have found no relationship between debrisoquin phenotype prevalence and lung cancer. One confounder appears to be race; the
association seen in Caucasians may not be extrapolated to African-American or Asians.  

In summary, the data addressing the relationships between CYP2D6 genotype, phenotype and lung cancer remains conflicting and inconclusive. There is some biological plausibility to more efficient activation of N-nitroso compounds such as NKK in tobacco smoke by the extensive metabolizer genotypes, but the variable reports of gene expression in the lung as target organ is problematic for the CYP2D6-lung cancer hypothesis.

Investigators have associated CYP2D6 polymorphism with other types of cancer. A French study suggested a relationship between tobacco smoke dose, enhanced CYP2D6 metabolic phenotype and the presence of laryngeal carcinoma.  A more recent publication reported no association of the CYP2D6 genotype with upper aerodigestive cancers in European populations.  

Topic et al. also studied the relation between CYP2D6 polymorphism and breast, head and neck cancer. They concluded that the polymorphic CYP2D6 gene does not play a role in carcinogenesis.  In addition, a study in breast cancer patients reported no association between the CYP2D6*4 allele frequency (allele encoding for the poor metabolizer phenotype) and breast cancer in a standard case-control design.  

Moreover, the analysis of bladder cancer patients for allelic variations in the CYP2D6 gene showed no significant differences between the two phenotypes.
Also, an association between the genotype representing the extensive metabolizer phenotype of CYP2D6 and hepatocellular carcinoma, particularly synergizing with the N-acetyltransferase 2 (NAT2) genotype, has been reported. It indicated an increased risk of liver cancer in homozygous extensive metabolizers.

Finally, susceptibility to childhood acute lymphoblastic leukemia was studied in relation to CYP2D6 genetic polymorphism. Results suggested that mutations in CYP2D6 alleles do not play a role in the etiology of acute lymphoblastic leukemia.

In light of the conflicting results reported between a variety of studies and cohorts of cancer patients, it seems difficult to confirm any association between CYP2D6 polymorphism and the risk of cancer. Additional studies in larger cohorts should be done to provide a clearer picture of the extent and correlation of CYP2D6 mutant genotypes in all types of cancer.

2.3.3.2 CYP2D6 and Parkinson’s disease

The etiology of the common forms of Parkinson’s disease remains unknown. Aside from age, the major risk factors are family history and elements of rural residence. Studies of unrelated patients have shown increased risk of Parkinson’s disease in first-degree relatives of patients, suggesting that genes contribute to the common forms of Parkinson’s disease. The observations that rural residence and pesticide exposure can increase the risk of developing Parkinson’s disease, and that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) can cause dopaminergic neuronal damage and parkinsonian symptoms, suggest that at least a subset of Parkinson’s disease may be caused by environmental toxins. The current thought is
that the common forms of Parkinson’s disease result from an interplay of genetic susceptibility and exposure to environmental toxins, making genes, which regulate bioactivation and detoxification of exogenous and endogenous compounds, strong candidates for Parkinson’s disease susceptibility.

The most extensively studied candidate gene for Parkinson’s disease is \textit{CYP2D6} because it regulates drug and toxin metabolism. The CYP2D6 enzyme is involved in the metabolism of over 25 exogenous drugs and neurotoxins including MPTP, \textsuperscript{199} as well as formation of endogenous compounds including dopamine. \textsuperscript{200} A variety of studies have examined the frequency of the mutant \textit{CYP2D6*4} allele and the poor metabolizer phenotype in patients with Parkinson’s disease and control subjects; some have observed a higher frequency of the allele in Parkinson’s disease and others failed to do so. \textsuperscript{183,201-216}

In conclusion, although some reports associating the CYP2D6 poor metabolizer phenotype with Parkinson’s disease have been published, the discrepancies between different study results obtained so far and the poor understanding of the population dynamics of the candidate genes hold back the possibility of confirming the relationship between the \textit{CYP2D6*4} allele and the susceptibility to Parkinson’s disease.
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CHAPTER THREE

BACKGROUND AND GENERAL CONSIDERATIONS

" Certain types of adverse reactions are due to unusual genetic pharmacokinetic variations: though it will rarely be possible to study such aberrant behavior in a prospective manner, every effort must be put into elucidating the pharmacokinetic mechanism(s) if there is any reason to suspect that the adverse reaction is caused by altered pharmacokinetics of the drug." 

3.1 IN-VITRO STUDIES

At least from a quantitative point of view, the liver is the most important organ involved in drug metabolism in humans. Thus *in-vitro* investigations commonly utilize human hepatic tissue. The most important advantage of using human material is direct applicability without necessity of adjustment for interspecies differences. Human liver tissue, however, is difficult to obtain, and ethical considerations apply. The most common source of human liver tissue is surgical waste obtained from partial hepatectomy, wedge biopsy and organ transplantation. However, since availability of human liver in general is limited, many investigations have been done with liver tissue from animals, especially from rats.
Different tools are used to investigate drug metabolism in liver tissue. Liver slices and hepatocytes provide the whole spectrum of enzymes responsible for hepatic metabolism, including phase I and phase II enzymes. Use of these systems is limited since both liver slices and hepatocytes have to be prepared from fresh human liver, and long-term storage remains to be a problem.  

In contrast, liver tissue can be deep-frozen immediately after surgery and stored at -80°C. Such samples can be used to prepare cell homogenates or subcellular fractions of human liver by differential centrifugation. Centrifugation at 9000 g provides a supernatant that contains the cytoplasm and the endoplasmic reticulum. Liver microsomes are obtained subsequently by centrifugation at 100000 g and contain the membranes of endoplasmic reticulum. The advantage of this system is that liver tissue can be stored for a long time at -80°C and microsomal fractions are readily prepared once needed.

The microsomal fraction contains the various P450 enzymes in their natural abundance. For some problems in metabolism it is mandatory, however, to use single enzymes (e.g. once assessing involvement of one enzyme in certain metabolic pathways). Such single P450 enzymes can be obtained by biochemical procedures or more popular using genetically engineered cells.
Advantages and disadvantages of the different in-vitro systems available are discussed below.

Precision-cut liver slices have been used to investigate drug metabolism in-vitro utilizing human and animal tissue. Liver slices provide all cell types representative for liver tissue (hepatocytes, Kupffer cells, endothelial cells) and tissues are still in their in situ topology. This system should therefore reflect enzymatic activity of the whole organ. However, only few papers have been published based on investigations done with slices from human liver and the system is therefore not thoroughly validated. 5-7

Hepatocytes are obtained by perfusion of fresh liver with collagenase and are subsequently cultured in appropriate media. Co-culture of hepatocytes with collagen and fibroblasts allows long-term culture. Cryopreservation of human hepatocytes has been used, but after thawing only a part of the cells survive and retain their functional capacity. 8,9 A problem in long-term culture is a shift in the relative P450 content in hepatocytes compared to native liver which hampers successful predictions. 10-12

Human hepatocytes have not been systematically used to evaluate CYP2D6 function in-vitro. A study of Maurice et al., which was primarily targeted to the evaluation of imidazole derivatives and rifampicin on hepatocyte function, also monitored debrisoquine 4-hydroxylase. 13 As expected from previous in-vivo experiments, 14 CYP2D6 was not inducible by rifampicin whereas CYP3A4 which was monitored by erythromycin demethylase and cyclosporine oxidase was induced by clotrimazole and inhibited by ketoconazole.
Michaelis-Menten kinetics for a number of substrates have been determined in human liver microsomes obtained from extensive metabolizers and compared with data from poor metabolizers. Microsomes obtained from poor metabolizer livers show lower turnover numbers for substrates cosegregating with sparteine/debrisoquine than microsomes from extensive metabolizers. 1'-hydroxylation of (+)-bufuralol shows a decrease in $V_{\text{max}}$ and an increase in $K_m$ in liver microsomes from poor metabolizers compared to extensive metabolizers indicating decreased enzyme affinity to its substrate. A similar observation was made for the biotransformation of codeine into morphine.

A study of sparteine oxidation in liver microsomes showed significant increases in $K_m$ in poor metabolizers demonstrating altered substrate-binding affinity; on the other hand, the $V_{\text{max}}$ values were similar in both phenotypes but also showed large interindividual variations. This suggests that the amount of cytochrome P450 involved in sparteine oxidation varies to a certain extent in different individuals independent of the phenotype.

Studies of dextromethorphan metabolism by CYP2D6 in human liver microsomes have also suggested a shift to a higher apparent $K_m$ in microsomes of poor metabolizers; the rate of dextorphan production differed to a lesser extent. Moreover, Bendriss et al. noted that when incubated with human liver microsomes, dextromethorphan was more specific for CYP2D6 than debrisoquin or sparteine. Therefore they concluded that in-vitro metabolic studies with dextromethorphan require limited amount of liver microsomes. This is a major advantage that can
contribute to reducing the need of healthy human liver samples, which become less available with the important advances in liver transplantation.

The value of Dark Agouti rats liver microsomes as a model for sparteine/debrisoquin polymorphism has been thoroughly investigated. Female Dark Agouti rats lack the CYP2D1 isozyme, which is the rat cytochrome analogous to human CYP2D6. It was found that debrisoquin hydroxylation was reduced in female Dark Agouti rats compared to Lewis rats\textsuperscript{20} or Fischer rats,\textsuperscript{21} however, phenacetin O-deethylation was also reduced in Dark Agouti rats, indicating that enzymes other than CYP2D6 may be impaired in these rats as well.\textsuperscript{20} Reduced biotransformation in female Dark Agouti rats was shown for (+)-bufuralol 1'-hydroxylation,\textsuperscript{22,23} sparteine oxidation,\textsuperscript{24} dextromethorphan O-demethylation\textsuperscript{25,26} and codeine O-demethylation.\textsuperscript{27}

Genetic engineering enables the expression of cDNA encoding for proteins in suitable cell systems such as yeast, COS cells (cells from a monkey kidney cell line) or human lymphoblastoid cells, and therefore allows the investigation of single P450s. A variety of investigations describe catalytic characteristics of stably expressed CYP2D6.

The mutagenic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone was investigated in human lymphoblastoid cells stably expressing CYP2D6 (2D6/Hol) or cell lines expressing CYP1A2, CYP2E1 and CYP2A3.\textsuperscript{28} Using a transcriptional cassette system containing two CYP2D6 cDNAs, higher CYP2D6 levels have been expressed in human lymphoblast cells (h2D6v2).\textsuperscript{29} (+)-Bufuralol 1-hydroxylase activity in microsomes from these cells was higher than in microsomes prepared from extensive metabolizer liver.
Biotransformation of the antipsychotic drug clozapine was investigated in microsomes prepared from V79 Chinese hamster lung fibroblasts stably expressing CYP2D6. In this system the main metabolites of clozapine, N-demethylclozapine and clozapine-N-oxide, were not found. In contrast, these two main metabolites were identified in presence of the microsomal fraction of human liver, whereas the genetically engineered cells formed metabolites were different from N-demethylclozapine and the N-oxide but not definitely identified. The same system was used to elucidate the involvement of CYP2D6 in biotransformation of tropisetron and ondansetron and in the metabolism of dextromethorphan and bufuralol.

CYP2D6 expressed in yeast has debrisoquin 4-hydroxylase activity and shows characteristics of the enzyme. This system was used to investigate the importance of CYP2D6 in the biotransformation of methylenedioxyamphetamine (ecstasy). It was shown, that this drug is converted to dihydroxymethamphetamine by CYP2D6.

A cDNA coding for CYP2D1, the rat isoform to CYP2D6, has been expressed in COS cells, which are then able to form 1'-hydroxybufuralol.

Any analytical method used for in-vitro investigations has to meet two requirements. First, it must be sensitive enough to enable determination of picomolar amounts of metabolites formed in the in-vitro systems. Second, since determination of the enzyme kinetics requires relatively high concentrations of the drug as substrate, such an excess concentration of the parent compound should not interfere with the assay for the metabolites.
Prototype reactions to monitor CYP2D6 activity *in-vitro* are debrisoquin 4-hydroxylation, \( ^{36} \) bufuralol 1'-hydroxylation, \( ^{36,38} \) dextromethorphan O-demethylation, \( ^{18,36,39} \) and sparteine oxidation. \( ^{40} \) For all these prototype reactions, analytical methods exist which exhibit a sensitivity that allows determination of low amounts of metabolites formed in *in-vitro* experiments.

The metabolites of sparteine, 2,3-dehydrosparteine and 5,6-dehydrosparteine, can be determined after alkaline extraction into dichloromethane by gas chromatography (GC) with a nitrogen selective detector, \( ^{40} \) or with mass spectrometric detection. \( ^{41} \) For the other reactions, debrisoquin 4-hydroxylation, bufuralol 1'-hydroxylation and dextromethorphan O-demethylation, high performance liquid chromatography (HPLC) methods with fluorescence detection are usually employed. Fluorescence detection is significantly more specific than ultraviolet (UV) absorbance detection \( ^{42} \) and allows the determination of the respective metabolites in the presence of numerous detergents, antibodies or specific inhibitors. \( ^{43} \) All substrates and their respective metabolites are basic compounds and ion suppression reversed-phase HPLC is not applicable, as the required alkaline pH would lead to deterioration of the stationary phase. Therefore, other HPLC techniques are applied. For analysis of dextrophan, triethylamine was added to the mobile phase, \( ^{18} \) for monitoring of bufuralol 1'-hydroxylation, normal-phase HPLC was employed. \( ^{37} \) The most common method applicable for all three prototype reactions used inorganic ion-pair reversed-phase HPLC with perchlorate as the counter-ion. The metabolites can be determined directly in incubate mixtures after protein precipitation with perchloric acid. \( ^{36} \) This procedure shows better sensitivity than extraction procedures described for bufuralol. \( ^{37,38} \)
In-vitro models of drug metabolism allow the determination of the enzyme systems involved in the biotransformation of a given drug, the kinetic behavior of a specific enzyme substrate as well as the identification of those compounds that might compete for the same enzyme and thus inhibit drug metabolism. Either human liver microsomes or specific isoenzymes expressed in various cell culture systems are used for this purpose. In addition, selective isozyme inhibitors (antibodies or chemical inhibitors) help to determine not only which isoforms are involved in a metabolic pathway but also the extent to which each isoform contributes to enzymatic activity. However, only in-vivo studies can ultimately establish the quantitative importance of the different metabolic pathways under therapeutic conditions.

It should be stressed again that in-vivo variability may be greater than that in-vitro and that a major part of the variability therefore might not be accounted for by in-vivo estimates. However, if a substantial amount of a drug is metabolized via a given pathway, or if a number of metabolites metabolized by the same enzyme accounts for 40-50% of the dose, then any variation in the enzyme's catalytic activity is likely to be of clinical significance.

If more than 30% of a drug is metabolized through one pathway, identification of the enzymes involved in the elimination is justified or advisable. The potential drug-enzyme interaction can be estimated from the inhibition constant $K_i$ of the inhibitor for a specific enzyme. If the $K_i$ of a drug is of the same order of magnitude of the steady-state concentration the inhibitor achieves in-vivo, interactions are likely to occur, and in-vivo interaction studies should be carried out to clarify the situation.
further. However, if the $K_i$ or $K_m$ are high compared to the concentrations of the inhibitor or drug reached in-vivo, the inhibition will be negligible and there will be probably no clinically significant interaction.

Antibodies directed against an enzyme can be used to inhibit the metabolism of a drug catalyzed by this specific enzyme. Inhibition by a well-characterized antibody then indicates the involvement of the respective enzyme in a drug's metabolism. As an example of this approach, LKM1 antibodies (liver kidney microsomes) bind to CYP2D6 and inhibit this enzyme.\textsuperscript{44-46} These antibodies have been used to inhibit *in-vitro* the metabolism of propafenone,\textsuperscript{47} mexiletine,\textsuperscript{48} imipramine\textsuperscript{49} and dextromethorphan.\textsuperscript{50}

Antibodies raised against purified rat debrisoquin 4-hydroxylase inhibited in human liver microsomes in-vitro the oxidation of debrisoquin, sparteine, encainide and propranolol,\textsuperscript{51,52,53} drugs which are known substrates of CYP2D6. They did not inhibit reactions known to be catalyzed by cytochrome P450 enzymes different from CYP2D6.\textsuperscript{54}

The relationship between in-vitro and in-vivo tests has not yet been fully investigated. When comparing selective serotonin reuptake inhibitors and their metabolites that inhibit CYP2D6 in human liver in-vitro and in-vivo, Brozen reported mixed results.\textsuperscript{55} Gram et al. demonstrated that there is a discrepancy between the inhibitory potency of moclobemide in-vitro and in-vivo.\textsuperscript{56} Moclobemide and/or its metabolites were weak inhibitors in-vitro and rather potent inhibitors of CYP2D6 in-vivo. Further investigations are needed to clarify the relationship between in-vitro and in-vivo
results. Furthermore, during the long-term treatment of depression, metabolites of an unknown nature might accumulate and have significant biological effects. This might not be predicted from *in-vitro* studies.

### 3.2 CYP2D6 PHENOTYPING: *IN-VIVO* STUDIES

#### 3.2.1 Phenotyping probes

Commonly used prototype substrate reactions to characterize cytochrome P450 2D6 activity include bufuralol 1'-hydroxylation, debrisoquin 4-hydroxylation, sparteine oxidation and dextromethorphan O-demethylation.\(^{57-60}\) Debrisoquin, sparteine and bufuralol are not readily available because they are marketed in a limited number of countries and some have been withdrawn from clinical use because of their associated side effects such as the occurrence of significant cardiovascular adverse effects including orthostatic hypotension especially in poor metabolizer subjects.

Dextromethorphan (DM) is the (+)-isomer of the codeine analog levorphanol; however, unlike the (-)-isomer, it has no analgesic\(^ {61}\) or addictive\(^ {62}\) properties and does not act through opioid receptors. The drug acts centrally to elevate the threshold for coughing\(^ {63}\) and is a constituent of many over-the-counter cough medications. Dextromethorphan exhibits half the potency of codeine and 60 mg dextromethorphan hydrobromide are equivalent to 30 mg codeine phosphate in terms of antitussive activity.\(^ {64}\)

The metabolism of dextromethorphan (Figure 6) consists primarily of O-demethylation to dextrorphan, a reaction that is catalyzed by the cytochrome P450
Dextromethorphan is also metabolized to 3-methoxymorphinan and 3-hydroxymorphinan, but these appear to be minor pathways mediated by CYP2D6 and CYP3A3/4, respectively. The metabolites are then further glucuronidated.

Figure 6: Metabolic pathways of dextromethorphan elimination

Once the metabolism of dextromethorphan was established to cosegregate with that of debrisoquin and other prototype substrates of CYP2D6, it has been unanimously accepted as an attractive alternative to the other phenotyping probes because of its recognized safety, its worldwide availability and its specificity to CYP2D6 since no co-inheritance between mephenytoin and dextromethorphan metabolism was found in human subjects. In a study of CYP2D6 polymorphic metabolism in a pediatric population, dextromethorphan was found to be a suitable substrate probe for phenotyping in children since it proved to be innocuous in this population with no occurrence of adverse events after dextromethorphan administration.
Furthermore, the large \( K_m \) difference observed between phenotypes \textit{in-vivo} and consequently the ease to distinguish between different phenotypes could be an argument in support of the use of dextromethorphan as phenotyping probe. Moreover, the fact that the dextromethorphan \( K_m \) in liver microsomes of extensive metabolizers is lower than the \( K_m \) reported for other prototype compounds such as bufuralol, \( \textsuperscript{15} \) debrisoquin, \( \textsuperscript{36,69} \) or sparteine \( \textsuperscript{17} \) is another argument favoring the use of dextromethorphan as an \textit{in-vivo} phenotyping probe: its higher affinity for the polymorphic isozyme allows for low drug doses to be administered while achieving target concentrations required, and on a theoretic basis, it may make dextromethorphan less sensitive to inhibition by other drugs given concomitantly. \( \textsuperscript{70-73} \)

Based on the aforementioned criteria, the choice of the phenotyping probe to be used in this study of CYP2D6 polymorphism fell upon dextromethorphan, a well-recognized safe drug, whose O-demethylation pathway is specific to cytochrome P450 2D6.

\textbf{3.2.2 Biologic fluids}

\textit{3.2.2.1 Plasma}

Dextromethorphan undergoes extensive first-pass metabolism when given orally. \( \textsuperscript{74} \) Dextromethorphan was detected in human serum in a concentration approximating 2 ng/ml after a single 20-mg oral dose. \( \textsuperscript{75} \) The levels obtained were too low in most cases to permit an accurate assessment of the kinetic behavior of the drug. Consequently, even large doses of dextromethorphan (\( \geq 750 \) mg/day) do not produce
predictable or in some cases even measurable plasma concentrations. Furthermore, the non-linearity in its elimination and the accumulation of its O-demethylated metabolite dextorphan under these conditions make adverse effects and toxicity from dextromethorphan more likely and less predictable.

Dextromethorphan levels in dogs were also found to be much lower following intraperitoneal administration compared to the intravenous route, suggesting significant first-pass hepatic metabolism.

Zhang et al. also reported significantly low plasma concentrations of dextromethorphan (5-40 ng/ml) even at the highest daily dose of 120 mg/day. They noted that although low plasma concentrations could indicate either extensive first-pass elimination of dextromethorphan or poor absorption, two separate studies in poor metabolizers supported the former explanation. They then demonstrated that a low-dose quinidine (150 mg/day) substantially increased the dextromethorphan plasma concentrations through inhibition of CYP2D6 resulting in enhanced bioavailability of dextromethorphan when given orally and improving the predictability of dextromethorphan plasma levels in relation to the dose administered.

The difficulty in detecting measurable amounts of dextromethorphan in plasma because of its extensive first-pass metabolism, in addition to the reluctance of volunteers to permit blood collection and the availability of more convenient phenotyping methods (discussed below) guided our choice of biologic fluid in this study towards avoiding blood samples.
3.2.2.2 Saliva

Hou et al. proposed that phenotypes could be determined by measuring drug to metabolite ratio in saliva collected three hours after an oral dose of dextromethorphan. Comparing the metabolic ratio in saliva to that in urine collected for eight hours after dosing yielded a Spearman rank correlation of 0.70. The investigators achieved 100% concordance: all of the poor metabolizers identified by urinary metabolic ratio were also identified by the metabolic ratio in saliva.

Drawbacks of this study included the need for higher dextromethorphan doses (50 mg) than those usually used for phenotyping using urine samples resulting in higher incidence of mild transient adverse effects mainly in poor metabolizers and Chinese subjects. In addition, the salivary assay is technically more time-consuming and more difficult than the urinary assay. Moreover, some subjects may be reluctant to provide saliva samples and prefer urine collection.

Although salivary analysis can identify all subjects with the poor metabolizer phenotype in a group of healthy volunteers, it is unlikely to replace urine collection for dextromethorphan metabolic phenotyping due to the widespread use of urinary analyses in healthy subjects and the major drawbacks of the salivary analysis aforementioned. It may however prove valuable in end-stage renal disease patients who are unable to provide appropriate urine samples and in clinical settings where the shorter time required for sample collection (3 hours versus 8 hours for urine) are important.
3.2.2.3 Urine

Administering a CYP2D6 substrate as a probe drug like bufuralol, dextromethorphan, debrisoquin and sparteine, and measuring the parent drug to metabolite ratio in the urine (metabolic ratio) allows differentiating extensive metabolizers from poor metabolizers. Poor metabolizers of these probe drugs excrete most of a dose as the non-metabolized parent compound, whereas extensive metabolizers excrete relatively little unchanged drug.

The metabolic urinary ratio (MR), in widespread use today, was developed empirically as a method for phenotyping. Only a decade ago has the pharmacokinetic basis for this and other indices of phenotype been examined. Using computer simulations, Jackson and Tucker determined that the metabolic ratio in urine is the best discriminator of bimodality and showed why area under the plasma drug concentration-time curve (AUC) is a poor index of polymorphism when the frequency of the gene defect is low. 81

Metabolic phenotype analysis of CYP2D6 polymorphism using dextromethorphan is carried out by administration of 30 mg of the probe drug and subsequent urine analysis after 8 hours to determine the ratio between the parent compound dextromethorphan and the primary metabolite dextrorphan. 19,59,82 This ratio varies 1000-10000 fold in the Caucasian population, and a metabolic ratio of 0.3 depicts the antimode between extensive metabolizers and poor metabolizers. 58 The bimodal distribution of the metabolic ratios in the Caucasian population can easily permit the distinction between poor metabolizers with mean MRs>0.3 and extensive
metabolizers of dextromethorphan (MR<0.3). The identification of intermediate or ultra rapid metabolizers however is more problematic and relies more on genotyping because some extensive metabolizers may exhibit low metabolic ratios similar to those observed in ultra rapid metabolizers and intermediate metabolizers show average metabolic ratios close to those observed in extensive metabolizers.

On account of the widespread use of urine assays for phenotyping of CYP2D6 metabolic activity and the less convenient and more cumbersome alternative analyses using plasma or saliva collection, urine analysis was found to be the most adequate choice of biologic fluid to be used for phenotyping purposes in this study.

3.2.3 Methods

Several methods have been reported for the quantification or screening of dextromethorphan and its metabolites in urine including high-performance liquid chromatography (HPLC),\textsuperscript{19,42,83-89} gas chromatography (GC) with selective nitrogen detection\textsuperscript{58,59,82,90} or electron-capture detection,\textsuperscript{75,91} capillary electrophoresis,\textsuperscript{92} and thin-layer chromatography.\textsuperscript{93}

The extensive use of HPLC in pharmaceutical analysis and in pharmacokinetics has led to a marked reduction of the practice of derivatization reactions, although these were extensively used 25 years ago to render volatile in GC analysis substances possessing carboxylic, alcoholic, phenolic, aminic, amido groups and/or to render such substances sensitive to selective detectors.\textsuperscript{94} In fact, HPLC now solves most analytical problems in pharmaceutics and pharmacokinetics without requiring derivatizations.
Bioassay sensitivity is being continuously enhanced, due to improvements in analytical apparatus, chromatographic columns and detectors. The mass spectrometry (MS) coupled with GC and HPLC and the tandem MS have significantly improved the sensitivity and the selectivity of bioassays. The GC procedure however involves lengthy sample preparation, requires more analysis time as compared with HPLC analysis. In addition, HPLC analysis is more precise for the quantification of dextromethorphan hydrobromide indicating that GC analysis is not the method of choice for metabolic phenotyping with dextromethorphan due to the difficulty in extracting the free base. ²⁹

Capillary electrophoresis (CE) is a technique that is more useful in pharmaceutics than in pharmacokinetics. This is due to the fact that, compared to HPLC, CE is in itself on average 100 times more sensitive but requires a volume 1000 times higher to be injected, so that it is ultimately not more sensitive than HPLC and thus is of little use when sensitivity is a rate-limiting step, as usually occurs in pharmacokinetics. However, in pharmaceutics and in some toxicokinetic applications, CE is extensively used, as it is more selective than HPLC. ²⁵ CE could also be useful in the metabolic screening of urinary metabolites of drugs, where their concentrations are high enough.

Because study results have proved the superiority of HPLC in determining dextromethorphan and its metabolites in urine during phenotyping for CYP2D6 metabolic polymorphism, an HPLC technique was adopted in this study.
Several factors however should be considered. First, the detector used is of great significance. The use of ultraviolet and fluorescence detectors has been mainly associated with HPLC procedures for CYP2D6 metabolic phenotyping using dextromethorphan as a probe drug.\textsuperscript{19,42,86,88} The detector converts any variation in the column effluent into an electrical signal that is subsequently recorded by the data system.\textsuperscript{96} Ideally, a detector is highly sensitive and selective; it should be able to measure small substrate and metabolites concentrations without the need to increase the injection volumes considerably. In addition, a detector should exhibit a stable baseline while being insensitive to variations in flow rate or in the temperature of the system.\textsuperscript{96}

Lam and Rodriguez indicated that fluorescence detection is superior to UV detection in both sensitivity and selectivity. Although comparable phenotype results were obtained, fluorescence detection produced cleaner chromatograms for quantification of dextromethorphan and dextrorphan with significantly less interference from endogenous substances.\textsuperscript{42} The choice of the detector to be used in this study was therefore decided. The detector was set on an excitation wavelength of 200 nm at which, based on their structures, dextromethorphan and its metabolites are believed to absorb light.\textsuperscript{19}

The chromatographic characteristics of dextromethorphan and its related alkaloids are dependent on both the pH and composition of the mobile phase. A mobile phase consisting of a mixture of water containing glacial acetic acid and diethylamine, and
acetonitrile was chosen because it permitted the baseline separation of
dextromethorphan and its three metabolites with reasonable analysis time and
sensitivity. In reverse-phase chromatography, the mobile phase used is usually based
on a polar solvent typically water, to which a less polar organic solvent such as
acetonitrile is added. Reverse-phase HPLC is capable of separating neutral molecules
according to their hydrophobicity. Therefore, a decrease in the polarity of the mobile
phase by mixing water, the major constituent of the mobile phase with less polar
compounds such as acetonitrile, will cause a decrease in the solute retention time. The
flow rate of the mobile phase affects the time to recovery of our parent compound and
its metabolites. As the flow rate increases, the time to recovery decreases thus the
length of the assay is reduced.

The nature of the chromatographic technique, whether normal or reverse-phase HPLC
is adopted, guides us to the choice of the column to be utilized. The column represents
the basic component of the HPLC system; it is responsible for the separation of the
mixture injected into different elements. Depending on the nature of its packing
material, and the size of the packing material as well as the length and diameter of its
container, a column can alter resolution, speed and efficiency of the HPLC system. 96
Based on the required column characteristics, two columns serially connected were
used in this study.
3.3 CYP2D6 GENOTYPING

One method that has proven useful in screening for genetic mutations associated with altered metabolism of drugs and/or cancer susceptibility \(^{97,98}\) is amplification of a specific region of the gene of interest by Polymerase chain reaction (PCR) followed by digestion of the amplified DNA product with restriction endonucleases. Restriction endonucleases have the capacity to digest DNA with a high degree of nucleotide sequence specificity. Thus, point mutations within the recognition sequence of a specific restriction endonuclease may be detected through determining whether the DNA of interest serves as a substrate for that endonuclease. These studies are routinely carried out by comparing the size of digestion products generated from a DNA substrate amplified from control subject DNA versus study subject DNAs. Differences in the size of DNA fragments generated as a result of endonuclease digestion is commonly referred to as a restriction fragment length polymorphism (RFLP). \(^{99-101}\) The size of the digestion products is easily evaluated by agarose gel electrophoresis with ethidium bromide staining and UV transillumination.

A second approach for detection of specific mutations within a gene of interest is through allele-specific PCR amplification where oligonucleotides specific for hybridizing with the common or variant alleles are utilized in parallel amplification reactions. Only the oligonucleotide that precisely hybridizes to the target sequences produces an amplification product. Analysis for the presence or absence of the appropriate amplified product is also accomplished by agarose gel electrophoresis. The best example of this approach is the identification of the CYP2D6*3 and
CYP2D6*4 variant alleles of the CYP2D6 gene. These genotyping methods require small amounts of blood or tissue, are not affected by underlying disease or by drugs taken by the patient, and provide results within 48-72 hours, allowing for rapid intervention.

The CYP2D6 gene deletion can be detected through RFLP analysis of genomic DNA and Southern transfer by using a cDNA probe. Digestion of genomic DNA with the restriction endonuclease XbaI followed by Southern transfer yields either a ≥29-kb fragment in subjects without the deletion or an 11.5-kb fragment in individuals with the CYP2D6*5 allele. The labor intensity of this approach makes this method much less attractive to clinical application compared with recently developed PCR-based methods. The most common null allele is CYP2D6*4, representing 23% of all CYP2D6 alleles and 70% of the null CYP2D6 alleles. The second most common null allele is the CYP2D6*5 allele, followed by the CYP2D6*3 allele, representing 5% and 2% of all CYP2D6 alleles respectively. Analysis of the CYP2D6*3 and CYP2D6*4 variants is typically carried out with a allele-specific amplification method developed by Heim and Meyer, which includes an initial amplification strategy to specifically amplify a region of the CYP2D6 gene, followed by allele-specific amplification to identify the CYP2D6*3 and CYP2D6*4 mutant alleles. The second step of this approach has been automated on the basis of ligase chain reaction technology, which should advance efforts to reduce the time and expense associated with testing. Studies that have utilized this method to prospectively identify poor metabolizers have demonstrated 95% sensitivity and 100% specificity, and the phenotype of 99% of randomly selected subjects can be determined by this genotyping strategy.

A few years ago, a faster method has been developed by Douglas et al. that allows for
the simultaneous evaluation of both CYP2D6*3 and CYP2D6*4 variants with a single amplification step and restriction digest.\textsuperscript{108} This approach requires careful interpretation of the restriction digestion patterns however, it is widely utilized.\textsuperscript{104}

From all these data, it is evident that for clinical purposes of identifying phenotypically poor metabolizer individuals, analysis of the CYP2D6*3 and CYP2D6*4 variants will predict the poor metabolizer phenotype with >95% accuracy and the extensive metabolizer phenotype with >99% accuracy.\textsuperscript{57,109} It is therefore very likely that these analyses could significantly affect the risk-benefit ratio and increase the therapeutic efficacy of drugs that are substrates for CYP2D6. When analysis of the CYP2D6*3 and CYP2D6*4 variants fail to corroborate the apparent phenotype, analysis for the CYP2D6*5 gene deletion would be the next logical step. The complete absence of an amplification product during the analysis of the CYP2D6*3 and CYP2D6*4 variants would lend support for confirming the gene deletion by Southern transfer. However, in the event that CYP2D6*3 and CYP2D6*4 analysis yields the apparently wild type PCR product, the best approach may be to pursue characterization of the other null or inactivating alleles for which PCR methods are available.\textsuperscript{104,110-112}

The CYP2D6 gene duplication may be detected by observation of a 42-kb fragment in the XbaI RFLP analysis of genomic DNA.\textsuperscript{113} Allele-specific PCR methods have been developed as a screening tool for diagnosis of the ultrarapid phenotype.\textsuperscript{104,110}
Interpretation of the PCR-based analysis of CYP2D6 genotype requires the laboratory to be aware that individuals homozygous for the gene deletion will not yield a PCR product in standard assays for the various mutations. Also, heterozygotes for the gene deletion may appear to be homozygous for the wild-type allele. On the basis of the metabolic criteria for phenotype assignment, individuals heterozygous for the gene deletion are categorized as extensive metabolizers and thus there is concordance between the observed genotype with phenotype assignment. Individuals heterozygous for the gene deletion and one of the other inactivating alleles will be correctly classified as poor metabolizers. However, the true genotype can be proven only through the Southern transfer approach or long-PCR techniques. For clinical purposes, this is not necessary, but assays for identification of the gene deletion allele CYP2D6*5 must be carried out in all population studies designed to characterize allele frequencies.

Traditional DNA analysis methods are laborious and costly and have decreased the collection of valuable data needed to achieve marked advances in the fields of pharmacogenetics and pharmacogenomics in addition to reducing the realization of the vast potential of these fields in treatment and prevention of diseases and adverse drug reactions.

DNA chips represent an evolving technology available that can markedly decrease the cost of genotyping while speeding the processing too. DNA chips consist of a complex matrix of oligonucleotide probes (short single-stranded DNA sequences) affixed to a solid support (typically glass) set in a manner to allow the identification of the presence or absence of genetic polymorphisms. The process involved resembles
the automated Southern transfer. The sample collected undergoes sometimes PCR amplification then is labeled with a fluorescent tag, applied to the DNA chip and incubated. The DNA chip probe has a complementary nucleic acid sequence to which the sample hybridizes during incubation. Afterwards, the information is analyzed by a computer-based system and the sequence of the applied DNA is determined.

Microassays for the identification of cytochrome P450 polymorphisms are already available and represent the beginning of a new era where the importance of pharmacogenetics and pharmacogenomics is becoming noticeable and screening of individual genotypes along with phenotyping will become part of preliminary treatment phases in order to optimize management of diseases by avoiding therapeutic failure and minimizing the incidence of adverse effects.

**3.4 PHENOTYPING VERSUS GENOTYPING**

Phenotyping has been a valuable research tool and for a long time it was the only possibility of assessing the genetic basis of a patient's metabolic capacity. Defining the individual's phenotype, relative to reference substrate, allows the drug metabolism phenotype for other substrates of that enzyme to be predicted.

In pharmacokinetic studies, phenotyping has the advantage over genotyping in revealing drug-drug interactions or defects in the overall process of drug metabolism. In clinical situations, it has not been greatly utilized because of several caveats. Phenotyping has several drawbacks in that it is hampered by complicated protocols of testing, cumbersome sample collection and biochemical analysis, risks of adverse
drug reactions because it requires the application of test drugs that are very often not approved and marketed for therapeutic purposes, problems with incorrect phenotype assignment due to coadministration of drugs, and confounding effects of disease. Because of interaction with many other clinically used drugs, in particular antidepressant, neuroleptic and antiarrhythmic medications, a washout period of several days is necessary to obtain meaningful results. This implies an inherent ethical problem, as patients seeking medical help for their diseases would have to stay untreated for several days.

CYP2D6 genotyping has proven to be a valid alternative to traditional phenotyping for determination of poor metabolizer status. Given the correlation between CYP2D6 genotype and phenotype, CYP2D6 genotyping should be used in the clinical trial setting.

Genotyping involves identification of defined genetic mutations that give rise to the specific drug metabolism phenotype. Genotyping methodologies can be easier to use than biochemical measurements in a clinical setting. Often a very small amount of material such as blood from a single finger prick or a buccal swab is all that is needed to complete genotyping assays. CYP2D6 genotyping methodology is well documented and should be easy for any molecular biology laboratory to adapt.

Genotyping should yield the same results across different laboratories, thus making it ideally suited to a diagnostic application; in contrast, phenotyping may yield more variable data between laboratories and at different time points for the same patient. Intraindividual variation does not exist in genotype data; on any given day a patient’s
genotype will be exactly the same. This constancy does not apply to CYP2D6 phenotypes because other factors, such as drug-drug interactions can cause inhibition of CYP2D6 metabolic capacity and thus result in an incorrect measure of CYP2D6 metabolic rate upon administration of the probe drug. This fact could be particularly important in clinical trials of neurotrauma drugs in which the patient may be unconscious. The nature of these compounds and their ability to penetrate the blood-brain barrier make them susceptible to CYP2D6 metabolism, and if the patient is unconscious, a physician may have no knowledge of drugs that act as CYP2D6 inhibitors that the patient may have been taking. Even though in such cases genotyping would give an accurate prediction of metabolic status (while phenotyping might not), a physician still needs to be aware of other factors that can introduce variability in CYP2D6 metabolism.

CYP2D6 genotyping is also a flexible methodology in that one can decide which alleles to investigate depending on the needs of each particular study. The advantage of screening for all known alleles is to increase our ability to safely test new therapeutic entities. However, screening for the most common poor metabolizer alleles (CYP2D6*3, CYP2D6*4, and CYP2D6*5) will typically identify about 95% of the poor metabolizers in a sample set. One strategy for increasing genotyping efficiency might be to develop a protocol that includes an initial screen for the more common poor metabolizer alleles and, in cases where none of these alleles are detected, a screen for the less common poor metabolizer alleles resulting in loss of enzyme activity and intermediate metabolizer alleles that cause altered enzyme catalytic capacity.
Genotyping results can often be obtained more quickly than phenotyping results, and future technologies may offer rapid "bedside" assessment. For assessment of CYP2D6 status, samples for genotyping can be obtained at the screening visit, and results can be obtained before the wash-out period is over; phenotyping usually cannot be performed until after the wash-out period. Because an individual's DNA sequence does not change over time, genotyping for a particular gene needs only to be performed once, whereas biochemical measurements may need to be taken numerous times.

Prospective CYP2D6 genotyping for enrollment purposes may be extremely useful in Phase I first-in-human (FIH) studies where safety and efficacy profiles are essentially unknown. For safety purposes, a clinician may initially choose to enroll only subjects with two active alleles. Genotype can be compared with data on pharmacokinetic variability, adverse events, and drug response to aid in the decision-making process for subsequent studies for the compound, eventually in clouding poor metabolizer individuals. This information is important for all drugs in development, no matter which metabolizing enzymes are involved, as it could help make clinical trials safer with respect to drug metabolism. In clinical trials in which significant variability in drug response or pharmacokinetic data are observed, retrospective genotyping is probably easier to use and more informative than retrospective phenotyping. A further benefit of genotyping is that the comparison of genotype and pharmacokinetic data is likely to indicate an individual's rate of metabolism for a particular drug, whereas the rates of metabolism of probe drug and test drug may be significantly different in a phenotype screen.
Genotyping is likely to assist clinicians in finding drug doses that are safe and efficacious for both extensive and poor metabolizers, or perhaps in tailoring drug dosage to an individual's needs, with extensive and poor metabolizers receiving differential dosing. Screening for potential nonresponders to drug therapy due to genetic polymorphisms of metabolizing enzymes directly addresses the pharmacokinetic basis of the problems of delayed response, non-response or adverse drug reactions. Whether such an approach prior to or at the beginning of therapy can be clinically as well as cost effective has still to be established. Increased prevalence of both poor metabolizers and ultrarapid metabolizers in hospitalized patients have been reported, possibly the consequence of adverse or insufficient drug effects and subsequent noncompliance. This would indirectly support the clinical relevance of the polymorphisms. Shorter hospital stays as well as replacing empiric trial and error therapy though a more rational approach could be the potential benefits of pretherapeutic genotyping. Possible consequences of the detection of a polymorphism with functional changes in a metabolizing enzyme could be avoiding therapy with substrates of this enzyme, prescribing either reduced doses in poor metabolizers or extremely elevated doses in ultrarapid metabolizers. Finally, genotyping is likely to be less expensive especially as new high-throughput technologies become available.

Although genotyping has many advantages over phenotyping in a clinical trial setting, it also has some limitations. First, the described PCR-RFLP methodology has some technical shortcomings. For example, some of the assays are prone to false negatives because they rely on the presence or absence of a PCR product. In addition, the RFLP banding patterns and band intensities may be confusing. Using this methodology, one could potentially miss some poor metabolizers because a rare polymorphism or a
novel functional polymorphism was not screened for. This illustrates one major drawback of CYP2D6 genotyping by the methodology described. By screening only for known CYP2D6 polymorphisms, poor metabolizers with novel or rare polymorphisms that result in loss of enzyme activity could be missed. However, the PCR-RFLP method should be reliable more than 99% of the time.

Second, even though genotype is an excellent predictor of CYP2D6 poor metabolizer status, it is probably not an absolute predictor of catalytic function. Patients of a single common genotype have dextromethorphan/dextrorphan metabolic ratios over a 1000- to 10000-fold range, yet there is a significant gene-dose effect for both debrisoquin and dextromethorphan for all poor, intermediate, extensive and ultrarapid metabolizers differences. The precise reason for this range is unknown but is probably due to diet, differential expression of the CYP2D6 gene, inherent backup metabolism systems, significant interlaboratory and intraindividual variability in phenotype data, and possible combinations of other minor undetected genetic polymorphisms in the CYP2D6 gene. CYP2D6 genotyping could, therefore, be challenging for therapies with narrow therapeutic indices, as intermediate metabolizers cannot be readily identified since some heterozygous individuals for the wild-type allele may have enzyme metabolic activity comparable to homozygous subjects.

Third, specific drug-drug interactions can convert extensive metabolizers to poor metabolizers. While this is a minor issue in the clinical trial setting where concomitant medications can be carefully screened, drug-drug interactions in a traditional medical care setting can have extremely important consequences because patients can be far
more difficult to monitor. CYP2D6 genotyping would be of little use in such cases where it is a drug-drug interaction, not metabolic status that is problematic.

A final limitation of genotyping is the ethical issue surrounding anonymous and non-anonymous genotyping. Patients feel that genetic studies are an invasion of their privacy, and some may worry that employers or insurance companies could get access to genotype data. Therefore, for legal and ethical reasons, genotyping in clinical trials requires new consent form and institutional review board approval.

In conclusion, phenotyping and genotyping complement each other, although phenotyping may provide the physician with information that is more relevant for the treatment of the patient. The most information is derived from knowing both the genotype and the phenotype. The genotype will provide extremely important information during the pretreatment phase in order to decide on the treatment strategy while phenotyping can offer valuable data if drug treatment has to be adjusted according to apparent phenotype if phenocopying occurs. Nevertheless, prospective studies have yet to demonstrate the practical usefulness of such assessments.

In light of the aforementioned observations, genotyping the subjects included in this study seems to be an attractive idea as a next step in order to compare phenotyping and genotyping results and identify the importance of both assessments in our study population.
3.5 STATEMENT OF THE PROBLEM

Cytochrome P450 2D6 is responsible for the clearance of 25-30% of all clinically used medications and has been associated with the metabolism of over 50 of the 100 best selling drugs in the US. Clinical implications of CYP2D6 polymorphism have been investigated and reviewed thoroughly with special attention paid to cardiovascular and neuroactive medications with narrow therapeutic indices such as antiarrhythmics, tricyclic antidepressants and neuroleptics, because a large proportion of these drugs are substrates of CYP2D6. 126,121

The metabolic activity of cytochrome P450 2D6 was found in many studies to be bimodally distributed in Europeans and other Caucasian populations with 5-10% of the population being poor metabolizers whilst in Orientals only 0-1% of populations display poor metabolizing behavior. 121,122 There is a great variation in prevalence of poor metabolizers in African populations ranging from 0% to 19% presumably an indication of the ethnic heterogeneity on the continent. 123 In Arab countries, such as Saudi Arabia and Ethiopia, the frequency of the poor metabolizer phenotype is low ranging from 1 to 2% of the population. 124,125

The marked interethnic variability in CYP2D6 polymorphism in addition to the absence of studies in the Lebanese population as well as the impact of CYP2D6 polymorphism on medical care, strongly support the need for a study to investigate CYP2D6 metabolic capacity and its polymorphic behavior in the Lebanese population.