CYP2D6 POLYMORPHISMS IN SELECTED SOUTH AFRICAN POPULATIONS

A thesis submitted in fulfilment of the requirements for the degree of Philosophiae Doctor in the Faculty of Health Sciences,
Department of Haematology and Cell Biology,
University of the Free State, Bloemfontein,
SOUTH AFRICA

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January 2005

Declaration

I, the undersigned, hereby declare that the dissertation herewith submitted for the degree of Philosophiae Doctor in the Faculty of Health Sciences, Department of Haematology and Cell Biology at the University of the Free State, contains my own independent work and has hitherto not been submitted for any degree at any other University. I furthermore cede copyright of this dissertation in favour of the University of the Free State.

Christa Coetsee
January 2005

The Past

"The rational use of drugs requires that patients receive medications appropriate to their clinical needs, in doses that meet their own individual requirements for an adequate period of time, and at the lowest cost to them and their community. However, no medicinal drug is entirely or absolutely safe for all people, in all places, at all times. We must always live with uncertainty."

WHO Conference of experts
Nairobi 1985

and

The Future

"It is anticipated that, in the future, genotyping could be used to personalize drug treatment for vast numbers of subjects, decreasing the cost of drug treatment and increasing the efficacy of drugs and health in general."

Magnus Ingelman-Sundberg
Trends in Pharmacological Sciences Vol. 25 No. 4 April 2004

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Acknowledgements

I want to express my sincere gratitude and appreciation to the following people and Institutions:

Prof Oubaas Pretorius, for his guidance, support and friendship during the course of this study.

The Department of Haematology and Cell Biology, UFS and Prof Badenhorst, for the use of equipment and facilities, as well as financial support.

The Faculty of Health, UFS, and the Department of Pharmacology for financial support

The NHLS for the gratification to finish my degree.

Prof Gina Joubert of the Department of Biostatistics for the statistical analyses.

The NRF for financial support to present some of the results from this study at an international conference.

Dr André de Kock for his help and guidance with the sequence and analyses.

All my colleagues and friends from the Faculty for their moral support and encouragement.

Also my scientific colleagues and friends from AiBST for guidance.

My parents who always believed in my abilities and encouraged me.

Dawie, my loving husband, for all his love, understanding and tolerance to finish this study. My six year old twins, Dawid and Christel, who did not always understand why mom is working late again – Thank you!

And lastly, to my Heavenly Father, who has made all of this possible and without whom I would not have been able to succeed.

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Term	Definition
Allele	One of the different forms of the gene or DNA segment that can exist at a single locus. One allele is from the mother, the other from the father. Allelic frequencies in populations: *p* is the major allele: *q* is the variant allele(s).
Crossing-over	The exchange of corresponding chromosome parts by breakage and reunion. The consequence of recombination.
Drug metabolising enzymes	Enzymes, numbering in the hundreds, which are capable of metabolising pharmaceuticals.
Genotype	Genetic (DNA) sequence of each individual.
Haplotype	The relationship of variant sites (SNPs) to one another along a single chromosome.
Metabolic ratio	Rate by whereby the enzyme metabolise the parent compound (dosage) determined as amount of parent compound divided by amount of metabolite formed
Mutation	Alteration/change in the genetic material, could be silent or functional
Pharmacogenetics	Study of heritable response to pharmaceutical agents. Study of gene-drug interactions.
Phenocopy	When the same trait exists in two patients as a result of different genes or environmental factors contributing to that trait.
Phenotype	Any observable characteristic such as biochemical, physiological, morphological or behavioural of an organism.
Polymorphism	Detectable variation in genome structure among individuals in a population
Pseudogene	A gene that does not produce a protein product
Single nucleotide polymorphisms (SNPs)	A single base difference in the DNA sequence of part of a population, which may or may not lead to different amino acid in the protein. Polymorphic SNPs has frequencies ≥ 1 %

List of abbreviations

CI	confidence interval
CYP450	Cytochrome P450
CYP2D6	Cytochrome 2D6
DNA	deoxyribonucleic acid
ds	double stranded
EM	extensive metaboliser
IM	intermediate metaboliser
MR	metabolic ratio
mRNA	messenger RNA
nt	nucleotide
PM	poor metaboliser
RNA	ribonucleic acid
SNP	single nucleotide polymorphism
UM	ultra-rapid metaboliser

Chapter 1

Literature Review

1.1 Introduction

Clinical therapy remains very much an art where the doctor or clinician selects a drug according to his or her experience, rather than according to scientific knowledge. The aim is to choose a drug most likely to produce the desired result with minimum adverse effects and cost to the patient (Arranz *et al.*, 2001). Disruption of the normal metabolism of drugs could result in a direct clinical effect, and not always the desired effect. Despite the improved methods for drug development by which many safer drugs have been produced, adverse drug reactions remain a major cause of morbidity and mortality worldwide. The WHO Collaborating Centre for International Drug monitoring received over three million reports in 2003, while, in the year 2000, adverse drug reactions were the fifth leading cause of death in the United States (the Uppsala monitoring centre (UMC); Mancinelli *et al.*, 2000). The WHO Centre also stated an increase of 160 000 drug adverse events per year. This, together with other drug related problems, has led to a renewed interest in the development of strategies for the prevention of adverse drug reactions.

Drug metabolising enzyme polymorphisms have become an essential part of the field of pharmacogenetics (Gonzalez & Meyer, 1991; Ingelman-Sundberg, 2001a; Kalow, 1997). It is a field of growing interest to both medical doctors and the pharmaceutical industry. Physicians see it as a worry or a nuisance when their patients are not responding as expected to drug therapy (Kalow, 1997). Pharmacogenetics involves the study of heritable genetic variations causing variable drug response and includes genetic polymorphisms of drug transporters, drug metabolising enzymes (Phase I and II) and drug receptors (Arranz *et al.*, 2001; Ingelman-Sundberg, 2001b; Roses, 2001). The occurrence of pharmacogenetic differences among populations is seen as typical events (Kalow, 1997). Many pharmaceutical companies have decided to minimise losses by pre-

testing their products *in vitro*, using polymorphic drug metabolising enzymes that may show variation in drug response (Kalow, 1997). Identification of individuals at risk could also minimise the occurrence of side effects, especially of treatments having a high risk for the patient, and also increasing patient compliance and the efficacy of the treatment (Arranz *et al.*, 2001). Prospective genotyping could ensure the safety of subjects in a clinical trial, while enhancing the efficiency, power and blindness of the study (Murphy, 2000; Ingelman-Sundberg, 2004). In a paper published by Johnson and Evans (2002), the authors proposed that subjects used during Phase II clinical trials should be genotyped. Genotyping and/or phenotyping should however not replace traditional therapeutic drug monitoring, but rather be used as a first line for dosage adjustment to decrease the variability observed during steady state on a standard dosing regime (Brøsen & Gram, 1989; Tamminga *et al.*, 2003).

Because cytochrome P450 enzymes are responsible for the metabolism of most drugs used, variations in their activity could have profound effects on the pharmacological and toxicological profiles of many drugs. Indeed, some of the known mutations in the cytochrome P450 genes have been associated with altered pharmacokinetics and severe adverse drug reactions of many important drugs (Paine, 1995). This is exemplified by the autosomal recessive mutation of the cytochrome P450 isoform CYP2D6, characterised by deficient hydroxylation of debrisoquine. This mutation results in the compromised metabolism of at least 25 drugs, sometimes even leading to life-threatening side effects (Gough *et al.*, 1990).

Unfortunately, despite this understanding, there is very little information on cytochrome P450 activity in the Southern African population. This is partly due to lack of tests for genotyping and phenotyping of cytochrome P450 in Africans. Most of the existing genotyping and phenotyping tests have been developed based on mutations arising among Caucasians and are therefore not always applicable to the Southern African population because of wide differences found in the mutations of Africans compared to those of Caucasians (Dandara *et al.*, 2001; Eichelbaum & Gross, 1990; Gaedigk *et al.*, 2002). In this study we intend to develop methods for genotyping of the most polymorphic cytochrome P450 enzyme, namely CYP2D6, and to apply these methods to screen three

representative Southern African population groups. We envisage that the development of accurate genotyping assays will significantly enhance the benefits of patients requiring drugs metabolised by CYP2D6, many of which have a narrow therapeutic index (Paine, 1995).

1.2 Genetic Polymorphisms

A polymorphism is seen as a heritable monogenic trait occurring with a frequency of not less than 1-2 % in a population. It is indicated by a bimodal frequency distribution curve of the metabolic ratio with the antimode between the two phenotypic (extensive and poor metabolisers; Figure 1) populations (Abraham & Adithan, 2001; Bertilsson, 1995; Gonzalez & Meyer, 1991; Llerena *et al.*, 1996; McKinnon & Evans, 2000). The metabolic ratio is usually determined as the amount of metabolite formed, divided by the amount (dosage) of parent compound administered (Gonzalez & Meyer, 1991).

Since each monomeric enzyme is a product of a specific gene, a change in genetic composition would influence the expression of iso-enzymes in different individuals, leading to a variation in the metabolism of drugs (Badyal & Dadhich, 2001; Weinshilboum, 2003).

The drug metabolising enzymes found in mammalians consist of several classes able to metabolise almost every chemical to which the body is exposed (Kalow, 1997). Approximately three dozen of these enzymes found in man have been shown to be genetically variable (Kalow, 1997). Mutations are very heterogeneous, ranging from single nucleotide polymorphisms (SNPs) to dozens of alleles (Nelson, 1999). Amino acid variations in the substrate binding domains could result in altered compound-, regio- or stereo-selectivity of the enzymes (Masimirembwa *et al.*, 1999). Inter-individual variability in the metabolism of xenobiotics, results from the influence of many factors, such as environmental impact (smoke, alcohol, pesticides, etc), current medication, clinical status and genetic factors (McKinnon & Evans, 2000; van der Weide & Steijns, 1999). This becomes very important for individual drug therapy, for clinical trials targeting a

specific ethnic group and also when searching for possible relationships between genotype and the susceptibility to cancer and other diseases linked to xenobiotic metabolising enzymes (Hasler *et al.*, 1999).

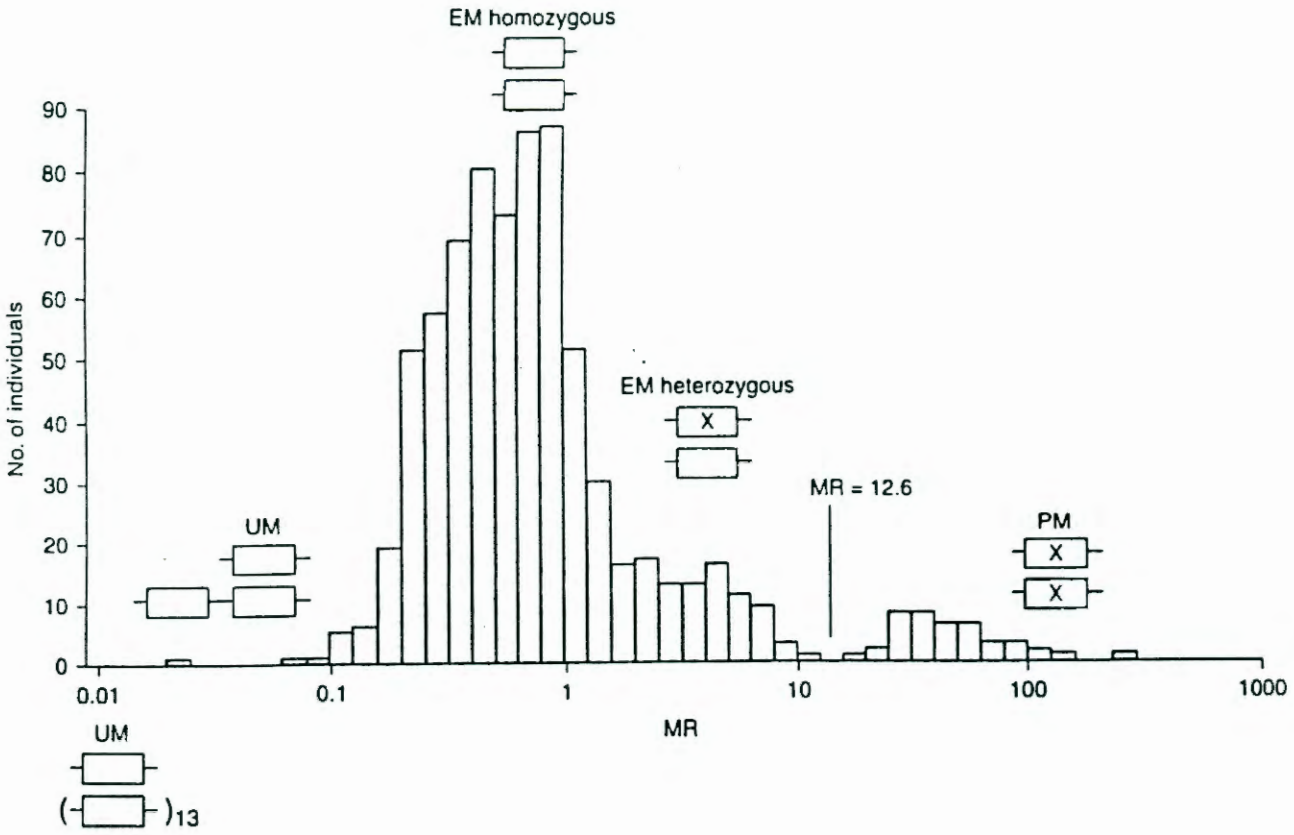


Figure 1. *Schematic presentation of the relationship between the debrisoquine metabolic ratio (MR = debrisoquine/4-OH debrisoquine) and the major CYP2D6 genotypes causing altered CYP2D6 activity in a Swedish population. MR = 12.6 indicates the antimode between the PMs and EMs. EM = Extensive metabolisers, UM = ultra-rapid metabolisers, PM = poor metabolisers. (Dahl, 2002).*

Genes encoding proteins involved in the response to an administered drug, may be polymorphic. These include a vast number of proteins such as all drug metabolising enzymes, drug receptors, drug transporters and even proteins involved in the pathophysiology of the disease treated (McKinnon & Evans, 2000; Weinshilboum, 2003).

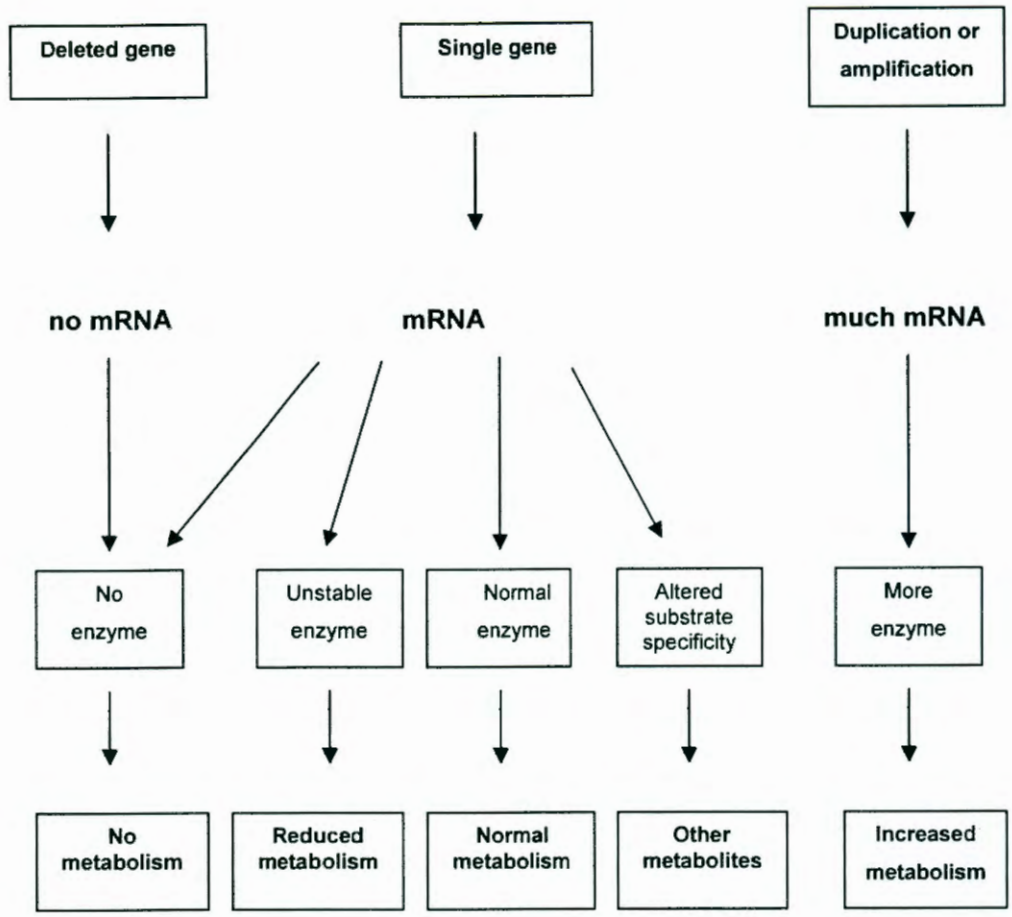


Figure 2. *Consequences of mutations occurring in the cytochrome P450* genes. *Reproduced from Ingelman-Sundberg, 2001a.*

The impact and importance of genetic variability in drug metabolising enzymes have been recognised by researchers (Gonzalez & Meyer, 1991; McKinnon & Evans, 2000; Ingelman-Sundberg, 2004). Previously these differences in the metabolism of drugs have been identified by the observance of unexpected responses to therapeutic dosages of drugs [Figure 2] (McKinnon & Evans, 2000). For the purpose of this study we will concentrate on the cytochrome P450 drug metabolising enzymes.

Genetic polymorphisms in the drug metabolising enzymes are indicated by a bimodal frequency distribution curve (McKinnon & Evans, 2000; Gonzalez & Meyer, 1991). Poor metabolisers have an inherited absent capacity to metabolise some drugs, while extensive metabolisers have a normal activity (Llerena *et al.*, 1996). Deficient debrisoquine hydroxylation is observed as a bimodal distribution curve of the metabolic ratios for a given population. Polymorphic expression of the drug-metabolising enzymes is one of the factors responsible for inter-ethnic and inter-individual variability in the metabolism of a variety of drugs, causing pharmacological and toxicological responses (Dandara *et al.*, 2001; Llerena *et al.*, 1996; Masimirembwa *et al.*, 1995).

1.3 Cytochrome P450 (CYP450) Enzymes

The cytochrome P450 enzymes are a superfamily of oxygen-reacting haeme proteins (Hasler *et al.*, 1999). More than 40 different members of the family have been identified in humans and they play a critical role in the bioactivation and detoxification of numerous xenobiotics (Hasler *et al.*, 1999; Omiecinski *et al.*, 1999). The cytochrome P450 enzymes are responsible for Phase I metabolism reactions such as the biosynthesis of steroids, metabolism of xenobiotics to reactive metabolites, oxidation of unsaturated fatty acids to intracellular messengers and the stereo- and regio- specific metabolism of fat soluble vitamins. Most of these CYP450 enzymes are polymorphic and some are also inducible, thus having the potential of abolished or altered drug metabolism (Ingelman-Sundberg, 2001a & b).

The human cytochrome P450 2D6 gene was mapped to the long arm of chromosome 22 nearby the P_1 blood group (Eichelbaum *et al.*, 1987; Gonzalez & Meyer, 1991). These enzymes are distributed throughout the whole body, however the highest concentration is found in the liver (Badyal & Dadhich, 2001; Bertilsson, 1995; Hasler *et al.*, 1999; Nebert & Russel, 2002; Paine, 1995). Since there are so many different substrates of the P450 enzymes, the occurrence of drug-drug interactions is frequent (Nebert & Russel, 2002). The CYP450 family also acts as a protective system of the human body, scavenging free radicals and

also detoxifying the body from xenobiotics. Since the essential role of these enzymes in the human body is the metabolism of numerous xenobiotics and drugs, it would be of great interest to understand the varied response to therapeutic drugs. Of all the CYP450 enzymes, only a few are involved in the metabolism of drugs, namely, CYP1A2, 2A6, 3A4, 3A5, 2C8, 2C9, 2C19, 2D6 and 2E1 (Badyal & Dadhich, 2001; McKinnon & Evans, 2000).

The cytochrome P450 enzymes were first recognised by Martin Klingenberg in 1958, when he was studying the spectrophotometric properties of pigments in a microsomal fraction prepared from rat livers. He observed a unique spectral absorbance band at 450 nm, which proved to be unique among haeme proteins and thus served as the signature of the P450 proteins. It was only later discovered that these proteins, present in liver microsomes, play a very important role in the metabolism of drugs and other xenobiotics.

The CYP450 enzymes occur widely in nature and different enzymes are found in plants, insects, some bacteria, fungi and mammals. The number of chemicals serving as substrates for the P450s is also numerous. The known properties of the mammalian P450 enzymes are as follows;

(a) the proteins contain about 500 amino acids. The amino end of the protein is hydrophobic and is thought to act as the domain for binding to membranes.

(b) these enzymes catalyse the NADPH and oxygen dependent oxidation reactions of many different compounds,

(c) the concentration of P450s is the highest in the liver, intestine and cortex of the adrenal glands,

(d) these enzymes are however distributed to most organs of the human body and

(e) the cellular expression of some P450s is regulated by transcriptional factors activated on exposure to various chemicals. These enzymes are responsible for the metabolic activation of many other enzymes. It should also be noted that CYP2D6 is not inducible.

About 700 P450s (74 CYP families) and 1000 genes have currently been characterised (Omiecinski *et al.*, 1999; van der Weide & Steijns, 1999). There are 57 functional CYP genes, 33 pseudogenes comprising 42 subfamilies and 18 families in humans (Nebert & Russel, 2002). A standardised nomenclature was put together to categorise the wide variety of P450 enzymes. The enzymes have been classified into families and subfamilies according to their amino acid sequence similarities. P450 enzymes showing more than 40 % protein sequence similarity are placed within the same family, while enzymes with more than 60 % sequence similarity are grouped into subfamilies (Nebert & Russel, 2002; McKinnon & Evans, 2000; Nelson, 1999; Omiecinski *et al.*, 1999). Subfamilies are indicated by a letter (CYP2**D**) following the family number (CYP**2**), while individual genes, coding for one specific isoenzyme, have a second arabic number after the letter e.g. CYP2D**6** (Van der Weide & Steijns, 1999).

Transcription factors activated by exposure to various chemicals regulate the cellular expression of some of the cytochrome P450 enzymes. Additional factors that could have a possible effect on expression levels of the CYP genes are age (decrease in activity of CYP with ageing), gender, hormones (e.g. testosterone deficiency decreases CYP activity), hepatic disease, inflammation (acute phase inflammation mediators could suppress CYP activity), nutrition (obesity & starvation inhibit CYP), pregnancy (possible induction of CYP) and genetic polymorphisms (Badyal & Dadhich, 2001; Llerena *et al.*, 1996; Weinshilboum, 2003). However, other researchers have found that CYP2D6 activity does not change with age, but rather that it is the pharmacokinetics of substrates of CYP2D6 changing due to age-associated changes in hepatic blood flow, volume of distribution and renal elimination of the metabolites formed (Schulman & Özdemir, 1997).

Currently, studies on polymorphic expression of the activity of CYP450 enzymes have become an essential part of the field of research termed "pharmacogenetics" (Gonzalez & Meyer, 1991). Pharmacogenetics comprises the study of the biological consequences of drug metabolising enzyme mutations [Figure 2] (Kalow, 2002). These mutations result in abolished and quantitatively or qualitatively altered, even enhanced, metabolism of drugs (Ingelman-Sundberg, 2001a). Since

these variations are inherited via monogenic or Mendelian trait (Kalow, 1982; Skoda *et al.*, 1988), development of methods for detection of cytochrome P450 mutations in humans will allow for the design of molecular epidemiological studies to determine if inter-individual differences in the gene sequences of these enzymes could confer toxicity or sensitivity to certain drugs (Gonzalez & Meyer, 1991; Paine, 1995). By this approach, many clinically significant variations in the activity of cytochrome P450 enzymes among different ethnic groups have been demonstrated, and inter-individual differences in drug response or toxicity have been explained.

Studies have shown that populations could be divided into two main groups, namely extensive (EM) and poor (PM) metabolisers in their ability to oxidise debrisoquine, sparteine or other drugs. Poor metabolisers are subjects with deficient oxidation as compared to extensive metabolisers with normal oxidation capabilities (Broly *et al.*, 1991). The observation of polymorphic P450 enzymes has proved to be of major clinical importance in the occurrence of adverse drug reactions upon drug administration. According to the WHO Technical Report No. 498 (1972), an adverse reaction is "a response to a drug which is noxious and unintended and which occur at doses normally used in man for the prophylaxis, diagnosis or therapy of disease or for the modification of physiological function." It is important to distinguish between rare disease-causing mutations and polymorphisms, which occurs in more than 1 % of a population (Nelson, 1999). There are many mutations known in P450s causing disease, such as those in CYP1B1, CYP17, CYP19, CYP21 and CYP2B7, but these differ from those polymorphic enzymes affecting the metabolism of drugs and disease susceptibility without causing diseases directly (Nelson, 1999).

1.4 The CYP2D Family

The CYP2D family is the largest and most diverse CYP family. More than 20 members have been identified for the CYP2D subfamily but only one major form, CYP2D6, is expressed in humans (Nelson, 1999).

The *CYP2D6* gene encoding CYP2D6 has been localised to the long arm of chromosome 22 in the region 13q.1, where it forms part of the *CYP2D* cluster, together with *CYP2D7* and *CYP2D8* (Bertillson *et al.*, 2002; Broly *et al.*, 1991; Gonzalez & Meyer, 1991; Gough *et al.*, 1993; Marez *et al.*, 1997). The *CYP2D6*, *CYP2D7* and *CYP2D8* genes are tandemly arranged, each containing 9 exons and spanning about 7 kb (Gonzalez & Meyer, 1991; Steen *et al.*, 1995). *CYP2D8P* is a pseudogene (non-functional) located upstream of the *CYP2D6* gene and is not expressed (Gaedigk *et al.*, 1991; Gonzalez & Meyer, 1991; Omiecinski *et al.*, 1999). Both the *CYP2D6* and *CYP2D7* genes are expressed, although only CYP2D6 produce an active protein (Endrizzi *et al.*, 2002; Gough *et al.*, 1993). *CYP2D8P* contains several gene-disrupting insertions, deletions and termination codons within its exons, while *CYP2D7* is full-length, except for a T insertion in the first exon at position 137, causing a disruption of the reading frame and thus a non-functional protein is encoded (Gonzalez & Meyer, 1991; Kimura *et al.*, 1989; Stüven *et al.*, 1996). Gene conversions occurring between *CYP2D8* and *CYP2D6* during recombination could introduce mutations into the *CYP2D6* gene (Kimura *et al.*, 1989). Loss or gain of genes could also be due to unequal crossover events during recombination [Figure 4] (Gaedigk *et al.*, 1991).

1.5 Cytochrome 2D6 polymorphisms

Previous research has shown that defective metabolism in some individuals are the result of the absence of CYP2D6 in the liver. Although CYP2D6 accounts for about 2 % of the total P450 content in the human liver, it is responsible for the metabolism of nearly one quarter of all prescribed drugs (Abraham & Adithan, 2001; Ingelman-Sundberg & Evans, 2001). The clinical significance of the CYP2D6 polymorphisms is more pronounced for the tricyclic antidepressants, certain neuroleptics, antiarrhythmics, antihypertensives, β-adrenoreceptor blockers and morphine derivatives (opioids) (Abraham & Adithan, 2001; Bertilsson *et al.*, 2002). Of these, quite a few have been used as probe substrates to determine the activity of CYP2D6 (Omiecinski *et al.*, 1999).

In 1977 it was observed that the hydroxylation of debrisoquine, an antihypertensive drug, was polymorphic, while another group observed similar results with the oxidation of sparteine. CYP2D6 is therefore also known as sparteine/debrisoquine hydroxylase (Bertilsson *et al.*, 2002; Gaedigk *et al.*, 1999).

CYP2D6 is non-inducible and the absence of post-translational regulation eliminates confounding factors for determination of its expression (Ingelman-Sundberg, 2001a & b). There are three major determinants for the expression and activity of CYP2D6 namely

1) the number of functional *CYP2D6* gene copies per genome,
2) promoter genotype and
3) the expressed allelic variant (Zanger *et al.*, 2001).

The *CYP2D6* gene is highly polymorphic and point mutations, small deletions or insertions, duplications and even entire 2D6 gene deletions occur regularly (Bertilsson *et al.*, 2002; Gaedigk *et al.*, 1999). This array of gene variations results in large variations in the enzymatic activity of CYP2D6 in populations, ranging from poor to ultra rapid metabolisers [Figure 3] (Gaedigk *et al.*, 1999). More than 75 allelic variants of CYP2D6 have been identified and characterised so far (Bertilsson *et al.*, 2002; Ingelman-Sundberg & Evans, 2001). The CYP2D6 deficiencies are only manifested during exposure to drugs (Gonzalez & Meyer, 1991).

Much research has been done to identify and classify all the possible polymorphic CYP2D6 alleles. Not all the SNPs have, however, a clinical impact and some are 'silent'. In 1996, Daly and co-workers put together a new nomenclature for the CYP2D6 alleles and subtypes. Since then, many more alleles have been identified and characterised (den Dunnen & Antonarakis, 2001; Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee).

Subjects are classified into EM (extensive), PM (poor), IM (intermediate) or UM (ultra rapid) metabolisers according to their ability to metabolise marker (probe) substrates. Most individuals (80 – 90 %) have at least one functional allele of CYP2D6 and are classified as EM (Pavanello & Clonfero, 2000). Enzyme activity

is highly variable among the extensive metabolisers, ranging from extremely high in ultra-rapid metabolisers to reduced activity in intermediate metabolisers (Bertilsson *et al.*, 2002). Intermediate enzyme activity is caused by a mutation of the *CYP2D6* gene causing decreased substrate specificity, while ultra-rapid activity is due to the duplication or amplification of the *CYP2D6* gene [Figure 2] (Pavanello & Clonfero, 2000). The poor metabolisers are identified by the presence of sequence variations within the *CYP2D6* gene leading to severely impaired enzyme activity (Pavanello & Clonfero, 2000). About 15 CYP2D6 alleles have been identified with low enzyme activity (CYP2D6*9, *10, *17) or the absence of activity (CYP2D6*3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16) (Human Cytochrome P450 (*CYP*) Allele Nomenclature Committees' webpage; Pavanello & Clonfero, 2000; Table 1).

Table 1. Some of the alleles responsible for the different CYP2D6 phenotypes

Phenotype	CYP2D6 Alleles
EM	*1, *2, *33, *35,
PM	*3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16, *18, *19, *20, *21, *38, *40, *42, *44,
IM	*9, *10, *17, *29, *36, *41,
UM	*1xN, *35x2, (amplification of allele)

Human Cytochrome P450 (CYP) Allele Nomenclature Committees' webpage

Inter-ethnic differences in the metabolic polymorphism of CYP2D6 have been extensively studied among Caucasians and Orientals, but little research has been done on the black populations of Africa. Studies performed on African populations using phenotyping and genotyping techniques have revealed new features of CYP2D6 genetic and phenotypic status (Dandara *et al.*, 2001; Masimirembwa *et al.*, 1996; Wennerholm *et al.*, 2002).

The prevalence of CYP2D6 PMs varies in different ethnic groups, ranging from 5-10 % in Caucasians, 0-1 % in Orientals, 2 % Afro-Americans and 0-5 % in some black Africans (Bertilsson, 1995; Dandara *et al.*, 2001; Fukuda *et al.*, 2000). The

occurrence of "population-specific" allelic variants has also been observed (Bertilsson *et al.*, 2002).

Dandara and co-workers (2001) found no difference between the CYP2D6 PM genotypes when CYP2D6*17 was excluded from their analyses. It was found that *17 occurs most frequently in Black population groups namely, 34 % Zimbabweans, 24 % Vendans and 20 % Tanzanians and it was concluded that this allele could be specific for black Africans (Dandara *et al.*, 2001; Masimirembwa *et al.*, 1996). A high frequency of allele *17 was found to be predictive of intermediate metabolisers in black African populations (Dandara *et al.*, 2001; Masimirembwa *et al.*, 1996; Wennerholm *et al.*, 2002).

In 1995, Bertilsson observed personality differences between poor and extensive metabolisers of CYP2D6 in Swedish and Spanish subjects. He postulated that CYP2D6 could be present in the brain, but in very low concentrations (Bertilsson, 1995). This hypothesis was confirmed when mRNA was found in certain regions of the brain. Further studies by Miksys and coworkers (2002) also showed an increase of CYP2D6 protein in brain regions of alcoholics and smokers (Miksys *et al.*, 2002). Further research also showed an association between the dopamine transporter and CYP2D6 in the brain, in that PMs developed adverse effects to neuroleptics much faster than EMs (Bertilsson, 1995). Caraco and co-workers (1996) found that the co-administration of serotonin-specific re-uptake inhibitors and CYP2D6 substrates is impossible. Research also showed that CYP2D6 is responsible for the *O*-demethylation of neurotoxic ß-carbolines alkaloids which are strong reversible inhibitors of monoamine oxidase (MAO) and thus protecting the brain against damage (Yu *et al.*, 2003). Poor metabolisers do not have the potential for this neuroprotective detoxification by CYP2D6. It was furthermore found that the inadequate therapies for depression were mostly due to CYP2D6 polymorphisms and that the determination of the patients' genotype and the subsequent individualised therapy could resolve this problem (Ereshefsky, 1998). The risk of tricyclic toxicity is greatly enhanced in poor metabolisers when nortriptyline and desipramine is co-administered, and lower doses of these drugs will, therefore, have to be prescribed. Fluoxetine and paroxetine are both inhibitors of CYP2D6 activity and an accumulation of drugs that are substrates of CYP2D6

would thus occur in the plasma, leading to toxicity. The administration of risperidone to poor metabolisers could lead to orthostatic hypotension (Ereshefsky, 1998). Gough and co-workers, 1990, have also observed that CYP2D6 polymorphism could be associated with an altered susceptibility to lung and bladder cancer in Caucasians.

1.5.1 Poor metabolisers

The poor metaboliser phenotype is almost always caused by the inheritance of two mutated, non-functional (null) CYP2D6 alleles and could be either homozygous or heterozygous carriers of inactivating mutations (Løvlie *et al.*, 2001; Marez *et al.*, 1997; Zanger *et al.*, 2001). As discussed previously, poor metabolisers of CYP2D6 are characterised by a low or absent enzyme activity, since no protein is formed (Gonzalez *et al.*, 1988). These subjects are at high risk for adverse drug reactions and toxicity. Studies have shown that PMs of CYP2D6 are more prone to oversedation, hypotension and even parkinsonism during treatment with classical antipsycotics (Dahl, 2002; Tamminga *et al.*, 2003).

The occurrence of PMs has been investigated in many populations (Figure 6). It was found that 7 – 10 % of Caucasians are poor metabolisers of debrisoquine, while only about 1 % of Chinese was found to be PMs (Bertilsson *et al.*, 1992; Ingelman-Sundberg, 2005; Zanger *et al.*, 2004). No clear bimodal distribution was observed for the Chinese although the MR was shifted to the right, indicating a lower CYP2D6 enzyme activity than for Caucausians (Llerena *et al.*, 1996). The different cut-off MRs for Caucasian PMs was found to be above 12.6 for debrisoquine, more than 20 for sparteine (Zanger *et al.*, 2001) and > 0.3 for Dextromethorphan (Brøsen & Gram, 1989).

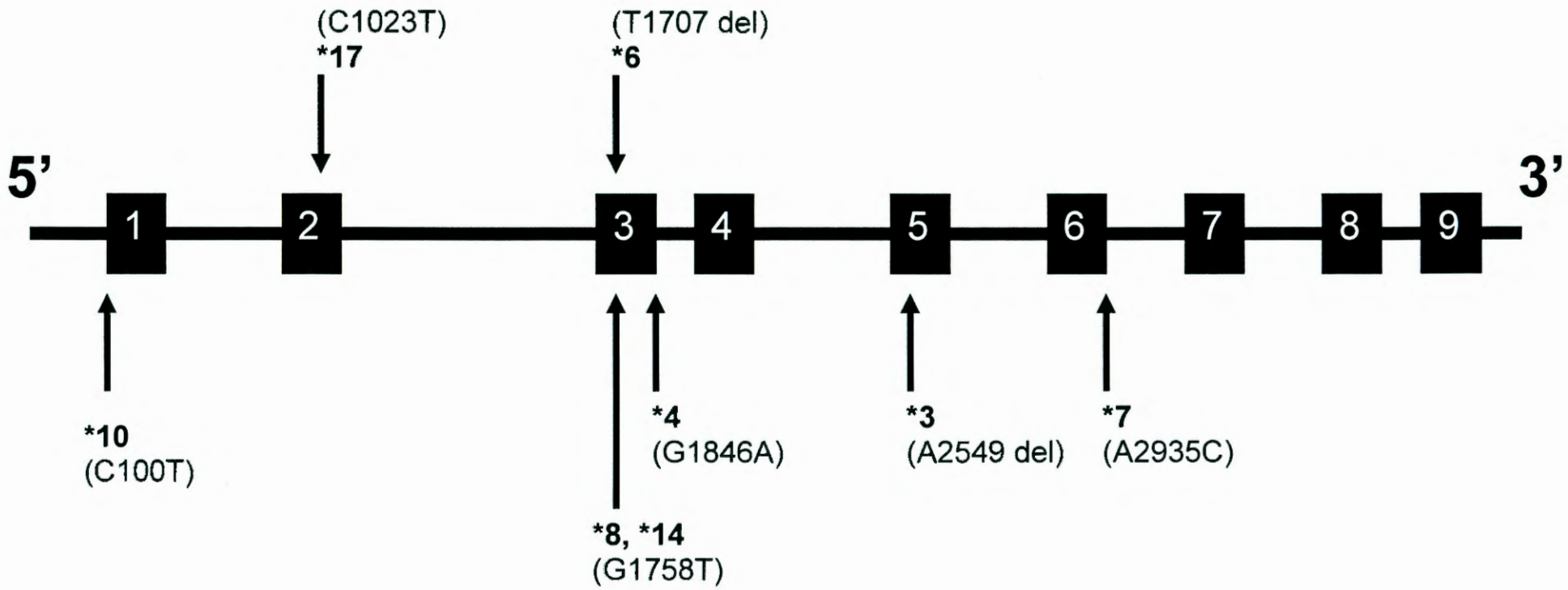


Figure 3. *Schematic representation of the CYP2D6 gene showing the nine exons and the polymorphic alleles, with the significant SNP responsible for each, detected in this study.*

Using Southern blot analysis after *Xba*I restriction enzyme analyses it was found that the deletion of the *CYP2D6* gene (*5) is characterised by an 11.5 kb restriction fragment (Gaedigk *et al.*, 1991; Gough *et al.*, 1990). Skoda and co-workers, 1988, observed that the PM phenotype is associated with either two mutated alleles, heterozygous for 44 kb and 11.5 kb, or homozygous for 44 kb.

Three major mutant alleles have been identified in Caucasians to cause a low activity, namely CYP2D6*3, *4 and *5, as well as a large number of low frequency alleles (Bertilsson *et al.*, 2002). Alleles *3 and *4 occur more frequently in Caucasians, while CYP2D6*5 occurred evenly in Caucasians, Orientals and Blacks (Aklillu *et al.*, 1996). CYP2D6*4 was found to account for more than 75 % of the mutant alleles in the Swedish population, occurring with a frequency of 22 %, 6.3 % among Ghanians, while this same allele is almost absent in Chinese (Bertilsson *et al.*, 2002; Griese *et al.*, 1999). The frequency of CYP2D6*5 was, however, found to be similar among different populations ranging from 4-6 % (Bertilsson *et al.*, 2002; Griese *et al.*, 1999). Allele 5 (*5) was found to be the second highest genetic defect responsible for the PM phenotype in Caucasians (Marez *et al.*, 1997). Recently another mutation, CYP2D6*41, causing decreased protein expression of CYP2D6 among Caucasians, was characterised (Raimundo *et al.*, 2004).

The number and specific alleles responsible for the poor metaboliser phenotype may however, differ between populations, and needs to be considered when performing genotyping (Bertilsson *et al.*, 2002).

1.5.2 Intermediate metabolisers

Impaired but still detectable enzyme activity is characteristic of intermediate metabolisers with a MR ranging from 1.2 to 20 for sparteine in Caucasians (Zanger *et al.*, 2001).

CYP2D6*17 was found to cause a right shift of the MR for debrisoquine hydroxylation in black Zimbabweans (Masimirembwa *et al.*, 1996). This right shift

was caused by an allele encoding an enzyme with decreased activity due to a decrease in substrate affinity (Oscarson *et al.*, 1997). CYP2D6*17 carries three functional mutations (C1023T, C2850T and G4180C) causing impaired hydroxylation of debrisoquine among Zimbabweans and one (G1638C) silent mutation. Studies showed the C1111T mutation linked with the C2850T and G4180C mutations on the same allele and the effect on enzyme activity only occurs when all three these mutations are present (Oscarson *et al.*, 1997). Despite the high frequency of CYP2D6*17 among black Africans (34 % Zimbabweans, 17 % Tanzanians, 28 % Ghanaians and 9 % Ethiopians), much variation was observed within these populations, demonstrating the heterogeneity of the African populations (Bertilsson *et al.*, 2002; Griese *et al.*, 1999). CYP2D6*17 was also observed in the Aborigines, but at a very low percentage of 0.2 % (Griese *et al.*, 2001). Gaedigk and co-workers (2002) also found CYP2D6*17 in a high frequency (0.213) among the African Americans.

Studies have revealed another allele (CYP2D6*10) causing reduced activity of the CYP2D6 enzyme among Orientals. Comparing Swedish and Chinese subjects showed a right-shift in the MR of Chinese extensive metabolisers (EMs) (Bertilsson *et al.*, 1992; Bertilsson *et al.*, 2002; Johansson *et al.*, 1994). Further research revealed a high frequency of CYP2D6*10 among Chinese, resulting in a reduced rate of debrisoquine hydroxylation. The CYP2D6*10 allele results from a SNP at position C100T, causing a Pro34Ser substitution resulting in an unstable enzyme with decreased catalytic activity (Johansson *et al.*, 1994). Although this allele was found in high frequencies among Orientals (Chinese, Koreans and Japanese), it occurs less frequently in Caucasians (Aklillu *et al.*, 1996; Bertilsson *et al.*, 2002). Ozawa and co-workers, 2004, observed this allele with a frequency of 0.408 in Japanese and 0.05 in Caucasian subjects. However, debrisoquine and nortryptyline CYP2D6-dependent elimination was limited to only a certain degree in Korean subjects with at least one CYP2D6*10 allele (Dalén *et al.*, 2003). Different results would perhaps have been obtained if a larger number of Korean subjects had been included in the study. CYP2D6*10 was also found to be responsible for the higher serum levels of tramadol in Malaysian patients, compared to CYP2D6*1 (Gan *et al.*, 2002). The allele occurred with a frequency of 0.43 in Malaysian subjects. CYP2D6*10 was also observed with a frequency of

0.8 % among the Aborigines (Griese *et al.*, 2001). Ramamoorthy and co-workers (2001) further found that subjects homozygous for CYP2D6*10 could develop drug dependence for amphetamines, more rapidly than CYP2D6*1 subjects.

It could thus safely be postulated that there are three alleles, CYP2D6*4 in Caucasians, CYP2D6*10 in Orientals and CYP2D6*17 in black Africans, that could be to some degree population specific.

1.5.3 Extensive metabolisers

Extensive or normal metabolisers are subjects with at least one active/functional allele, characterised by a 29 kb *Xba*I restriction fragment (Johansson *et al.*, 1993). A wide variation of metabolic ratios (MR) has been observed for EMs in different ethnic groups, even up to 1000 fold for debrisoquine metabolism (Marez *et al.*, 1997; Masimirembwa *et al.*, 1996). The mean MR determined for Caucasians (12.6) was found to be lower than that for black Africans, when using debrisoquine (Masimirembwa *et al.*, 1996).

1.5.4 Ultra-rapid metabolisers

Ultra-rapid metabolisers (UMs) are characterised by an extremely fast and effective enzyme activity caused by a duplication or amplification of the *CYP2D6* gene (Bertilsson *et al.*, 2002; Løvlie *et al.*, 2001). The UM phenotype is the result of a dominantly inherited amplification of the functional *CYP2D6* genes in the CYP2D locus (Johansson *et al.*, 1993; Steen *et al.*, 1995). Duplication of the *CYP2D6* gene has occurred by homologous, unequal crossover involving the 2.8 kb CYP-REP-7 and CYP-REP-6 units [Figure 4] (Løvlie *et al.*, 1996). Amplification of the functional *CYP2D6* genes is observed as a 12.1 kb fragment after *Eco*RI or a 42 kb fragment after *Xba*I digestion of genomic DNA (Johansson *et al.*, 1993; Lovlie *et al.*, 1996; Steen *et al.*, 1995). Subjects with up to 12 extra copies of the functional CYP2D6*2 gene have been found (Johansson *et al.*, 1993). Ultra-rapid metabolisers are identified by a MR below 0.2 for debrisoquine and less than 0.15

for Sparteine (Løvlie *et al.*, 2001; Zanger *et al.*, 2001). The *CYP2D6* gene duplication effect is more apparent when using debrisoquine compared to dextromethorphan as probe drugs. Identification of UM subjects could be clinically important for dosage adjustments as well as to avoid misclassification of patient non-compliance (Steijns & van der Weide, 1998). Also, a high incidence of *CYP2D6* gene duplication was found among Swedish patients with persistent depression (Kawanishi *et al.*, 2004).

It is important to note that gene amplification is rare in normal human cells, except where it is for the benefit of the organism (Ingelman-Sundberg, 2001b; Johansson *et al.*, 1993). An increase in expression and function of the CYP2D6 protein was observed with an increase in the number of functional copies of the gene (Zanger *et al.*, 2001). The duplicated genes are still functional and therefore the increased enzyme activity. The level of enzyme activity is however limited by the amount of enzyme expressed (Johansson *et al.*, 1993).

Subjects characterised as UMs may encounter therapeutic failure during treatment, since the drug is rapidly metabolised after administration and sub-therapeutic plasma concentrations are observed [Figure 7] (Johansson *et al.*, 1993; Steen *et al.*, 1995). The frequency of occurrence of UMs differ among populations and frequencies of 1 % for Scandinavians, 1-2 % for Swedes, 3.6 % Germans, 7-10 % Spaniards, 10 % Italians and people in Turkey, 20 % Saudi Arabians and 29 % Ethiopians have been observed (Aklillu *et al.*, 1996; Bertilsson *et al.*, 2002; Ingelman-Sundberg, 2004; Løvlie *et al.*, 2001; Sachse *et al.*, 1997). The ultra-rapid metaboliser phenotype was found to be uncommon in Northern Europe (1-2 %) and absent in Asia (Ingelman-Sundberg, 2004). It seems that there is a European-African, North-South gradient in the occurrence of *CYP2D6* gene duplications (Bertilsson *et al.*, 2002). It was also hypothesised that dietary stress among Ethiopians cause an increased selection for gene duplications (Ingelman-Sundberg, 2001b).

Duplication of the gene does not, however, always result in ultra-rapid metabolism, as had been shown by Aklillu and co-workers in 1996. They found that black Ethiopians having multiple *CYP2D6* genes did not always have an ultra-rapid

metabolic activity. Eichelbaum and co-workers, 2001, also observed that the duplication of the *CYP2D6* gene only explains 10-30 % of UM phenotypes occurring in Caucasians. They found two mutations, 31G > A (*35), as well as a –1584C > G (*2) promoter polymorphism, that result in a CYP2D6 duplication-negative ultra-rapid metaboliser phenotype. Both these mutations could be responsible for a decrease in the metabolic ratio and were found at a high frequency among the UM Caucasians. CYP2D6*2 is associated with an increase in protein expression and metabolic activity, but not specifically with an increase in gene transcription as with duplication-positive UMs (Zanger *et al.*, 2001). CYP2D6*35 was found to be comparable with normal metabolic activity similar to the wild type CYP2D6*1 (Allorge *et al.*, 2001; Raimundo *et al.*, 2004). Duplication of the CYP2D6*10 allele was also observed in Hong Kong Chinese and in Japanese subjects, however the metabolic capacity of CYP2D6 was not increased (Garcia-Barceló *et al.*, 2000; Ishiguro *et al.*, 2004).

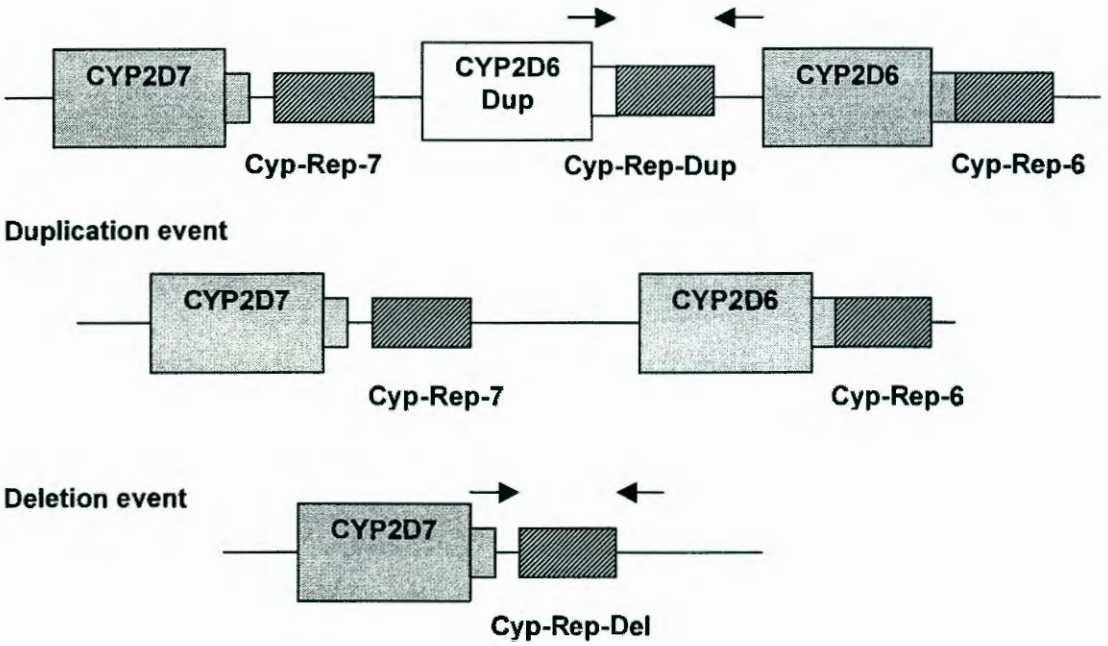


Figure 4. *Possible mechanisms for the* generation *of duplication or deletion alleles of the CYP2D6* gene *during recombination (without CYP2D8P) (Løvlie et al., 1996; Steen et al., 1995).*

Care should be taken when interpreting results from duplication tests for UMs, as it has been observed that non-functional alleles could also be duplicated, especially in black American and African subjects (Aitchison *et al.*, 1999; Løvlie *et al.*, 1996; Sachse *et al.*, 1997). Duplication of the *CYP2D6* gene in a Zimbabwean population has been found to be associated with the CYP2D6*4 mutation (Garcia-Barceló *et al.*, 2000). Aitchison and co-workers (1999) also found the CYP2D6*4 duplication to be present in Caucasians. The 42 kb *Xba*I genotype could thus be associated with both PMs, EMs and UMs (Aitchison *et al.*, 1999; Løvlie *et al.*, 1996; Sachse *et al.*, 1997).

1.6 The Pharmacogenetics of Cytochrome P450

Genetic variation within the CYP450 family of enzymes has been well researched and many allelic variants for most of the polymorphic P450 genes have been characterised [Table 2] (Omiecinski *et al.*, 1999). The occurrence of polymorphisms in the expression of some human CYP450 enzymes have been known to cause an alteration in the pharmacokinetic profile of drugs metabolised by the specific enzyme, leading to adverse drug reactions and even toxicity (Dandara *et al.*, 2001; Masimirembwa *et al.*, 1995; Paine, 1995). These mutations could lead to enzyme products with abolished, reduced, altered or increased enzyme activity (Ingelman-Sundberg, 2001a). Some of these genetic variants involve base substitutions in non-coding regions of the respective genes, such as in the introns and flanking regions (Omiecinski *et al.*, 1999).

Two types of P450 variability are found, namely structural and regulatory. A structural polymorphism occurs in the coding region of a gene stipulating the structure of the encoded enzyme and could therefore alter the activity of the enzyme (McKinnon & Evans, 2000). Variations in the regulatory regions (non-coding) of the P450 gene could cause a change in the amount of enzyme produced, but not in the enzyme structure (McKinnon & Evans, 2000). About 50 functional P450 genes have been identified. However, the enzymes predominantly responsible for the metabolism of most drugs are CYP1A2, CYP2C9, CYP2C19,

CYP2D6, CYP2E1 and CYP3A4 [Table 2] (Badyal & Dadhich, 2001; McKinnon & Evans, 2000).

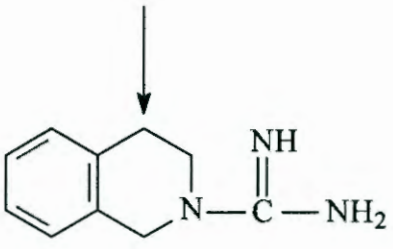
Pharmacogenetics will enable more accurate predictions of the response to drugs and could therefore have a profound impact on the future of therapeutics and drug development (Ingelman-Sundberg, 2001b; McKinnon & Evans, 2000). By using genotyping or phenotyping techniques, each and every individual could be classified as either a poor, an intermediate, extensive or an ultra rapid metaboliser (van der Weide & Steijns, 1999). It is important to be able to correlate the genotype to the resultant phenotype in subjects (McKinnon & Evans, 2000). Performing these tests before onset of drug treatment could ensure the correct selection of drugs and dosage for the patient to reach therapeutic plasma levels with minimum adverse reactions (Gaedigk *et al.*, 1999; van der Weide & Steijns, 1999). The advantage of prior genetic knowledge about a patient is thus obvious. Top of the list is the reduced risk of adverse drug reactions, reduced costs of treatment, increased efficacy of the treatment (the right drug for the right patient), and also prediction of drug compliance.

Screening every patient before pharmacotherapy could prove to be very expensive, however, the frequency of use and the duration of therapy should be considered, especially in the case of CYP2D6 substrates (Table 3), such as tricyclic antidepressants, certain antipsychotics and cardiovascular compounds (van der Weide & Steijns, 1999). It therefore seems useful to screen patients before onset of therapy, because the differences in drug disposition could be compensated for by adjustment of the dosage according to the metabolic capacity of the enzyme. However, it is important to keep in mind that the administration of a drug according to the genotype is no guarantee that therapeutic plasma levels would be obtained (van der Weide & Steijns, 1999). To safeguard the patient and the physician, the best option would be to screen the patient beforehand, forewarning the physician of possible risks involving a specific drug treatment.

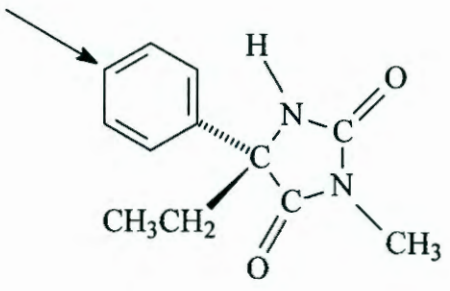
Subjects are characterised by either the absence of the 2D6 gene, (poor metaboliser, PM), or by the overexpression of the enzyme, (ultra-rapid metaboliser, UM). It is especially in the poor metabolisers that adverse drug

reactions and toxicity could occur, since the drug plasma levels would be too high due to a deficiency in the metabolism of the drugs, leading to a build-up of drug. The CYP2D6 polymorphism has also been associated with a number of specific human diseases in that the extensive metaboliser phenotype occurs more frequently in lung cancer patients, while the PM phenotype have been found in patients with Parkinson's disease (Paine, 1995). It has already been established that some toxicological responses could be due to the autosomal recessive polymorphisms of the CYP2D6 debrisoquine hydroxylase enzyme, resulting in compromised metabolism of a number of drugs (Gough *et al.*, 1990). Recent research has also shown that it is possible to detect CYP2D6 polymorphisms post-mortem, enabling screening for possible PM status after accidental death caused by a specific medicine (Levo *et al.*, 2003).

The CYP2D6 pattern is however not unique and other cytochrome P450 enzymes have been found to be polymorphic (Figure 5). In 1996, Goldstein and Blaisdell observed large interracial differences in the frequency of the poor metaboliser phenotype for CYP2C19. CYP2C19 together with CYP2D6 was found to be the most polymorphic cytochrome P450 enzymes (Badyal & Dadhich, 1995). Currently, 11 CYP2C19 alleles have been characterised (Goldstein, 2001; Kalow, 2002). CYP2C19 has shown the most striking inter-ethnic variation so far for a cytochrome P450 enzyme with 2-7 % Caucasians, 14-25 % Orientals and 60 % Vanuatu being poor metabolisers (Dandara *et al.*, 2001).



Debrisoquine



S- Mephenytoin

Figure 5. *Chemical structures of debrisoquine and S-mephenytoin, substrates of CYP2D6 and CYP2C19. Hydroxylation sites are indicated by the arrows (Bertilsson, 1995).*

Table 2. Characteristics of the major human cytochrome P450s (Hasler *et al.*, 1999).

CYP450	% of liver[a]	Polymorphic	First pass metabolism	Metabolism of carcinogens	Representative substrates
CYP1A1	-	Yes	No	Yes	Carcinogenic polycyclic aromatic hydrocarbons e.g. benzxopyrene
CYP1A2	13	Yes	Yes	Yes	Arylamines, nitrosamines, aflatoxin B1, caffeine, paracetamol, theophylline, imipramine, fluvoxamine
CYP2A6	4	Yes	No	Yes	Coumarin, nicotine
CYP2C9	18	Yes	Yes	No	tolbutamide, ibuprofen, mefenamic acid, tetrahydrocannabinol, losartan, diclofenac7
CYP2C19		Yes	Yes	No	S-mephenytoin, amitriptyline, diazepam, omeprazole, proguanil, hexobarbital, propanolol, imipramine
CYP2D6	2	Yes	Yes	No	Debrisoquine, metoprolol, sparteine, propranolol, encainide, codeine, dextramethorphan, clozapine, desipramine, haloperidol, amitriptyline, imipramine
CYP2E1	7	Yes	No	Yes	Ethanol, nitrosamines, paraceytamol, chlorzoxazone, halothane
CYP3A4	29	No	Yes	Yes	Erythromycin, ethinyl estradiol, nifedipine, triazolam, cyclosporine, amitriptyline, imipramine, aflatoxin B1

a approximated content of liver comprised by specific P450 enzyme

1.7 Ethnic differences in Cytochrome P450 (CYP2D6)

Substantial differences in drug response and the activities of drug metabolising enzymes have been observed between different ethnic groups [Figure 6] (Abraham & Adithan, 2001; Lambert & Minas, 1998; Lin & Poland, 1995; Ozawa *et al.*, 2004). These metabolism reactions include the acetylation and hydrolysis of drugs, as well as the oxidation of ethanol (Bertilsson *et al.*, 1992). Inter-ethnic and interindividual variability in the metabolism of drugs and the subsequent occurrence of side effects are important factors in the clinical use of many drugs (Llerena *et al.*, 1996). The inter-individual distribution of the CYP450 enzymes varies much and the extensive polymorphism can be greatly contributed to dietary adaptation over evolutionary time of different populations around the world (Ingelman-Sundberg, 2001b).

Currently all treatments and dosages are the same for all ethnic groups, without the consideration of inter-ethnic variability (Llerena *et al.*, 1996). The effects of a drug in different people is seldom similar and drastic variations could occur (Kalow, 2002). Most new drugs designed and developed could be good for some, but not all, especially since mostly Caucasian subjects are considered for clinical trials (Kalow, 2002; Llerena *et al.*, 1996). This while the Oriental and Negroid populations make up a much larger fraction of the world population (Llerena *et al.*, 1996). Extrapolation of data obtained from Caucasians to other ethnic groups could be dangerous and sometimes even misleading (Eichelbaum & Gross, 1990; Gaedigk *et al.*, 2002; Iyun *et al.*, 1986). Each racial group should be studied separately for evidence of polymorphic drug metabolism.

Genetic factors are mainly responsible for the interindividual variability, while the differences between two populations could be the result of many non-genetic influences, such as environmental factors (Llerena *et al.*, 1996; Lin & Poland, 1995; Kalow, 1982). Drug response controlled by even a single gene differs between ethnic groups. The frequency of the allele, as well as the type of mutation, will vary between different populations (Kalow, 2002). Clinical response and side effects could sometimes vary by as much as 30 – 40 fold between individuals (Lambert & Minas, 1998). Different populations live in different

environments, have different diets, lifestyles, climates, herbal remedies, some populations could even have been exposed to toxic chemicals (Llerena *et al.*, 1996; Lambert & Minas, 1998). A number of clinical studies have already shown large cross-ethnic variation in drug metabolism caused by environmetal influences e.g. diet, smoking, pesticides and climate to name but a few (Lin & Poland, 1995). Changes in pharmacokinetic and/or pharmacodynamic factors such as absorption, distribution, biotransformation and excretion, could also have an effect on inter-ethnic variability (Llerena *et al.*, 1996; Lambert & Minas, 1998). It must be kept in mind that although wide intergroup variations are observed, considerable intragroup variation could also occur (Lin, 2001).

Comparison of ethnic groups revealed substantial differences in the relative frequency of poor metabolisers (Lambert & Minas, 1998; Lin & Poland, 1995). Studies showed that the number of PMs, with exceptions, in different Black populations is generally low (Aklillu *et al.*, 1996). A low number of PMs was also observed for Arabian and other Middle Eastern populations (Aklillu *et al.*, 1996). The incidence of poor metabolisers for debrisoquine among the Japanese was also found to be lower than that for white subjects (Bertilsson *et al.*, 1992).

Griese and co-workers (2001) observed that the CYP2D6 allele frequencies of the Aborigines differed significantly from those of the Caucasians, but are similar to the Orientals. These observations showed a close relationship between the Orientals and the Aborigines. It could be that the Aborigines originated from East Asia. The only PM found among the Aborigine subjects tested was homozygous for CYP2D6*5 (Griese *et al.*, 2001). Even though it seems that the Aborigines originated from the Orient, CYP2D6*10 was found in a very low percentage (0.8 %) as well as CYP2D6*17, 0.2 % (Griese *et al.*, 2001).

A bimodal distribution was observed for the deficient debrisoquine hydroxylase ratio in Caucasian populations, while studies performed on black populations showed no bimodality [Figures 1 & 6] (Iyun *et al.*, 1986). This unimodal distribution, also observed in African Americans, could possibly be explained by the dissociation between the phenotyping probes (Gaedigk *et al.*, 2002). Griese and co-workers, 1999, observed a trimodal distribution for sparteine and unimodal

for debrisoquine among Ghananians. Major differences in the frequencies of more common alleles were also observed between the white and African American populations (Gaedigk *et al.*, 2002). CYP2D6*41 described by Raimundo and co-workers (2004) in 92 % of Caucasians tested, was also found in African Americans although at a very low frequency of 0.2 (Gaedigk *et al.*, 2005 submitted). However, while the G2988A SNP seems to correlate with an IM phenotype using sparteine, this is not necessarily the case using dextromethorphan. Another allele, CYP2D6*40, was discovered in discordant African Americans although at a low frequency of 0.008, but absent in Caucasians (Gaedigk *et al.*, 2002). This allele was originally genotyped as CYP2D6*17 and is associated with reduced or absent CYP2D6 activity.

The CYP2D6 polymorphism has been extensively studied in the Caucasian and Oriental populations. Pronounced differences have been observed for the hydroxylation of debrisoquine between Caucasians (5 - 10 % PMs) and Orientals (< 1 % PMs) (Abraham & Adithan, 2001; Llerena *et al.*, 1996). A very high correlation of metabolic ratios for CYP2D6, using different probe drugs, was found in these populations (Abraham & Adithan, 2001). Chinese were found to metabolise antidepressants at a slower rate than Caucasians. Lower doses of antidepressants are also prescribed for Asian patients (Johansson *et al.*, 1994). CYP2D6*1 (wt) was found to be relatively uncommon in Chinese, while CYP2D6*10 was observed in high frequencies in Chinese, while rarely in Caucasians (Huang *et al.*, 1999; Marez *et al.*, 1997; Ozawa *et al.*, 2004). Chinese individuals have been found to be more sensitive than Caucasians for drugs metabolised by CYP2D6 (Johansson *et al.*, 1994). Further research revealed two novel alleles in a Japanese population, namely CYP2D6*44 and CYP2D6*21B, causing a splicing error and a frameshift, respectively, resulting in impaired CYP2D6 function (Yamazaki *et al.*, 2004). In 2004, a further 5 new Japanese CYP2D6 alleles (CYP2D6*47, *48, *49, *50 and *51) were characterised (Soyama *et al.*, 2004). The occurrence of these alleles has not yet been tested in other populations.

Studies in African populations have yielded inconsistent results covering a wide range of 0 - 19 % PMs (Abraham & Adithan, 2001; Masimirembwa *et al.*, 1996). A

regional variation was observed among the African populations, suggesting that the black African population is not genetically homogeneous (Abraham & Adithan, 2001; Bertilsson *et al.*, 2002). Studies performed on the Venda and San Bushmen using metoprolol and debrisoquine as probe drugs, have shown that there is no co-segregation for PMs between these two populations for the two drugs (Sommers *et al.*, 1989). Studies performed on African populations have revealed a lower CYP2D6 activity in relation to genotype, indicating that environmental factors such as diet for example, could have an effect on the CYP2D6 enzyme in addition to genetic factors (Bertilsson *et al.*, 2002).

CYP2D6*17, responsible for the lower CYP2D6 activity among Africans, was also observed among African Americans even though this allele is rarely seen in populations outside Africa, thus reflecting their West African origin (Gaedigk *et al.*, 2002; Wan *et al.*, 2001). CYP2D6*17 was also observed among Malaysian subjects, a first for Asia (Teh *et al.*, 2001).

The occurrence of ultra-rapid metabolisers (UMs) was found to be 1.5-29 % among different ethnic populations (Abraham & Adithan, 2001). The *CYP2D6* gene duplication with the highest occurrence, namely 29 %, was found in the Ethiopians followed by 21 % in Saudi Arabians, 12 % in Turkish subjects and 2-3 % in most European populations (Abraham & Adithan, 2001; Aklillu *et al.*, 1996). Differences in the MRs have also been seen for different populations. A MR (debrisoquine/dextromethorphan) ranging from 0.01 to 0.15 has been obtained for Caucasians with a *CYP2D6* gene duplication (Johansson *et al.*, 1993; Sachse *et al.*, 1997), while Akillu and co-workers (1996) determined a MR (debrisoquine) between 0.1 and 1 for Ethiopians with multiple *CYP2D6* genes. It was however found that the Ethiopian subjects did not have the ultra-rapid metabolism of debrisoquine as have been found for the Caucasians with multiple genes. Environmental factors could have been responsible for the decreased activity among the Ethiopians, even in the presence of CYP2D6 multiple genes (Bertilsson *et al.*, 2002).

Studies among Indian subjects have revealed a wide variation in PMs using different probe drugs, with MRs falling in between those for Caucasians and

Orientals. DNA marker studies have shown that European and Indian populations have a common Caucasoid ancestor and are therefore genetically distinct from the Orientals. CYP2D6 activity studies have, however, shown that the Indian population is a separate group with enzyme activity in between the Caucasian and Oriental groups (Abraham & Adithan, 2001).

Five human races are currently recognised, comprising of two small groups, Bushmen and Australian aborigines, and three large groups, Caucasians, Mongoloids and Negroids (Llerena *et al.*, 1996). When considering these five races, many populations are made up of racial mixes. The term "ethnic differences" refers to differences relating to races or large groups of people grouped together according to common traits and customs (Kalow, 1982). Classing people together geographically would make more sense than grouping them together regarding tribal, linguistics or political nature according to Kalow, 1982. Using genetic distances and gene frequencies, pertaining to a specific deficiency, could prove to be the ultimate choice of separation between populations (Kalow, 1982; Kalow, 2002; Llerena *et al.*, 1996).

Although remarkable progress has been made in the clarification of the mechanisms by which ethnicity and culture influence drug response, there is still much that remains unresolved (Lin & Poland, 1995). Available information, however, clearly indicates that ethnicity and culture could have a major impact and should not be neglected (Lin & Poland, 1995). A growing number of studies have clearly indicated the importance of race and ethnicity in the treatment of depression and anxiety disorders (Lin, 2001). The incidence of poor metabolisers among the Japanese was lower than found in Caucasian subjects. A similar trend was also observed in Chinese subjects, where it was found that these subjects are more sensitive to desipramine and haloperidol than Caucasian subjects (Bertilsson *et al.*, 1992). The first task would be to determine and record the ethnic differences in the drug metabolising enzymes for clinical purposes, before taking other influences into consideration (Kalow, 1982). Gaining knowledge about the specific metabolic pathways or reactions are influenced by differences between populations and also which populations are similar and which differ with respect to a specific pathway. Differences between populations are important because they

convey some element of predictability which is absent when considering individuals (Kalow, 1982). It would thus be sensible and practical to categorise patients into genetically definable groups having similar drug effects, especially when choosing the right drug for a patient (Kalow, 2002).

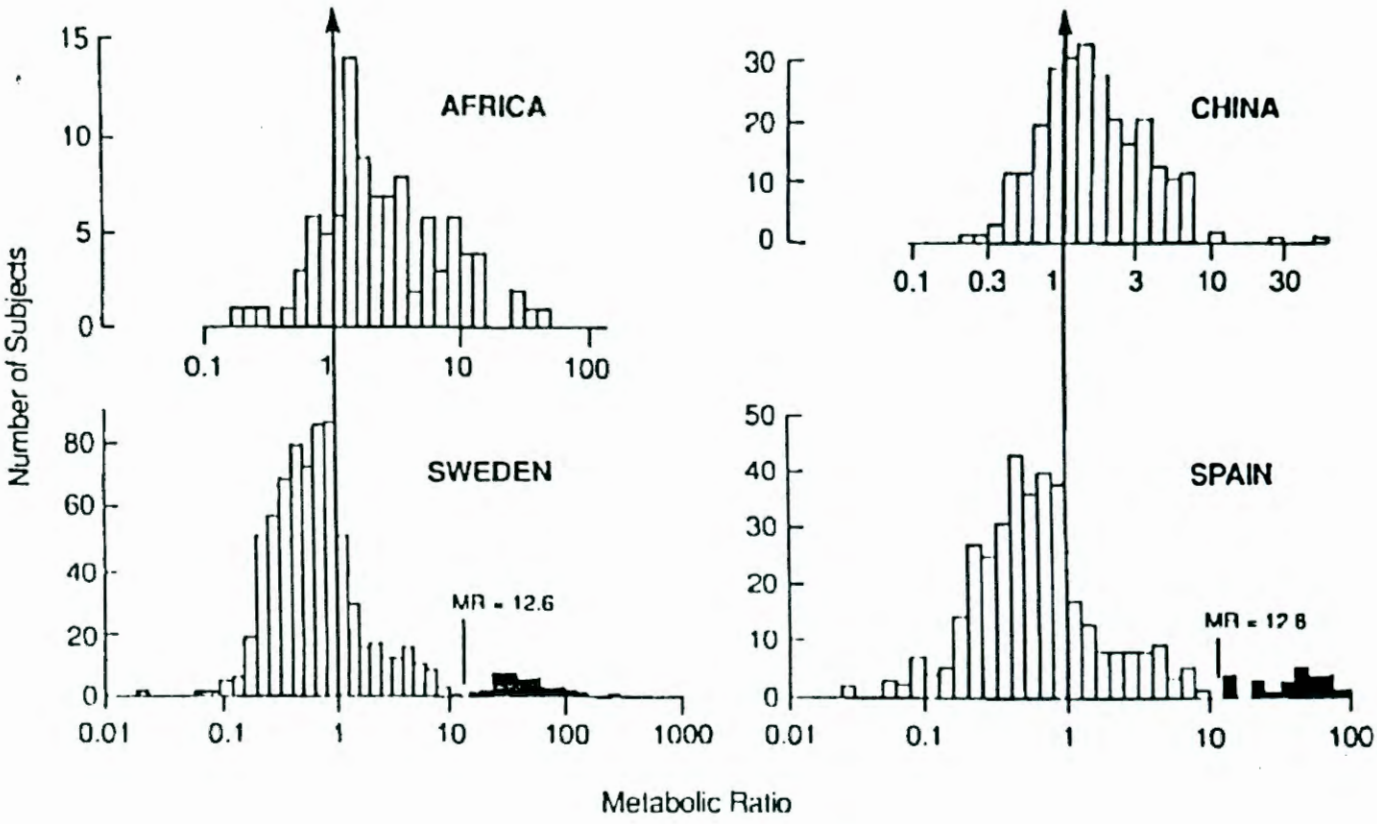


Figure 6. *Frequency distributions of the CYP2D6 activities in four human populations. The ratio of debrisoquine/4-OH-debrisoquine in urine has been plotted on a logarithmic scale after a single dose of debrisoquine. The increasing ratios reflect decreasing metabolic ratios (Kalow, 1997).*

Continued research in these areas is clinically important as patients with increasingly divergent ethnic and cultural backgrounds are seeking treatment for a range of psychotic disorders. The Japanese authorities have already decided not to depend on pharmacokinetic studies performed in Caucasians for drug optimisation, but prefer to have similar studies performed in Japanese (Hasler *et al.*, 1999).

1.8 Clinical importance of CYP2D6 polymorphisms

The clinical implications of genetic variability in cytochrome P450 genes are dependent on the function of the encoded enzyme (McKinnon & Evans, 2000). This means that the clinical significance of a polymorphic drug metabolising enzyme in relation to a particular drug would require that, firstly, the kinetics of the drug be mainly dependent on one CYP450 enzyme, secondly, the altered pharmacokinetic profile is of clinical importance and thirdly, that the inter-individual drug response could not be determined using clinical or paraclinical parameters (Brøsen & Gram, 1989). This clinical impact is usually found in the response outliers observed during drug treatment [Figure 7] (Kalow, 2002). These differences in the P450 genes may have an impact on many human health aspects, from drug and chemical sensitivity to cancer (Marez *et al.*, 1997; McKinnon & Evans, 2000). Allelic variation in these genes encoding for the drug metabolising enzymes may therefore increase the risk of sensitivity of an individual to the harmful effects of drugs and chemicals, without causing any pathological changes (McKinnon & Evans, 2000). The clinical relevance of genetic variability in the P450 genes is dependent on various factors, namely, the presence of alleles, the therapeutic index of the drug administered, the smoking habits of the subject, concomitantly administered drugs as well as the clinical status of the patient (Bertilsson *et al.*, 2002; McKinnon & Evans, 2000).

Numerous investigations have highlighted the clinical importance of the debrisoquine polymorphism since its first discovery in 1977 (Abraham & Adithan, 2001). CYP2D6 is responsible for the stereospecific metabolism of many drugs (Table 3) with a narrow therapeutic range, such as the antiarrythmics, antidepressants and neuroleptics (Badyal & Dadhich, 2001; Bertilsson *et al.*, 1992; Bertilsson, 1995; Omiecinski *et al.*, 1999). The kinetics of nortriptyline, for example, depends on CYP2D6 and subsequent dosages required to reach similar plasma levels varying from 30-50 mg in PMs to 500 mg in UMs (Ingelman-Sundberg, 2004). Poor metabolisers of CYP2D6 will reach toxic levels of the drug in their plasma on administration of normal dosages, while too low plasma levels will be observed for the ultra-rapid metabolisers, resulting in therapeutic failures (Llerena *et al.*, 1996). The consequences of the polymorphism depend on the

compound metabolised. Failure of drug elimination could lead to an exaggerated, sometimes fatal response (perhexiline, sparteine), a lack of response when a pro-drug has not been activated (codeine), or a negligible response when there are other available pathways for metabolism (Kalow, 1997).

The clinical importance of the CYP2D6 polymorphism depends on a number of scenarios, namely

1) whether the parent compound, metabolite or even both are solely metabolised by CYP2D6,
2) whether the parent compound and/or metabolite are active,
3) the potency of the active species and
4) the overall contribution of the CYP2D6 pathway in the clearance of the drug (Bertilsson *et al.*, 2002; Dahl, 2002).

The therapeutic index of the drug, saturation of the CYP2D6-dependent pathway and the possible use of other pathways for elimination, need to be taken into account as well (Bertilsson *et al.*, 2002). It is therefore very important to investigate the clinical impact of CYP2D6 polymorphisms for each substrate. About 7 % of Caucasians have been found to be poor metabolisers of debrisoquine. Family studies have revealed that the gene is inherited by a monogenic autosomal recessive defect (Bertilsson *et al.*, 1992; Gonzalez *et al.*, 1988; Gonzalez & Meyer, 1991). Studies also showed that the duration of drug treatment has to be extended for PMs due to lower dosages to avoid toxicity or side effects, thereby increasing costs (Ingelman-Sundberg, 2004).

The classical antipsychotics have a narrow therapeutic window and concentration dependent adverse effects could occur at the clinically effective concentrations or higher concentrations (Dahl, 2002; Tamminga *et al.*, 2003). The newer antipsychotics do have a broader therapeutic range, but interactions still occur, especially during polytherapy (Dahl, 2002; Tamminga *et al.*, 2003). Brockmöller and co-workers, 2002, found that CYP2D6 genotype is an important determinant of haloperidol, reduced haloperidol disposition, and also for the risk of adverse drug events.

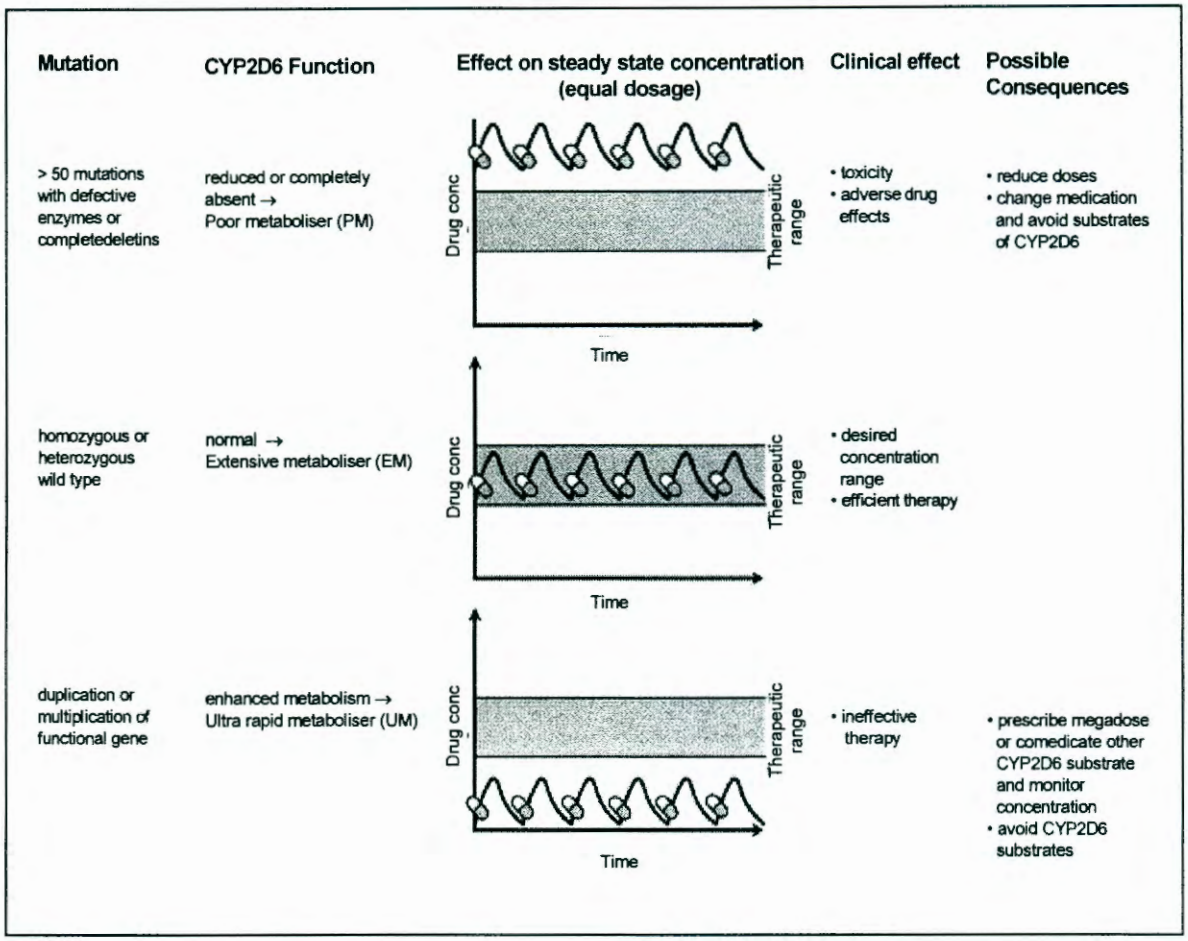


Figure 7. *Representation of clinical effect of CYP2D6 polymorphisms on therapeutic effect of drugs that are substrates of CYP2D6. (with permission from Olavi Pelkonen)*

Codeine has no analgesic effect in poor metabolisers because of the impaired formation of the morphine metabolite (Broly *et al.*, 1991). The determination of the CYP2D6 genotypes of individuals might prevent the onset of severe extrapyramidal effects in about 5 % of patients undergoing haloperidol treatment (Brockmöller *et al.*, 2002; Kalow, 2002). This may not seem to be a high percentage but every patient who is in the position to benefit from better treatment should be able to receive the benefits thereof. Studies have shown that the cost of treating patients having PM or UM status were much higher than for those having intermediate or extensive metaboliser status (Bertilsson *et al.*, 2002).

More than 80 compounds have already been identified as substrates of CYP2D6 (Table 3) (Badyal & Dadhich, 2001; Gaedigk *et al.*, 1999; Pavanello & Clonfero,

2000). Since CYP2D6 plays a major role in the metabolism of many drugs, genotyping could provide a valuable tool for the prevention of adverse drug reactions (Gaedigk *et al.*, 1999). The loss of enzyme activity could have serious clinical effects and in some cases even lead to death, especially in the case of narrow therapeutic drugs (Dandara *et al.*, 2001; Gaedigk *et al.*, 1999; Pavanello & Clonfero, 2000). Genotyping is however not commonly used in the clinical setting due to the high number of CYP2D6 alleles and their frequencies in various populations (Gaedigk *et al.*, 1999).

The advantage of genotyping with therapeutic drug monitoring is that the enzymatic status of a patient could be predicted before onset of treatment, resulting in alternative drug treatment or dosage adjustments. Routine genotyping may however not be economical in all instances, especially developing countries. On the other hand, it could be of essence to monitor CYP2D6 enzymatic status in patients undergoing treatment with drugs that are substrates of this enzyme (Table 3). If one knows which gene is active in a specific patient, the correct drug could be prescribed for the best therapeutic outcome. The medical advantage would be the avoidance of many drug failures as some drugs would be useful in some but not all patients (Kalow, 2002).

Deficient debrisoquine hydroxylation is bimodally distributed in a population and more than two phenotypes have been described. Apart from EMs and PMs, IM s and UMs have also been described. The clinical picture for PM and UM patients are sometimes similar causing problems in the understanding of the specific adverse drug reaction that occurred. Furthermore, since most of these drugs have long half-lives, the toxic effect could take up to 5-7 weeks to develop (Abraham & Adithan, 2001). It was therefore suggested that the enzymatic status of patients be determined before onset of treatment with drugs mainly metabolised by CYP2D6 (Abraham & Adithan, 2001; Gaedigk *et al.*, 1999).

Table 3. Substrates of CYP2D6 (Badyal & Dadhich, 2001; Llerena *et al.*, 1996; Omiecinski *et al.*, 1999).

Antihypertensives			
Alprenolol	Bufuralol	Bunitrolol	Bupranolol
Carteolol	Clonidine	Debrisoquine	Guanoxan
Indoramin	Losartan	Metoprolol	Nimodipine
Nitrendipine	Oxyprenolol	Propranolol	Timolol
Antiarrhythmics			
Amiodarone	Aprindine	Encainide	Flecainide
Mexiletine	Procainamide	N-propylajmaline	Propafenone
Sparteine			
Antidepressants			
Amiflavine	Amitriptyline	Brofaromine	Citalopram
Clomipramine	Desmethylcitalopram	Desipramine	Fluvoxamine
Fluoxetine	Imipramine	Maprotiline	Minaprine
Moclobemide	Nefazodone	Nortriptyline	Paroxetine
Tomoxetine	Tranylcypromine	Trimipramine	Venlafaxine
Neuroleptics			
Clozapine	Haloperidol	Levomepromazine	Olanzapine
Perphenazine	Pimozide	Risperidone	Sertindole
Thioridazine			
Opiates			
Codeine	Dihydrocodeine	Dextromethorphan	Ethylmorphine
Hydrocodone	Norcodeine	Oxycodone	Tramadol
Chemotherapeutic agents			
Clotrimazole	Doxorubicin	Ketoconazole	Mefloquine
Pyrimethamine	Rifampicin	Ritonavir	Roxithromycin
Sulfasalazine			
Antihistamines			
Azelastine	Cinnarizine	Loratadine	Promethazine
Miscellaneous			
Apigenine	Budesonide	Chloral hydrate	Cyclobenzaprine
Dexfenfluramine	Dibucaine	Dihydroergotamine	Dolansetron
Ethinyloestradiol	Fenoterol	4-hydroxyamphetamine	Formoterol
Laudampsone	MDMA (ecstacy)	Methoxamine HCl	MPTP
Methoxyamphetamine	Methoxyphenamine	Methoxypsoralen	Metoclopramide
Nigergoline	Odansetron	Perhexiline	Phenformin
Phenylpropanolamine	Quercitin	Serotonin	Tacrine
Tamoxifen	Tolterodine	Tropisetron	

Despite all the available knowledge, the existence of a link between the debrisoquine phenotype and susceptibility to certain diseases is still uncertain (Broly *et al.*, 1991). However, Marez and co-workers postulated in 1997 that CYP2D6 polymorphisms could be associated with some cancers, chronic inflammatory diseases and some neurodegenerative disorders. Poor metabolisers have, however, been found in association with susceptibility to bladder and lung cancer especially squamous cell carcinoma and adenocarcinoma (Gonzalez & Meyer, 1991). Hirvonen and co-workers (1993), have found that extensive metabolisers of CYP2D6 have an increased risk of developing lung cancer. Dysfunction of the CYP2D6 gene could also minimally increase the risk of developing ankylosing spondylitis (Brown *et al.*, 2000). A strong association between the *CYP2D6* gene and susceptibility to leukaemia has also been observed, due to the close proximity of the platelet-derived growth factor β subunit gene (PDGFB) and CYP2D6 on chromosome 22 (Gough *et al.*, 1993). Gérard and co-workers, 2002, found that the risk of developing Parkinson's disease increased with the presence of CYP2D6 non-functional alleles. Genotyping could maybe result in a clearer picture of the linkage between CYP2D6 activity and the predisposition to certain diseases (Broly *et al.*, 1991). However there is still no concrete link or association between susceptibility to diseases and CYP2D6 enzymatic status.

Table 4. Some Inducers and Inhibitors of CYP2D6 (Badyal & Dadhich, 2001; Goshman *et al.*, 1999; Llerena *et al.*, 1996; Omiecinski *et al.*, 1999)

Inducers	Inhibitors	
Not inducible	Amiodarone	Carbamazepine
	Amitriptyline	Fluoxetine
	Buproprion	Dexamethasone
	Celecoxib	Paroxetine
	Chlorpheniramine	Phenobarbital
	Chlorpromazine	Phenytoin
	Cimetidine	Quinidine
	Clomipramine	Quinine
	Clozapine (weakly)	Rifampicin
	Cocaine	Ritonavir
	Desipramine	
	Doxorubicin	
	Fluoxetine	
	Fluphenazine	
	Fluvoxamine	
	Halofantrine	
	Haloperidol	
	Indinavir	
	Levomepromazine	
	Lomustine	
	Methadone	

1.8.1 Drug interactions

CYP2D6 is absent in PMs and has a finite metabolising capacity in EMs. This means that CYP2D6 could become saturated during first pass metabolism, causing the clinical effect of drugs such as propafenone to become dose dependent in EMs. A number of clinical significant drug interactions occur with this enzyme *in vivo* due to the finite metabolism. The binding of high affinity substrates to CYP2D6 will inhibit the metabolism of other lower affinity compounds. Drug

interactions therefore occur in all phenotypes of CYP2D6. Some drugs may also increase their rate of metabolism or even that of other drugs, by inducing the activity of the enzyme responsible for its metabolism [Table 4; Figure 8] (Kalow, 2002). However, since CYP2D6 is not inducible the administration of enzyme-inducing agents will have no effect on its activity (Eichelbaum *et al.*, 1987; Eichelbaum & Gross, 1990).

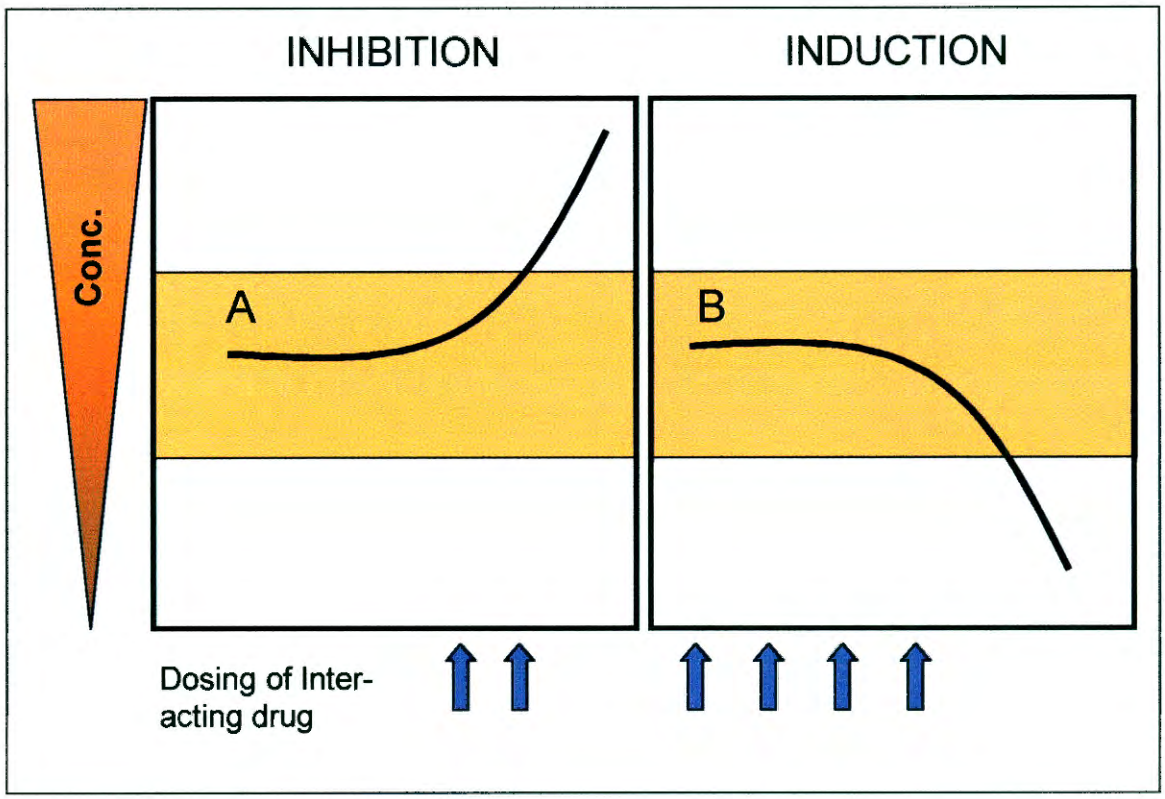


Figure 8. *Graphic demonstration of the effect of metabolic inhibition and induction on plasma concentration.*

Chronic administration of high affinity drugs would cause EMs to behave as PMs metabolically and EMs could be misclassified as PMs during phenotyping. This phenonomenon is called phenocopying and complexes the prediction of phenotype from genotype (Eichelbaum & Gross, 1990; Marez *et al.*, 1997). Phenotypes could therefore never be predicted with 100 % accuracy, as there could also still be unknown or rare mutations for CYP2D6 (Marez *et al.*, 1997).

However, not all drugs binding with high affinity to CYP2D6, are substrates of the enzyme. For example quinidine, a potent inhibitor of CYP2D6 *in vivo* (Table 4), is not metabolised by the enzyme (Eichelbaum & Gross, 1990). When potent inhibitors, such as propafenone and quinidine, are administered together with a CYP2D6 substrate drug, the pharmacokinetics of the patient is indistinguishable from those of a PM, meaning that the plasma concentration of the CYP2D6 drug could have increased about five-fold resulting in clinically relevant interactions [Figure 8] (Brøsen & Gram, 1989; Tamminga *et al.*, 2003). The prevalence of poor metabolisers was found to be higher in an adverse event (AE) group than in the non-AE group of patients undergoing psychotropic treatment (Tamminga *et al.*, 2003). Different drugs were also prescribed for patients with normal metabolism than for those with impaired metabolism. Prescriptions of CYP2D6 drugs were also lower in the PM than EM group, indicating that CYP2D6 drugs were less frequently used for PMs. A significantly higher number of drugs for Parkinsonian-like side effects were also prescribed for the PM group. CYP2D6-impaired metabolism thus seems to be a contributing factor to the onset of extrapyramidal side-effects during psychotropic treatment (Tamminga *et al.*, 2003). It was also found that the pharmacotherapy of UM patients were comparable with the EM group and less affected than for the PM group (Tamminga *et al.*, 2003). This could be due to the fact that some anti-psychotics are metabolised to an active metabolite, thus resulting in normal pharmacological activity. In a study performed by Aitchison and co-workers in 1999, they also found that failure to respond to anti-spychotics are not associated with the UM genotype. Individuals with higher CYP2D6 activity was found to be at increased risk for multiple chemical sensitivity (MCS), also known as environmental sensitivity, compared to individuals with two non-functional alleles (McKeown-Eyssen *et al.*, 2004)

Drug interactions could also occur when a polymorphism is present. Pharmacokinetic interactions could be anticipated using the following equation:

If drug A affects P450 enzyme X and if P450 enzyme X metabolises drugs B, C and D,

then drug A should affect the metabolism of drug B, C and D.

The above gained knowledge could also be used to decide which drugs to develop (Abraham & Adithan, 2001).

We know that genes control the effect of drugs. It is now also clear that drugs could affect the function of genes. Most drug effects are not regulated by a single gene but by many genes interacting with each other and also with environmental factors (Kalow, 2002). The antibiotic, rifampicin, caused a four fold increase of CYP2C9 and even an increase as high as 55 fold of CYP3A4 mRNA expression (Kalow, 2002).

1.8.2 HIV and CYP2D6

As already discussed, many factors could affect the metabolism of a drug. The reaction of a healthy subject to a drug could differ from that of a ill subject. Previous research has already shown that the activity of NAT2 (N-acetyltransferase 2) was decreased in HIV patients. A report from the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), September 1999 in San Francisco, stated that pharmacokinetic studies for HIV drugs should be conducted on HIV-negative, as well as HIV-positive people. More than 50 % of the drugs prescribed for HIV patients are metabolised by liver enzymes, especially CYP3A4 and CYP2D6. Researchers found that the rate of metabolism by CYP3A4 of HIV drugs in HIV-positive patients remained unchanged from that of HIV-negative people. The same was however not found for CYP2D6 since this enzyme (for reasons yet unknown) processed the HIV drugs more slowly in HIV-positive cases, especially in patients with high viral loads. These findings suggest that patients with high viral loads receiving standard doses of anti-HIV drugs, metabolised by CYP2D6, may experience serious side effects.

O'Neill and co-workers, 2000, found a subsequent shift from the CYP2D6 EM to PM phenotype, in HIV-positive patients. Some HIV drugs (quinidine) are also inhibitors of CYP2D6, thus decreasing the activity of the CYP2D6 enzyme. HIV treatment involves polypharmacy and thus there is a risk for the occurrence of metabolic drug interactions. Knowledge of a patients' genotype would decrease

the risk of adverse events, as HIV patients tend to have slower CYP2D6 activity, than healthy subjects (O'Neill *et al.*, 2000).

1.8.3 Tuberculosis and CYP2D6

Tuberculosis (TB) is still one of the highest ranked diseases in the world and South Africa is no exception. Although the disease has declined in more developed countries it is still found to occur among individuals with mood disorders (Trenton & Currier, 2001). A survey of 100 hospitalised TB patients in South Africa indicated that 68 % had some degree of clinical depression. Pharmacological treatment of TB is well established with patient adherence to drug regimes of utmost importance. Patient compliance is sometimes lacking especially in patients also receiving psychiatric medication.

The use of at least two drugs is recommended for TB treatment, with isoniazid and rifampicin in the forefront. Additional treatment may also be necessary for HIV-positive patients. Cytochrome P450 enzymes have been found to be responsible for the metabolism of many of the drugs used in the treatments of TB, HIV and mood disorders. Isoniazid was found to competitively inhibit CYP2D6, while citalopram inhibits the enzyme only mildly. This may be of importance for a clinician to consider when prescribing medication for the treatment of TB, as well as HIV and mood disorders (Trenton & Currier, 2001).

1.9 Genotyping versus Phenotyping

The enzymatic status of a drug metabolising enzyme of an individual could be determined using either phenotyping and/or genotyping techniques.

Phenotyping has been a valuable research tool and for a long time it was the only means of assessing the genetic basis of a patient's metabolic capacity (Steimer *et al.*, 2001). The procedure requires the administration of a probe drug of which the metabolism should only depend on CYP2D6, followed by urine collection over a

period of time (Broly *et al.*, 1991; Hamelin *et al.*, 1999). During phenotyping, the subject is not allowed to ingest any other medication or interfering compound. A flush-out period, usually one week, prior to onset of study, is also required. It is thus not possible to perform phenotyping on subjects already undergoing treatment. The metabolic ratio (MR) of the excreted metabolite and parent compound in the urine (0-8 + 8-32 hours, sometimes 4 hours as in the case of dextrometorphan) is then determined and used as a measure for the CYP2D6 activity of the individual (Abraham & Adithan, 2001; Bertilsson *et al.*, 1992). Subjects with a MR > 12.6 for debrisoquine hydroxylation, was classified as PMs (Llerena *et al.*, 1996). Many probe drugs have already been used, such as debrisoquine, sparteine, dextromethorphan, metoprolol and codeine (Abraham & Adithan, 2001; Llerena *et al.*, 1996).

The use of different probe drugs for phenotyping caused, however, a poor correlation among subjects of the same group, especially among the black populations (Dandara *et al.*, 2001). MRs obtained for dextromethorphan did not correlate with the MRs obtained for debrisoquine and sparteine in a Ghanaian population. Studies in Tanzanians also suggested that phenotypic status could depend on the genotype and probe drug used (Gaedigk *et al.*, 1999; Gaedigk *et al.*, 2002; Wennerholm *et al.*, 2002). Poor metabolisers of debrisoquine did not always test as PMs of sparteine or metoprolol (Sommers *et al.*, 1989). These data suggest a dissociation of the metabolism of the probe drugs used in the African populations. A close pheno- and genotype correlation was, however, observed for Oriental and Caucasian populations.

Renal clearance as well as the activity of the enzyme could influence the metabolic ratio. Environmental factors could also play a role by causing differences in the antimode of the MR between different ethnic groups (Abraham & Adithan, 2001; Kalow, 1982). Patients undergoing phenotyping while receiving other treatments, especially psychotropics, could lead to increased MRs as the drugs inhibit CYP2D6 activity (Bertilsson *et al.*, 2002; Steimer *et al.*, 2001). However, some studies have shown that metabolic ratios are stable during a lifetime, indicating that environmental factors have a limited influence on CYP2D6 activity (Dahl, 2002). The protocols for testing and the risk of adverse reactions due to co-

administration of drugs or even the perplexing effect of disease, limited the use of phenotyping (Broly *et al.*, 1991). Recent results also showed that caution should be taken when selecting a probe drug for phenotyping, especially in black populations due to variations in substrate specificity for CYP2D6 (Dandara *et al.*, 2001; Sommers *et al.*, 1989; Wennerholm *et al.*, 2002). Discordance between genotype and phenotype has so far been found to be unique among the black populations and has also resulted in the discovery of new alleles in Japanese (Gaedigk *et al.*, 2002; Johnson *et al.*, 2000; Soyama *et al.*, 2004; Yamazaki *et al.*, 2003).

In 1990, Gough and co-workers, already found limitations to the phenotyping assays for the polymorphic CYP enzymes. In some cases it is not possible to perform phenotyping, particularly in Psychiatry and Oncology, since these patients are already on medication and the possibility of drug interactions are therefore too high (Broly *et al.*, 1991; Hamelin *et al.*, 1999). A higher accuracy can be expected when using genotyping methods (Gan *et al.*, 2002).

Correct prediction of phenotype from genotype data has been performed at 90 to 99 % accuracy in Caucasian Europeans populations, depending on the number of alleles tested for. It could however be a complex process (Dahl, 2002; Gaedigk *et al.*, 1999; 2002; Marez *et al.*, 1997; Tamminga *et al.*, 2003). The homozygous wild type individuals have significantly lower MR values than the heterozygous individuals, although a large overlap has been observed between the two groups (Broly *et al.*, 1991; Gaedigk *et al.*, 1999; 2002; Sachse, 1997). Molecular genetics could provide information about the conditions for different doses for patients, or even drug treatments before onset of therapy (Abraham & Adithan, 2001; Ensom *et al.*, 2001). The need for hospitalisation could also be reduced due to the occurrence of less adverse drug reactions and the costs associated there with.

Genotyping identifies the specific genetic mutation resulting in the specific drug metabolism phenotype. The genotyping methods require very small amounts of blood or even tissue and are not affected by concurrent diseases or co-conmitant drugs taken by the patient. Further advantages of genotyping include; the assumption of steady-state is not required; small amounts of blood can be

collected less invasively; the data stays constant over time and provides a predictive value for multiple drugs (Ensom *et al.*, 2001; Steimer *et al.*, 2001). And results can also be provided within 48 hours (Abraham & Adithan, 2001). Genotyping of subjects could be performed independent of current drug treatments and it is possible to assess several alleles at a time (Bertilsson *et al.*, 2002; Hersberger *et al.*, 2000; Hamelin *et al.*, 1999). The one main drawback is the number of alleles to be tested to obtain a 95-99% certainty for each ethnic group. The question is for which alleles do we have to test routinely for, to allow sufficient and reliable, but still practical, genotyping. Steijns and Van der Weide (1998) found that testing for CYP2D6*3, *4 and *5 would account for the identification of 95 % PMs in Caucasian subjects. Identification of PMs, homozygous and heterozygous EMs, as well as duplication-positive UMs, could be obtained using genotyping (Bertilsson *et al.*, 2002; Dahl, 2002). However, not all UMs carrying duplicated genes could be identified using genotyping and more accurate prediction of the catalytic activity of these subjects would increase the effectiveness and clinically usefulness of genotyping (Bertilsson *et al.*, 2002). Another group that needs more exploration, is the intermediate metabolisers with impaired metabolism. This group is also very prone to adverse reactions such as toxicity. Further analysis of functionally important mutated alleles of CYP2D6 would improve the genotype-phenotype correlations among the extensive metabolisers and may improve the prediction of concentration-dependent adverse effects or therapeutic failure (Bertilsson *et al.*, 2002).

It is important to consider ethnicity when genotyping, as some alleles occur in higher frequencies in some populations. CYP2D6*10 for instance is found in high frequency in Orientals and CYP2D6*17 in black Africans (Bertilsson *et al.*, 2002; Dahl, 2002; Ozawa *et al.*, 2004; Sachse *et al.*, 1997). However, the advantage of genotyping lies therein that no drug intake, followed by urine/blood collection, is necessary (Gonzalez & Meyer, 1991). A patient can also be genotyped irrespective of his/her current treatment (Dahl, 2002). Accuracy and reliability of the assays are very important and depend on the ability to selectively amplify the 2D6 gene alone, and not the 2D7 and 2D8 genes (Gonzalez & Meyer, 1991).

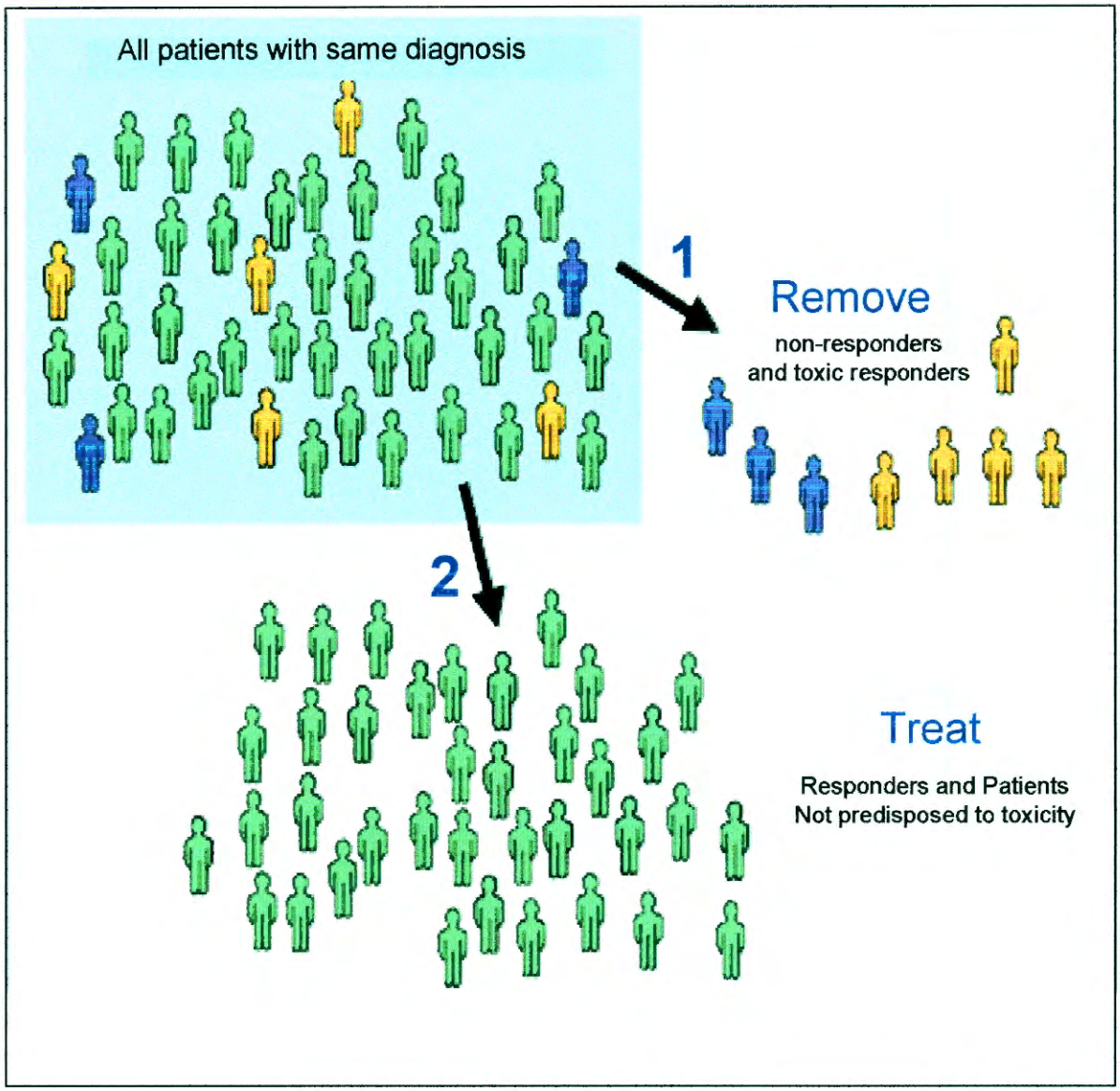


Figure 9. *Demographic representation of the potential of pharmacogenetics. The population could be subdivided into high and low risk subjects according to their genotypes (Johnson & Evans, 2002).*

Genetic heterogeneity appears to be a significant source of variability observed in the response to drugs (Mancinelli *et al.*, 2000). We know that information pertaining to inter-ethnic and inter-individual genetic differences can be used to facilitate rational drug discovery and development and to avoid or minimise the incidence of adverse events in clinical trials. One could generate criteria for selecting patients most likely to benefit from a drug, without incurring unnecessary risk. Early or preventive therapy guided by genotyping could significantly enhance the clinical outcome, especially when aberrant metabolic activity of CYP2D6 is

suspected (Dahl, 2002). The need for a new, individualised approach to drug development and therapy appears to be a necessity in future (Mancinelli *et al.*, 2000). The promise of pharmacogenomics lie therein, that by determining a patient's genotype, physicians would be able to make better prescribing decisions (Katz, 2000). Patient compliance with drug treatments could also potentially be improved (Ensom *et al.*, 2001).

In addition to genetics, an individual's response to a drug is also influenced by a host of factors including state of disease at diagnosis, concomitant illnesses, environmental interactions (smoking, diet etc.), the patient's physique and dosage compliance (Katz, 2000). Genomics could therefore be used to identify genes susceptible to new drugs and also to predict which patients will respond to which drugs, thus 'personalised' medicine (Mancinelli *et al.*, 2000; March, 2000). The whole concept rests on the correlation of phenotypic biomarkers, such as drug induced toxicity, with genetic characterisation association studies (Mancinelli *et al.*, 2000).

1.10 Review of the methods for CYP2D6 Genotyping

Much research has already been performed in the genotyping of CYP2D6. A wide variety of different techniques for the detection and characterisation of CYP2D6 mutations have been employed, such as cloning; allele specific PCR; ARMS PCR (amplification refractory mutation system); extra-long PCR (XL-PCR); RFLPs (restriction fragment length polymorphisms); SSCPs (single strand conformational heteroduplex analysis; pyrosequencing; primer extension based genotyping or minisequencing and realtime PCR. These assays, together with sequencing have also been used for the identification of new mutations. Many difficulties have been encountered with the number of possible assays, their complexity and also the cost implications. Add to that the number of different alleles to be tested for, and the enormity of genotyping, comes to the fore (Table 5). No wonder that researchers have developed their own strategies for genotyping large populations. There is also no consensus on how many alleles to test for to accurately determine the enzymatic status of a subject in conference with its phenotype. Gaedigk and

co-workers (1999) therefore, decided to devise a protocol, adhering to the following criteria: (1) accurate identification of a subject's genotype using minimum input; (2) 'easy to use' methods namely PCR and RFLPs; (3) two tests for one nucleotide position, wild type and mutation; (4) protocol must be expandable and flexible for the accommodation of new alleles and (5) must be adaptable for small or large sample numbers and cost effective. In this study we will be employing methods available in our laboratory to conserve costs and time.

Gaedigk and co-workers, 1999, constructed a genotyping protocol consisting of XL-PCRs and PCRs followed by restriction enzyme digestion. In their protocol of 1999, they could test for the presence of *5, duplication of CYP2D6, *3, *4, *6, *7, *8, *9, *10, *11, *12, *15, *16 and *18. They were also able to determine the specific point mutation giving rise to a specific allele. Although this strategy proved to be easy, reliable and quick, many of the restriction enzymes used are not available in South Africa or very expensive. It was therefore decided to investigate other protocols using XL-PCR followed by allele-specific PCRs.

Roberts and co-workers (2000) used genomic DNA isolated from peripheral blood collected in EDTA tubes, for the amplification of the different 2D6 alleles. To avoid false-positive detection of the CYP2D6 mutations through the possible co-amplification of pseudogenes, initial amplification of the entire *CYP2D6* gene was first performed. Primers (P100 & P200) complementary to unique intron sequences of CYP2D6 were used. These products were then used in a robust PCR reaction that simultaneously detected CYP2D6*3, *4, *6, *8, *11, *12, *14, *15, *19 and *20 alleles, using ARMS (amplification refractory mutation system) (Roberts *et al.*, 2000). They found the method reliable, rapid and relatively cheap.

In their study, Hersberger and co-workers (2000) developed three tetra-primer PCR assays for the detection of the CYP2D6*3, *4 and *6 alleles. The *CYP2D6* gene was amplified directly, followed by the allele-specific amplification of the obtained template. In addition, a multiplex long PCR method for the simultaneous detection of the *CYP2D6* gene as well as the deleted CYP2D6 (*5) allele was also developed (Hersberger *et al.*, 2000). The method was found to be rapid and reproducible for the majority of CYP2D6 poor metabolisers.

CYP2D6*17 was identified in 1996 by Masimirembwa and co-workers. This allele was found to be responsible for a decrease in enzyme activity of most black Africans. They developed an allele-specific PCR reaction using primers specific for *17 and the *CYP2D6* gene, amplified in a long PCR, as template (Masimirembwa *et al.*, 1996).

All the above methods entail a lot of work and a much quicker method, a CYP450 microchip, was developed by Roche and Affymetrix. However, the FDA has blocked the sale of this chip in November 2003 stating that a "higher level of review is required because it is of substantial importance in preventing impairment of human health, and the DNA microchip uses sophisticated technology" (Nebert & Vesell, 2004).

Since there are so many different genotyping protocols available, it is very important first to decide on the specific alleles to be tested; the size of the population sample; and the cost implications. In this project we decided to make mostly use of allele-specific PCR as it is more cost effective than PCR followed by restriction digests.

1.11 Ethical Issues

Scientific progress always seems to outrun our capacity for realising the consequences of the newly acquired knowledge. Pharmacogenetics is no exception and there are a number of issues that need to be addressed before we proceed headlong into applying our capabilities. Most of the ethical concerns currently raised concerning pharmacogenetics are of a hypothetical nature. The overall objective of pharmacogenetics is to determine the genetic variability in drug efficacy and safety and to use this knowledge to the benefit of the patient (Breckenridge *et al.*, 2004; March *et al.*, 2001). In September 2003, the Nuffield Council on Bioethics, funded by the MRC, the Nuffield Foundation and the Welcome Trust, published a document on the ethical issues involving pharmacogenetics.

Mishandling and confidentiality of 'sensitive and personal data' and also patient consent are main concerns (Roses, 2001). To which extent would a patient be able to understand and be fully informed about the details of a proposed pharmacogenetic study? Also, should patients be able to have access to certain medications if they refuse to undergo genetic testing? It is also important to keep in mind that no simple answers are provided by pharmacogenetic tests as to whom should receive which medicine (Breckenridge *et al.*, 2004). This means that the role of genetic counselling is indispensable for this field to develop to its full potential.

Another problematic issue concerns the allocation of resources. Should we use scarce resources to find that 5 % of patients suffering from a rare disease may benefit from genotyping-derived dosage adjustment? Would it be cost effective to prospectively genotype 100 patients, only to find that a maximum of 5 would benefit? Maybe not, but on the other hand, those 5 could perhaps receive more effective treatment. The major benefit of pharmacogenetics lies in the prospect of being able to provide more effective and safer treatments. Risk is tolerable, but benefit has to have priority (Breckenridge *et al.*, 2004). One also needs to keep in mind that there are important differences between classifying a disease into different genetic types and classifying people by their different genotypes.

Pharmacogenetic data could also vary according to ethnic origin, as in the case of CYP2D6. This gene coding for an enzyme responsible for the metabolism of many important drugs, has been shown to vary between different racial groups. Also, 95 % of genetic variation occurs within a racial/ethnic group, while 5 % occur between different groups. Genetics must not be used as a substitute for 'race'. Race and ethnicity cannot be given precise biological or genetic definitions. Stratification of subjects should be carefully considered to avoid stigmatisation and it is not sure if this stratification could be scientifically justifiable (Bamshad et al., 2004; Breckenridge *et al.*, 2004; Lipton, 2003). Problems could arise when one group benefits from a medication, while another ethnic group suffering similar conditions are denied the administration of the same medication due to their genetic make-up.

To increase the scientific understanding and the utility of drug treatments, national and international regulators have promoted the use of genetic data. The Committee for Proprietary Medicinal Products (CPMP) guidelines on drug interactions (1997) recommends that "subjects...should be appropriately genotyped and/or phenotyped if any of the active enzymes mediating the metabolism are polymorphically distributed in the population. In some cases, clinically relevant interactions may only occur in a subset of the total population, for instance, slow metabolisers...". Clinical trials conducted on pharmacogenetically tested subjects could result in smaller numbers, better protection of the subjects and lower costs. However it could also result in an increase in adverse drug reactions once the drug is released on the market (Figure 9). The FDA has also decided to stratify clinical trials according to genotype (Breckenridge *et al.*, 2004; Ingelman-Sundberg, 2004; Lash *et al.*, 2003; Lipton, 2003; Nuffield Council on Bioethics, 2003).

The day-to-day decisions concerning pharmacogenetics will mostly be medical and not ethical considerations and the benefit for the patient must always be of first concern. Ultimately, who should decide whether or not a patient should receive medication? Who is responsible when a patient takes medication against the advice of a doctor? Despite all the inconsistencies in the regulations of various countries, the long-term goal of pharmacogenetic research should always be to ensure that patients receive the best possible treatment and not be unnecessarily exposed to drugs to which they are genetically unable to respond to (March *et al.*, 2001).

1.12 The Southern African situation

South Africa's population is very diverse and mostly descended from immigrants over the course of several centuries, except for the Khoi and San groups who were the original inhabitants. There are at least 20 ethnic groups and cultures, divided into four main race groups: Africans (77%), whites (11%), Indians (3%) and Coloureds (9%). The African group consists of an extraordinary variety of cultural and tribal identities. The Nguni subgroup includes Xhosa, Zulu, Swati, Ndebele

and Xitsonga cultures, while the Sotho subgroup consists of Pedi, Sotho and Tswana. The Venda is closely related to the Shona of Zimbabwe. Then there is the unique Khoi-Khoi group consisting of the Khoi, Nama and San. These Bushmen still exist as an ethically distinct group. The white population is comprised of people from Northwestern European, British, Portuguese, Jewish and East European origin. The Coloureds are people of mixed ancestry such as European, Khoi-Khoi and slaves from Malaysia and West Africa.

The separation of the Caucasians from the Orientals occurred fairly recently, about 40–60 000 years ago, while the separation of the Blacks was much earlier, about 150 000 years ago. It could therefore be possible that Blacks will show even greater differences than the Caucasians and Orientals (Bertilsson, 1995). However, not enough studies have yet been done on the Black populations to prove or disprove the hypothesis. Recent studies have shown that there is a population shift towards lower enzyme activity in the black populations relative to the Caucasians (Gaedigk *et al.*, 2002). It was also suggested that there could be novel CYP2D6 mutations among the black populations, especially those from Africa (Gaedigk *et al.*, 2002). Also, the Coloureds are unique to South Africa and since they are comprised of such a mixed ancestry, it seems imperative to include them in this study. Numerous new alleles have only been identified in Oriental populations giving probable cause to test for the oriental alleles in the Coloureds as well (Soyama *et al.*, 2004; Yamazaki *et al.*, 2003). Including the Coloureds in this study concurs with a study performed by Jurima-Romet and co-workers, 1997, wherein they concluded that CYP2D6 polymorphisms occur in a Canadian Inuit population (an aboriginal Canadian population) with allele frequencies rendering this population unique.

South Africa is a multiracial nation and we have found that there could be a need to genotype CYP2D6 in Southern African populations, since there are already many different allelic variations identified for other black populations. Also, the populations have to be screened for all the known mutations as well as unknown mutations in order to see the whole picture.

Although there are many methods currently available, most were expensive in that restriction enzyme digestions are used and time consuming. We therefore decided to develop a protocol for our laboratory utilising a multiple allele specific screening method or approach for the Southern African populations.

Chapter 2

Materials and Methods

2.1 Ethical clearance and consent

Approval for the study was obtained from the Ethics committee of the Faculty of Health Sciences, University of the Free State (Etovs nr 150/02). Care has been taken to ensure the privacy of all the subjects used in the study.

2.2 Samples

Two hundred and fifty anonymous blood samples, from 100 Coloured and 100 black African subjects, as well as 50 Caucasian subjects for the control group, were taken from the sample archives of the Department of Haematology and Cell Biology, University of the Free State. Blood was collected in EDTA Vacutainer blood-collecting tubes and stored at 4 °C. These archived samples are from previous studies in which the respective individuals had already voluntarily disclosed their ethnic status. The samples were handed over by a third person, not involved in the project, to ensure breakage of the link between the identity of the subject and the blood sample. No knowledge about the health, age or disease status of the subjects was thus known. We also had no knowledge of medication being taken by subjects. However, the above knowledge is of more importance for phenotyping, which we did not perform in this study. We only had knowledge about self defined ethnicity and sex of each sample.

2.3 Genotyping procedure

2.3.1 DNA Extraction

All chemicals used were molecular grade. DNA was extracted from 400 µl whole blood, using a method adopted for the laboratory. The red blood cells were lysed using 1.8 ml red blood cell lysis buffer (RCLB; 0.144 M NH_4Cl, 1 mM $NaHCO_3$). The samples were thoroughly mixed for 5 minutes at room temperature by inverting the eppendorf tubes followed by centrifugation for 10 minutes at 1000 G (Eppendorf Mini Spin, Merck). The supernatant was discarded. If the obtained pellet still contained red blood cells, the above process was repeated. Thereafter, the nuclei were lysed with the addition of 240 µl nuclear lysis buffer (NLB + SDS; 10 mM Tris-HCl pH 8.0, 0.4 M NaCl and 2 mM Na_2EDTA; 0.06 % SDS) for 5 minutes at room temperature. The DNA was precipitated by the addition of 80 µl 6 M NaCl to the suspension and vortex. Chloroform (160 µl) was added and the suspension was mixed until milky. After further centrifugation of the sample for 10 min at 1000 G, the upper clear phase (160 µl) was transferred to a 1.5 ml eppendorf tube. The chloroform extraction was repeated if the upper phase was not clear. Two volumes of 95 % Ethanol (400 µl) was added and the tube content was mixed by inverting, where-after it was centrifuged for 5 min at full speed. The supernatant was discarded and the resulting pellet washed with 400 µl 70 % Ethanol and centrifuged at full speed for 5 minutes. The supernatant was discarded and the tubes inverted on paper towels to dry. The DNA was hydrated by the addition of 100 µl TE buffer (10 mM Tris-HCl pH7.6, 1 mM EDTA). The quality and quantity of the genomic DNA were checked by analysing it on a 0.8 % Agarose gel and TBE buffer.

2.3.2 **Polymerase Chain Reaction** (Tables 5 & 6; Figures 10 - 16)

All thermal cycling reactions were performed in a volume of 25 µl on a Gene Amp PCR System 2400 or PE2700 from Applied Biosystems. Due to costs, some of the allele-specific PCRs was performed in 10 µl volumes. All oligonucleotide

primers (Table 6; Figure 10) were synthesised by Inqaba Biotech, South Africa. The alleles tested for with their characteristic mutations, are depicted in Tables 5 and 7.

The CYP2D6 gene (5.1 kb) and the deletion of the gene (CYP2D6*5; 3.2 kb) were determined by amplification using about 100 ng genomic DNA and the primers, 5DPKup, 5DPKlow, 5Dup and 5Dlow (Hersberger *et al.*, 2000). The reaction mixture consisted of 2.5 mM $MgCl_2$, 200 μM dNTP mixture, 1.75 units Expand long template *Taq* polymerase (Roche Diagnostics) and primer mix (1.25 μM). The cycling conditions were as follows: initial denaturation at 95 °C for 1 min, 35 cycles of 30 s at 95 °C and 68 °C for 7 min, and a final extension of 7 min at 68 °C. The CYP2D6 gene and the PCR products were analysed on a 0.8 % agarose gel (Figure 11A).

Difficulty in the amplification of the CYP2D6 gene was encountered in some samples, especially in the Coloured group. After further investigation, two new primers, 5Kup and 5Klow, were designed and ordered (Table 6). The ratio of the primers in the primer mix (1:10; del:wt) was also drastically increased to ensure a more even balance between the 3.2 (deletion fragment) and 5.1 (whole gene) kb fragments. Non-specific bands were however, observed and we subsequently altered the cycling parameters as follows: an initial denaturation at 95 °C for 1 min, 35 cycles of 10 s at 95 °C (denaturation) and 68 °C for 5 min (annealing) with a final extension of 7 min at 68 °C. The above parameters were then used to amplify the CYP2D6 gene of the problematic samples (Figure 11B). The PCR products were diluted five times with the addition of TE buffer and stored at 4 °C. These products were used as templates in the multiplex and allele-specific PCR reactions for the detection of the other alleles.

Alleles *3, *4, *6, *8 and *14 were detected simultaneously by using a multiplex amplification refractory mutation system (ARMS) as described by Roberts and co-workers (2000). The reactions were performed in a total volume of 25 μl, containing 1 μl template (diluted PCR product from CYP2D6 gene amplification), 200 μM $MgCl_2$, 200 μM dNTP mix, 0.2 μM ARMS1 or ARMS2 primer mix and 2.5

units *Taq* DNA Polymerase (Promega). Thermal cycling was performed with an initial denaturation of 30 s at 94 °C, followed by 14 cycles of 65 °C for 20 s, 72 °C for 40 s and a final elongation at 72 °C for 2 min. Mutations were observed by analysing the obtained PCR fragments on 2.5 % Agarose MS gels (Roche Diagnostics) [Figure 12].

Alleles 8 and 14 are of the same size (236 bp) and separate amplification reactions have to be performed when bands (mutations) were observed in the ARMS 2 lane for these two alleles. Further analyses were performed in separate amplification reactions for each allele. We had to titrate the Magnesium concentration in the amplification reactions for alleles 8 and 14, due to the occurrence of non-specific bands in the original reactions. A final concentration of 1.3 mM $MgCl_2$ was decided on, using the same reaction conditions and cycling parameters as described earlier. PCR products were analysed on a 2.5 % Agarose MS gel (Roche Diagnostics).

Separate PCR reactions were performed to determine the status of alleles *7 (Roberts *et al.*, 2000) and *17 (Masimirembwa *et al.*, 1996). Allele 7 (Figure 13) was detected using the same reaction conditions as described by Roberts *et al.*, 2000. We had, however, to adjust the $MgCl_2$ concentration for the detection of *17 to 1.2 mM $MgCl_2$ instead of 0.6 mM, as used by Masimirembwa and co-workers (1996). The number of cycles was also increased to 20 (Figure 14). An annealing temperature of 60 °C was used.

The occurrence of CYP2D6*10 was first determined by amplifying a fragment of the CYP2D6 gene (310 bp) followed by a restriction enzyme digestion with *HphI*. The presence of *10 is indicated by the loss of one of two *HphI* sites (personal comm. with A.K. Daly). We have also found that using the allele-specific primers, L10mut and L10wt described by Ji and co-workers gave similar results (Ji *et al.*, 2002). The allele-specific primers were found to be more cost effective and results were obtained in less time. Subsequent screening for *10 was performed using the allele-specific primers of Ji and co-workers together with the forward

primer, 1C, from Ann Daly and the Touchdown program on the PE2700 (Figure 15, Table 6).

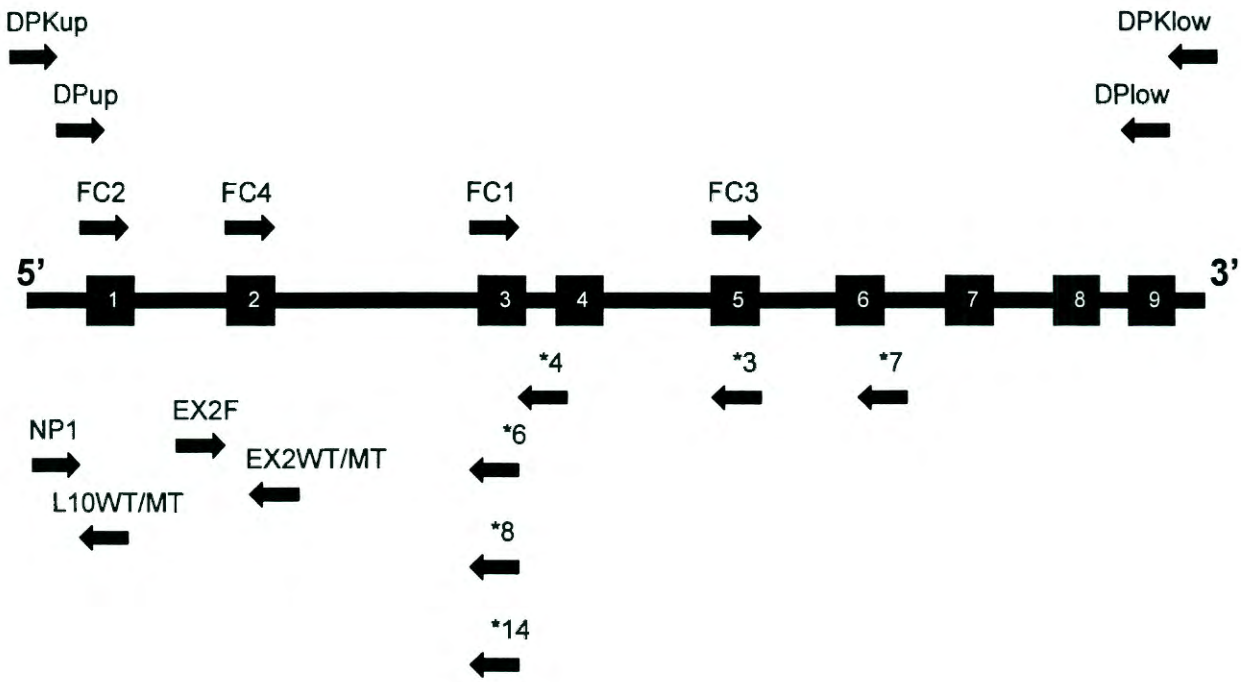


Figure 10. *Layout of CYP2D6 gene showing the 9 exons, the primers (Table 6) used to amplify the whole gene (5.1 kb), as well as the subsequent multiplex and allele-specific primers for each allele determined. DPKlow, DPKup, Dlow and Dup were used to amplify the entire gene, as well as to detect the CYP2D6 gene deletion. FC1 – FC4 are forward primers and the mutation-specific primers are indicated below the map. Primers EX2F, EX2WT, EX2MT and NP1, L10mut and L10wt are for the detection of *17 and *10 respectively.*

We have also determined the duplication of the 2D6 gene using the primers 2n-32 and 2n-17 as described by Løvlie and co-workers in 1996 (Figure 16). Using this method, we could determine if duplication of the CYP2D6 gene has occurred, but unfortunately not which specific allele was duplicated nor the copy number.

Table 5. The CYP2D6 polymorphisms determined in this study, their characteristic mutations and the subsequent effect on CYP2D6 enzyme activity.

Allele	**Characteristic mutation(s)**	**Effect**	**Enzyme activity**
CYP2D6*1 [b]	Wt	none	Normal
CYP2D6*3	A_{2637} deletion	Frameshift	Deficient
CYP2D6*4[b]	$G_{1934}A$	Splicing defect	Deficient
CYP2D6*5	Gene deletion	No protein formation	Deficient
CYP2D6*6	T_{1795} deletion	Frameshift	Deficient
CYP2D6*7	$A_{3023}C$	H324P	Deficient
CYP2D6*8	$G_{1846}T$	Stop codon	Deficient
CYP2D6*10 [b]	C_{188}**T**, $G_{1749}C$, $G_{4268}C$	Unstable enzyme	Decreased
CYP2D6*14	$G_{1846}A$		Deficient
CYP2D6*17	C_{1111}**T**, $C_{2938}T$, $G_{4268}C$	Reduced affinity for substrate	Decreased
CYP2D6*2n	Duplication[a] of 2D6 gene		Increased / Normal

a in rare cases the gene could also be multiplicated

b these alleles could also be duplicated

Table 6. Primers used in this study to determine the occurrence of different CYP2D6 alleles in the Caucasian, Black and Coloured Southern African population groups, as well as for sequencing. Bases depicted in bold shows base substitutions for specific SNP.

Alleles	*Primer name*	*Sequence (5'-3')*	*bp*	*Reference*
CYP2D6*5	Dup	CACACCGGGCACCTGTACTCCTCA	24	Hersberger *et al.*, 2000
	Dlow	CAGGCATGAGCTAAGGCACCCAGAC	25	
	DPKup	GTTATCCCAGAAGGCTTTGCAGGCTTCA	28	
	DPKlow	GCCGACTGAGCCCTGGGAGGTAGGTA	26	
	5Kup	*GTTATCCCAGAAGCCTGTGTGGGCTTGG*	*28*	*designed 2003*
	5Klow	*GCCGACTGAGCCCTGGGAGGTAGCCC*	*26*	
*3,*4,*6,*8,*14	FC1	GATGGTGGGGCTAATGCCTTCATGGCCACG	30	Roberts *et al.*, 2000
	FC2	**CAAGAACCTCTGGAGCAGCCCATACCCGGCC**	**30**	
	FC3	GCAAGGTCCTACGCTTCCAAAAGGCTTTCC	30	
	FC4	TCTCCTCCTTCCACCTGCTCACTCCTGGTA	30	
	3WT	GGGGGGCTGGGCTGGGTCCCAGGTCATCG**T**	30	
	3MU	GGGGGGCTGGGCTGGGTCCCAGGTCATCG**G**	30	
	6MU	ACAAAGGCAGGCGGCCTCCTCGGTCACCG**C**	30	
	6WT	ACAAAGGCAGGCGGCCTCCTCGGTCACCG**A**	30	
	4MU	CAAGAGACCGTTGGGGCGAAAGGGGCGTGT	30	
	4WT	CAAGAGACCGTTGGGGCGAAAGGGGCGTG**C**	30	
	8WT	CGCTTTGTGCCCTTCTGCCCATCACCCAG**C**	30	
	8MU	CGCTTTGTGCCCTTCTGCCCATCACCCAG**A**	30	
	14WT	CGCTTTGTGCCCTTCTGCCCATCACCCAG**C**	30	
	14MU	CGCTTTGTGCCCTTCTGCCCATCACCCAGT	30	
CYP2D6*7	7WT	GCTGCACATCCGGA**T**	15	Roberts *et al.*, 2000
	7MT	GCTGCACATCCGGA**G**	15	
CYP2D6*17	EX2F (f)	CCAAGGTTCAAATAGGACTA	20	Masimirembwa *et al.*, 1996
	EX2WT (r)	CCCGAAACCCAGGATCTGG**G**	20	
	EX2MT (r)	CCCGAAACCCAGGATCTGG**A**	20	
CYP2D6*10	1C (f)	TAGTGAGGCAGGTATGG	17	A.K. Daly (personal communication)
	NP1 (r)	AACCTGCTTCCCCTTCT	17	
	L10wt	GGGGGCCTGGTGG	13	Ji *et al.*, 2002
	L10mut	GGGGGCCTGGTGA	13	
CYP2D6*2n	2n-32	CACGTGCAGGGCACCTAGAT	20	Løvlie *et al.* 1996
	2n-17	TCCCCCACTGACCCAACTCT	20	

2.4 Data Analysis

Mutations would appear as bands at the corresponding positions in the lane opposite from the wild type. Scoring of the bands was done by the absence or presence of bands in the respective lanes. The allele and genotype frequencies, as well as enzymatic status, were summarised by percentages and compared between the three population groups.

2.5 Statistical Analysis

The Chi-squared test was used to compare the allele frequencies between the populations. Where a cell in the contingency table had an expected frequency of less than five, the Fisher's exact test was used. A *P* value of less than 0.05 was seen as statistically significant. The 95 % Confidence intervals for differences in proportions between the three population groups were also determined. All above statistical analyses were performed by the department of Biostatistics at the University of the Free State. Hardy-Weinberg Equilibrium tests were also performed for each population.

2.6 **DNA sequencing** (Figures 19-21)

Four coloured samples were chosen randomly for sequence analysis. DNA sequencing was done, using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The Run parameters used were: Seq POP6 (1 ml) E.mol4 module with a 30 seconds injection at 2.5 kV and a 120 minutes runtime at 50 °C and 12.2 kV. Samples were run using a POP6 polymer and a 61 cm x 51 μm capillary. Sequences were analysed using DNA Sequence Analysis Software version 5.1 from Applied BioSystems and the KB Basecaller parameter. Editing of sequences was done using Chromas v 2.3 and ChromasPro v 1.21 (Technelysium) and alignments of exon sequences were performed using

ClustalW. The obtained sequence data was verified by performing a Blast search.

The *CYP2D6* gene was amplified using only the two primers amplifying the gene itsself, cleaned using the Centri Sep columns (Princeton Separations) and used as template. The forward primers, FC1, FC2, FC3 and FC4 (Table 6), also used for the genotyping, were used for direct sequencing of the first six exons of the *CYP2D6* gene. We only sequenced six exons, as the polymorphisms we were interested in for this study only occurred in exons 1-6 of the *CYP2D6* gene. The sequence of the human *CYP2D6* gene (M33388) from GenBank was used as a reference sequence. Numbering of bases was started at the ATG start codon, with A defined as the first base (+1).

Chapter 3

Results and Discussion

3.1 Genotyping

A total of 193 subjects (49 Caucasian, 71 Blacks [Sotho] and 72 Coloured [mixed origin]) were genotyped in this study. We tested for the occurrence of alleles CYP2D6*3, *4, *5, *6, *8, *10, *14, *17, as well as for the duplication of the gene CYP2D6*2n (Figures 11-16). The CYP2D6 genotypes and allele frequencies are represented in Tables 8, 9, & 10.

Based on the advice from the Biostatician of the Faculty, it was decided on 100 subjects per population (Coloured and Black) because the confidence interval will then be 10 %. The sample sizes were however reduced (71 Coloured and 72 Black) due to technical problems, time constraints and financial reasons. Interim analyses performed indicated clear trends and therefore the statiscian advised that no further samples would be needed.

An initial long PCR (XL PCR) was performed for amplification of the CYP2D6 gene of 5.1 kb and the deletion fragment (3.2 kb) (Figure 11A). The 5.1 kb fragment served as template for subsequent multiplex and allele-specific PCRs, as well as for sequencing. Many problems were encountered with the XL PCR and it was concluded that the quality of the genomic DNA was very important. Although the extracted DNA seemed to be of good quality on agarose gels we still encountered problems probably due to a low level of single strand breaks and therefore recommend the use of a reliable DNA extraction method. We also found that the XL PCR product used as template for the subsequent allele-specific PCRs could not be stored for extended periods. Storage was done at 4 °C for longer than 8-10 months for some samples and when subsequent PCRs were done, no results were obtained. This proved to be a considerable problem since the genotyping was performed over a period of three years due to time constraints. Separation of the

primer pairs into 2 separate reactions as used by Hersberger and co-workers, 2000, in the XL PCR also contributed to more reliable results (Figure 11B).

The ARMS PCR (Roberts *et al.*, 2000) was adapted for our laboratory. Some optimisation was required ensuring that no "ghost bands" interfered with he interpretation. After optimisation this method was found to be reliable, quick and specific (Figure 12). We decided to perform genotyping using allele-specific PCR rather than RFLPs, since restriction enzymes are more expensive in South Africa. Also, it was not always clear when a digestion was uncompleted or the subject a heterozygote.

In Figure 11A, samples 8C, 21C and 23C shows a deletion (3.2 kb fragment) of the CYP2D6 gene (*5). Alleles CYP2D6*6, *7 and *8, were not detected in the Caucasian and Black groups, while CYP2D6*7 was detected in the Coloured group, although at a very low frequency of 0.014.

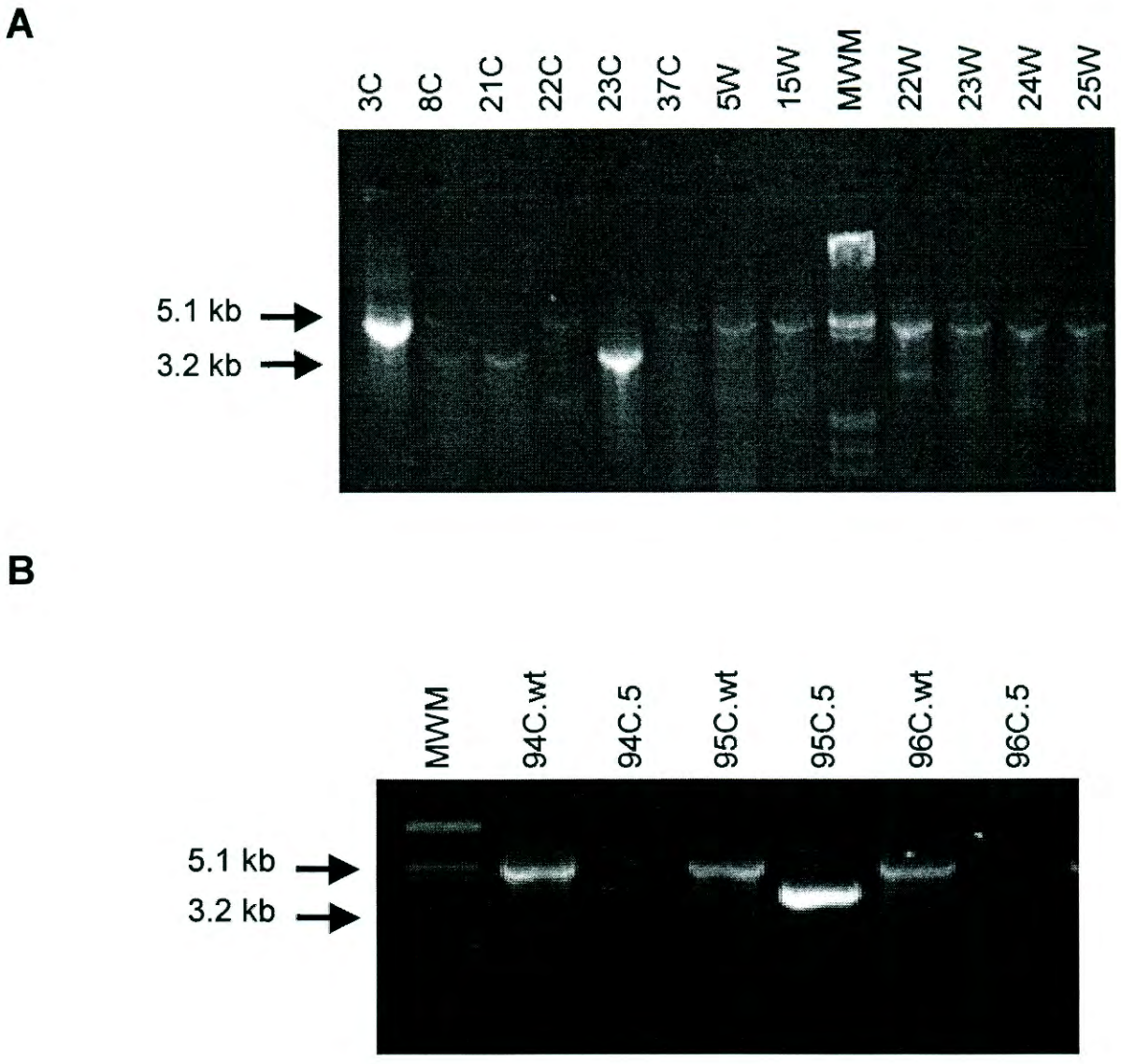


Figure 11. *0.8 % Agarose gel showing the PCR products for the CYP2D6 gene deletion allele (*5), using primers Dup, Dlow, DPKlow and DPKup. The 5.1 kb fragment represents the CYP2D6 gene, while the smaller 3.2 kbp fragment is obtained when a deletion of the CYP2D6 gene occurred. MWM represents the molecular weight marker used, in this case phage lambda digested with EcoRI and HindIII.*

- ***A.*** *Long PCR for CYP2D6*5 detection and amplification of whole gene, performed in one PCR tube.*
- **B.** *Long PCR for amplification of CYP2D6 gene and detection of deletion in separate tubes (wt & 5.)*

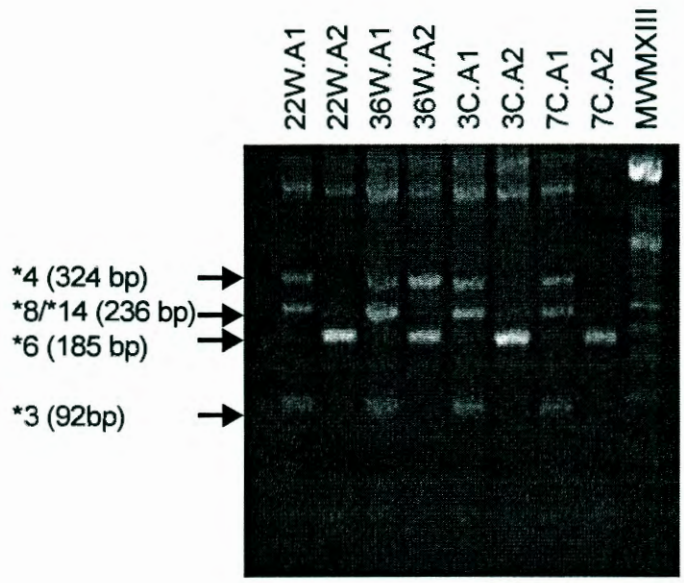


Figure 12. *2.5 % MS Agarose gel showing the PCR products from the multiplex allele specific PCR reactions performed for subjects in the Caucasian (W) and Coloured (C) groups. PCR reactions were performed in two tubes, A1 (ARMS1) and A2 (ARMS2).* Subjects were genotyped as follows: 22W = CYP2D6*1/*1, 36W = CYP2D6*1/*4, 3C = CYP2D6*1/*1 and 7C = CYP2D6*1/*5.

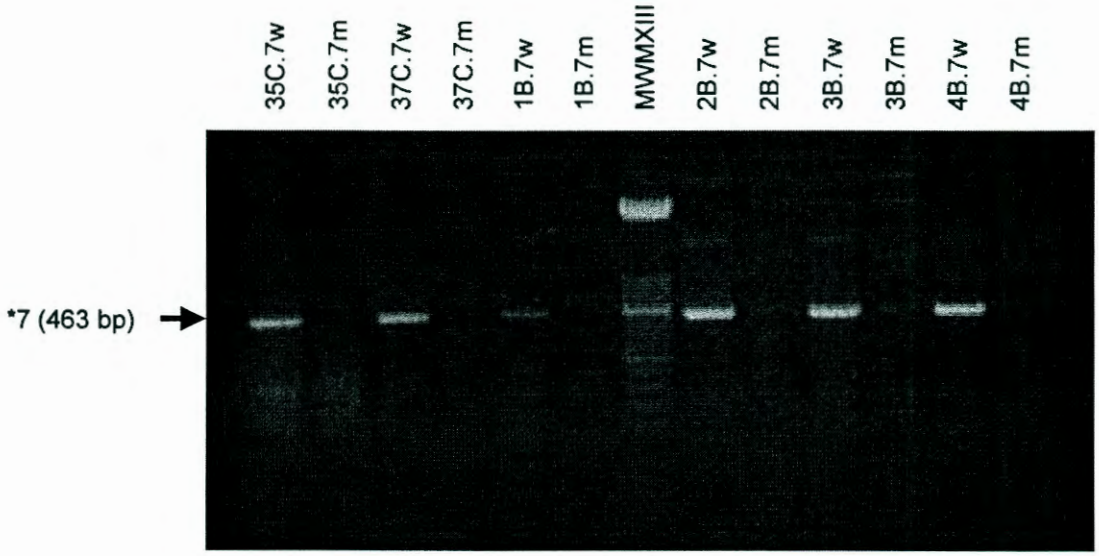


Figure 13. *1.5 % Agarose gel showing the PCR fragment obtained for *7 (463 bp). Subjects labelled C are from the Coloured group, while subjects labelled B are from the Black group. No subject tested positive for the presence of *7.*

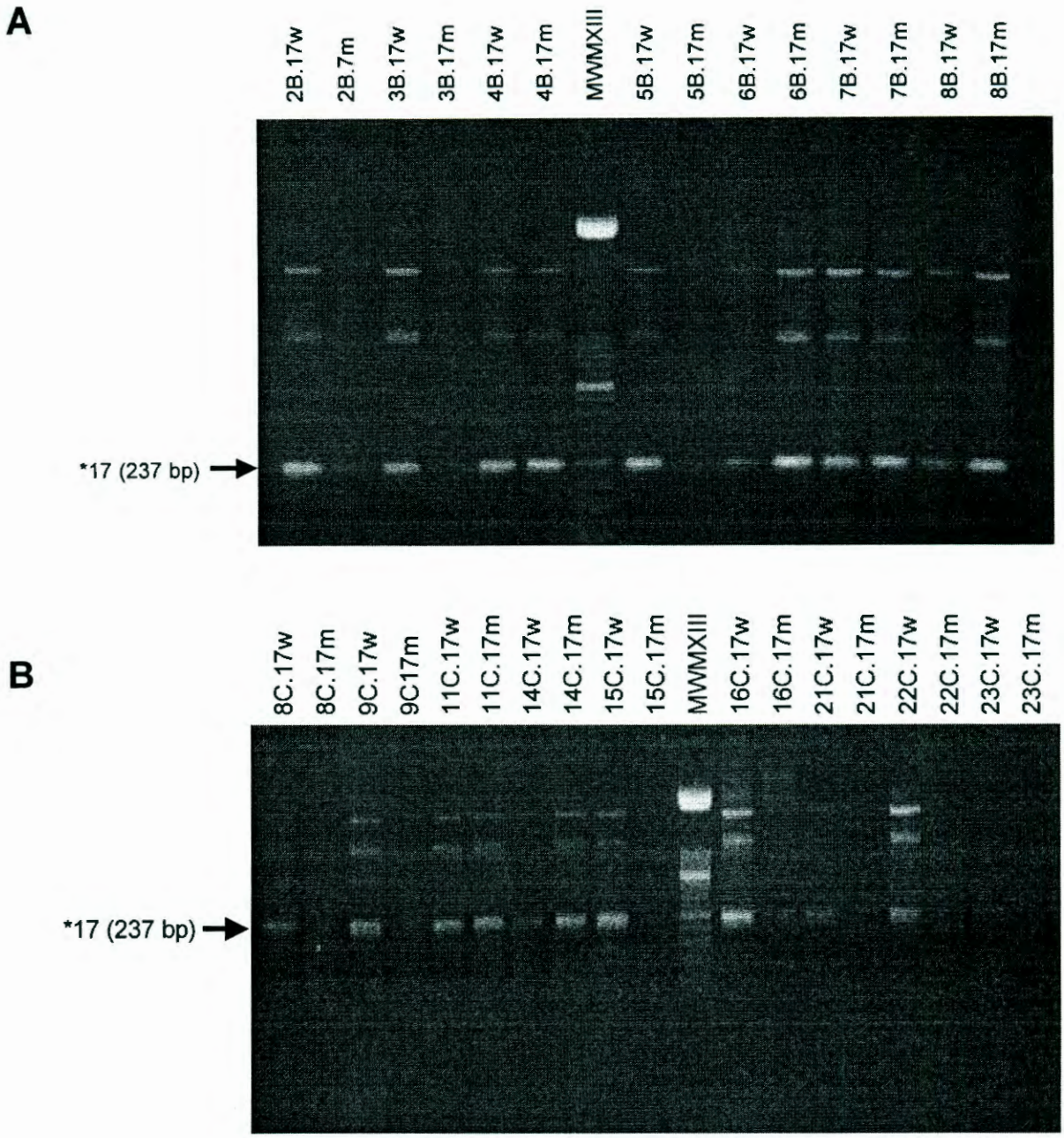


Figure 14. *PCR fragment obtained for genotyping subjects for *17 (237 bp). PCR products were analysed on a 1.5 % agarose gel.*

A. *Some subjects from the Black group. From the gel it is clear that subjects 4B, 6B, 7B and 8B tested positive for the presence of *17. All subjects were heterozygous for *17 except for subject 8B whom also tested positive for *14, thus having aCYP2D6 *14/*17 genotype.*

B. *Some subjects from the Coloured group. Subject 11C and 14C tested positive for *17.*

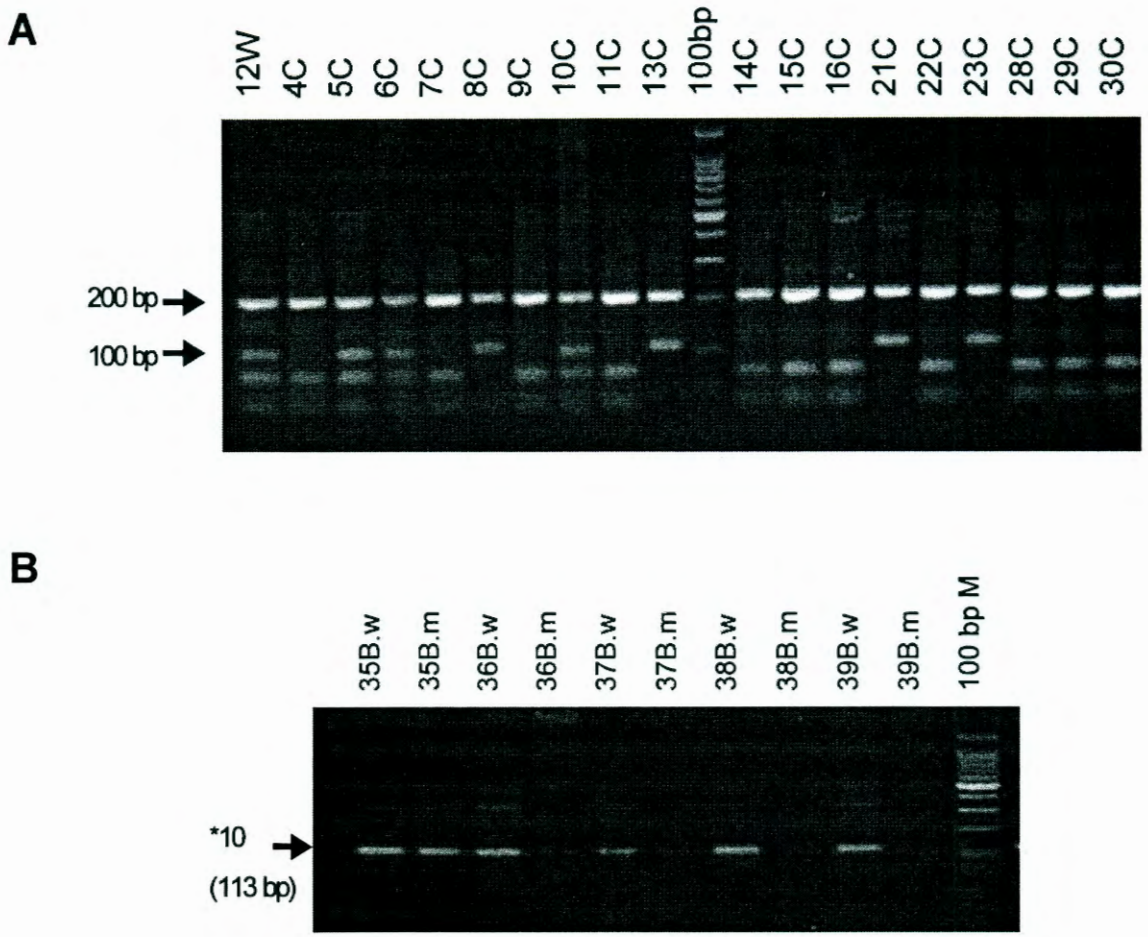


Figure 15. *PCR products obtained for subjects genotyped for *10.*

A. DNA band pattern obtained after PCR and subsequent restriction enzyme digestion using HphI.

B. PCR product obtained after using Allele-specific PCR (113 bp).

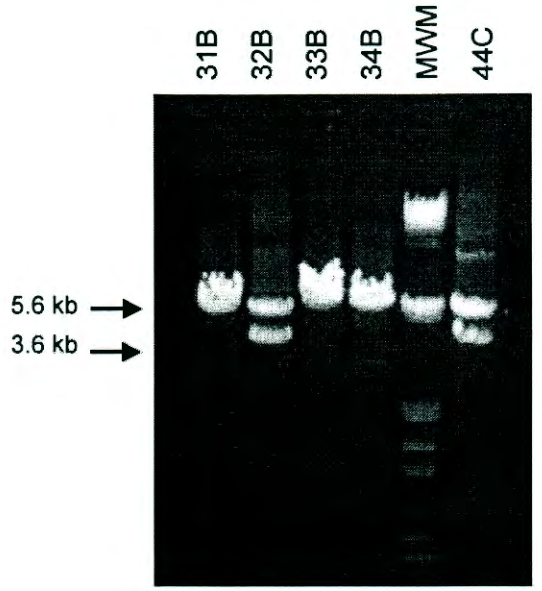


Figure 16. *0.8 % Agarose gel depicting the PCR products observed for the determination of CYP2D6 gene duplication using the primers 2n-17 and 2n-32 (Løvlie et al., 1996). If only fragment 5.6 kb is seen, no duplication of the gene has occurred. The occurrence of both fragments (5.6 kb + 3.6 kb) in a subject (32B) would then be positive for the duplication of the CYP2D6 gene. This method, however, does not identify which part of the gene that has been duplicated. MWM depicts the molecular weight marker (phage lambda digested with EcoRI and HindIII).*

Previous researchers observed pronounced differences between Caucasians and Orientals and postulated that it is possible that PCR tests standardised on Caucasians would not be specific enough when used to genotype Orientals (Garcia-Barceló *et al.*, 2000). We made similar observations in the current study, since PCRs standardised on the Caucasian and Black groups did not yield similar results when applied to the Coloured group. We encountered many problems, especially with the CYP2D6*17 allele-specific PCR, for the Coloured group. It could be that there were unidentified polymorphisms in the DNA primer binding sites of the Coloured subjects and the primers could therefore not bind properly. After sequencing of some samples, we observed that we did not always identify

the C1023T mutation correctly (Figure 19B). Samples 95C and 73C showed a base substition at position 1023 of a C to a T.

3.2 **Data Analysis** (Tables 8 - 11)

Subjects were classified as extensive metabolisers when there was at least one functional allele present in the individual. Poor metabolisers were those who tested positive for the presence of two null (deficient) alleles, while a duplication of the wt allele was indicative of the ultra-rapid metabolisers. Heterozygotes with one wild type and one polymorphic allele were thus classified as extensive metabolisers, although their enzyme activity may be lower than when compared to the homozygous wild type (two functional alleles present) individuals. In the scope of this study, however, no phenotype analysis was performed and the phenotypes were therefore estimated from the genotypes determined for each subject (Table 10; Figure 18).

Problems could arise when a subject tested positive for a deletion of the gene (CYP2D6*5). It is difficult to distinguish homozygous individuals from subjects carrying one copy of a polymorphism and the gene deletion (hemizygous). These individuals could sometimes be wrongly classified. If no mutations were identified in a subject, the allele was designated CYP1D6*1 (wt) by default, since we did not test for allele *2 (wt). In this study, we only screened for a selection of mutations and the designation of CYP2D6*1 must not be seen as absolute. Further studies involving more alleles would result in a more comprehensive picture.

The Chi-squared test was used to compare the allele frequencies between the three populations. The Hardy-Weinberg Equilibrium tests were also performed for each allele identified in the three Southern African populations. The Results obtained for each of the statistical analyses performed, compared well.

Table 7 . Haplotypes of the different CYP2D6 alleles identified in this study. Characteristic mutation for each allele is designated in bold. (bases numbered from translation initiation site; *+88 = transcription initiation site*)

Allele	Characteristic Mutations
CYP2D6*1	Wt
CYP2D6*3	**A_{2549} deletion**
	*+88 = **A_{2637}***
CYP2D6*4	**$G_{1846}A$**, $C_{100}T$, $C_{974}A$, $A_{984}G$, $C_{997}G$, $G_{1661}C$, $G_{4180}C$
	*+88 = **$G_{1934}A$**, $C_{188}T$, $C_{1062}A$, $A_{1072}G$, $C_{1085}G$, $G_{1749}C$, $G_{4268}C$*
CYP2D6*5	Gene deletion
CYP2D6*6	**T_{1707} deletion**
	*+88 = **T_{1795}***
CYP2D6*7	**$A_{2935}C$**
	*+88 = **$A_{3023}C$***
CYP2D6*8	**$G_{1758}T$**, $G_{1661}C$, $C_{2850}T$, $G_{4180}C$
	*+88 = **$G_{1846}T$**, $G_{1749}C$, $C_{2938}T$, $G_{4268}C$*
CYP2D6*10	**$C_{100}T$**, $G_{1661}C$, $G_{4180}C$
	*+88 = **$C_{188}T$**, $G_{1749}C$, $G_{4268}C$*
CYP2D6*14	**$G_{1758}A$**, $C_{100}T$, $C_{2850}T$, $G_{4180}C$
	*+88 = **$G_{1846}A$**, $C_{188}T$, $C_{2938}T$, $G_{4268}C$*
CYP2D6*17	**$C_{1023}T$**, $G_{1638}C$ (silent), **$C_{2850}T$**, $G_{4180}C$
	*+88 = **$C_{1111}T$**, $G_{1726}C$ (silent), **$C_{2938}T$**, $G_{4268}C$*
CYP2D6*2n	**Duplication of gene**

Table 8. CYP2D6 Allele frequencies determined for the Caucasian, Black and Coloured Southern African groups.

Allele	Caucasians	*f*	Blacks	*f*	Coloureds	*f*	Alleles detected for total SA population				
							Caucasian	Black	Coloured	Total	*f*
X	n=49	x=98	n=71	x=142	n=72	x=144				n=193	x=384
*1	72	0.7347	67	0.4718	70	0.4861	72	66	70	209	0.5415
*3	1	0.0102	0	0	1	0.0068	1	0	1	2	0.0052
*4	17	0.1735	3	0.0211	5	0.0342	17	3	5	25	0.0648
*5	7	0.0714	31	0.2183	29	0.2014	7	31	29	67	0.1736
*6	0	0	0	0	0	0	0	0	0	0	0
*7	0	0	0	0	2	0.0139	0	0	2	2	0.0052
*8	0	0	0	0	0	0	0	0	0	0	0
*10	1	0.0102	6	0.0423	16	0.1111	1	6	16	23	0.0596
*14	0	0	2	0.0141	2	0.0139	0	2	2	4	0.0104
*17	0	0	33	0.2324	19	0.1319	0	33	19	52	0.1347

X = CYP2D6 allele
x = number of alleles; for each subject there are 2 alleles (n x 2)
N = number of subjects
f = frequency

Table 9. Statistical analysis of the CYP2D6 allele frequencies determined in this study. 95 % Confidence Interval (CI) and p values are shown. A p value < 0.05 was considered significant.

Allele	Caucasian	Black	Coloured	Caucasian vs Black		Caucasian vs Coloured		Black vs Coloured	
	f	*f*	*f*	P value	95 % CI	P value	95 % CI	P value	95 % CI
*1	0.7347	0.4718	0.4795	<0.0001	0.1389; 0.3771	0.0001	0.1251; 0.3627	0.8090	-0.1291; 0.1010
*3	0.0102	0	0.0069	0.4083#	-0.0129; 0.0388	1.000#	-0.0228; 0.0353	1.000#	-0.0258; 0.0123
*4	0.1735	0.0211	0.0347	<0.0001	0.0732; 0.2313	0.0002	0.0579; 0.2200	0.7227#	-0.0533; 0.0267
*5	0.0714	0.2183	0.2014	0.0022	-0.2277; -0.0571	0.0053	-0.2090; -0.0423	0.7253	-0.0773; 0.1108
*6	0	0	0	–	–	–	–	–	–
*7	0	0	0.0139	–	–	0.5161#	-0.0359; 0.0146	0.4983#	-0.0368; 0.0096
*8	0	0	0	–	–	–	–	–	–
*10	0.0102	0.0423	0.1111	0.2454#	-0.0703; 0.0129	0.0026	-0.1534; -0.0398	0.0289	-0.1296; -0.0061
*14	0	0.0141	0.0139	0.5148#	-0.0364; 0.0146	0.5161#	-0.0359; 0.0146	1.000#	-0.0298; 0.0304
*17	0	0.2324	0.1319	<0.0001	-0.2968; -0.1559	0.0002	-0.1841; -0.0702	0.0277	0.0106; 0.1878

Fisher's exact test (sample size smaller than 5)
f frequency

Table 10. Phenotypes extrapolated from the genotypes determined for each population group in this study. The associated p values and 95 % Confidence Intervals (95 % CI) for comparison between populations are also shown.
A p value < 0.05 is considered significant.

Pheno-type	Caucasian		Coloured		Black		Cau vs Black		Cau vs Coloured		Black vs Coloured	
	Total	%	Total	%	Total	%	p	95 % CI	p	95 % CI	p	95 % CI
UM	0	0	2	2.740	1	1.408	1.0000*	-0.0506; 0.0347	0.5140*	-0.0707; 0.0285	1.000*	-0.0660; 0.0397
EM	47	95.918	50	69.444	50	70.423	0.0005	0.1223; 0.3637	0.0003	0.1315; 0.3733	0.8985	-0.1393; 0.1582
IM	0	0	17	23.611	14	19.718	0.0009	-0.2820;-0.0905	0.0002	-0.3248; -0.1232	0.5722	-0.1718; 0.0962
PM	2	4.082	3	4.167	6	8.451	0.4695*	-0.1261; 0.0517	1.0000*	-0.0726; 0.0819	0.3260*	-0.0340; 0.1237

* Fisher's exact test

Cau = Caucasian

UM = ultra-rapid metabolisers, EM = extensive metabolisers, IM = intermediate metabolisers and PM = poor metabolisers.

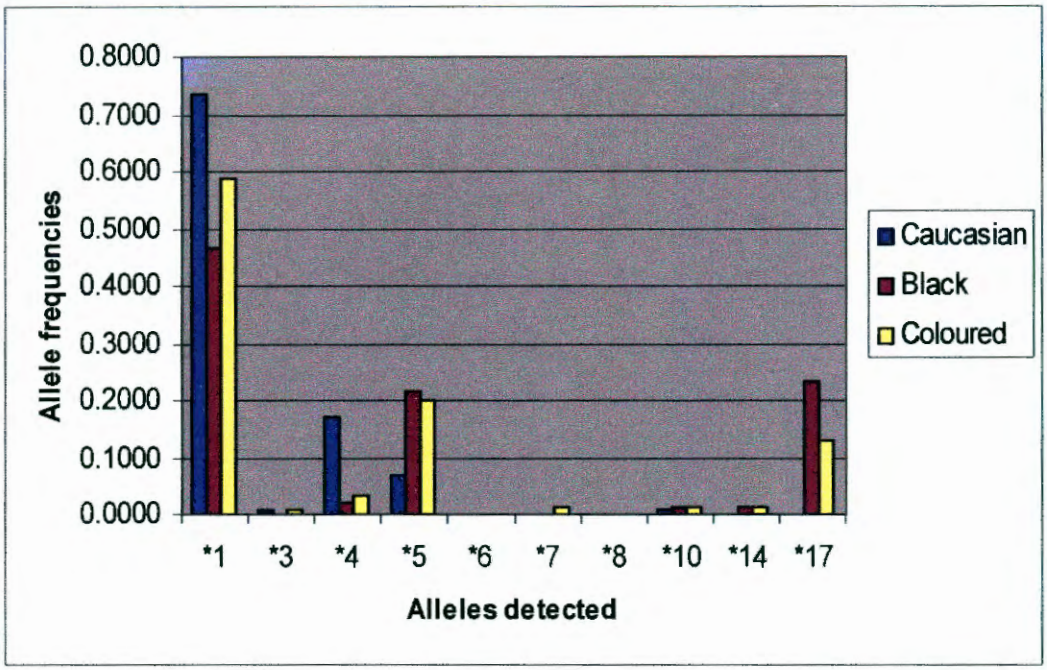


Figure 17. *Distribution of the allele frequencies of CYP2D6 in the three Southern African population groups.*

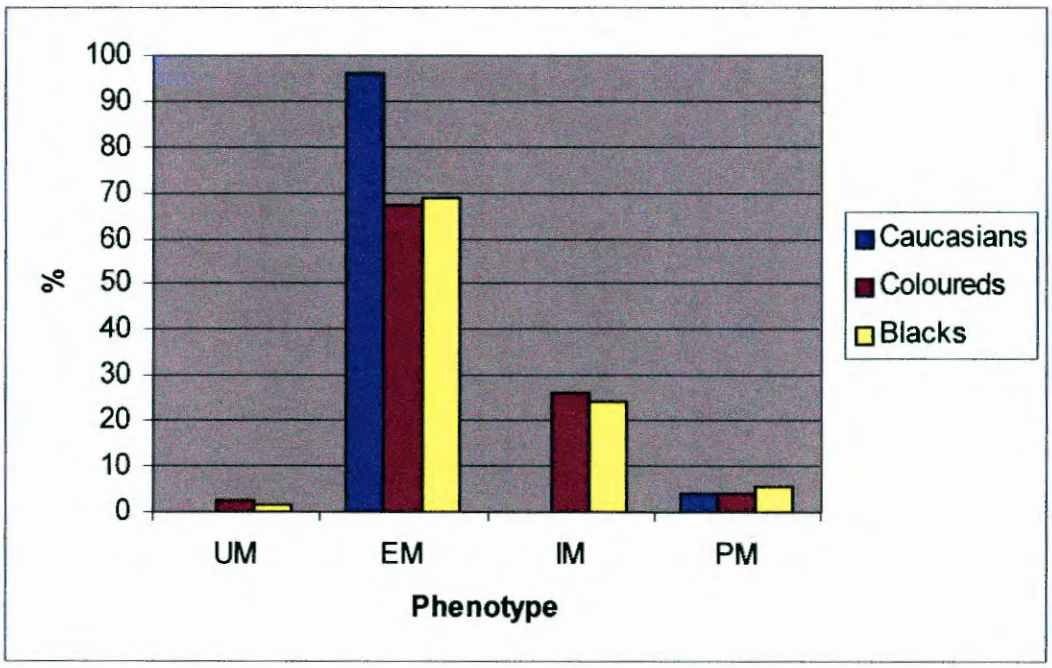


Figure 18. *Histogram showing the distribution of the different phenotypes extrapolated from the genotypes determined for each subject in the population group.*

Table 11. Some allelic variants of CYP2D6 and their frequency distribution in different ethnic groups.

Population	Alleles and allele frequencies (*f*)											Reference
	*1	*2	*4	*5	*6	*7	*8	*10	*17	*29	*40	
Caucasian (n=408)	0.37	0.33	0.18	0.04	0.01	0	0	0.02	0.002	0.002	0	Gaedigk *et al.*, 2002
Chinese												
Russians (n=290)	0.708	0.105	0.182	0.024	0.012	ND	ND	0.042	ND	ND	ND	Gaikovitch *et al.*, 2003
African American (n=502)	0.297	0.191	0.054	0.066	0.004	0	0	0.036	0.213	0.072	0.006	Gaedigk *et al.*, 2002
Tanzanian (n=212)	0.278 0.96[a]	0.203	0.009 0.04[a]	0.061	0	ND	ND	0.038	0.17	0.198	ND	Wennerholm *et al.*, 2001 Bathum *et al.*, 1999[a] (n=196)
Ethiopeans (n=122)	ND	ND	0.012	0.033	ND	ND	ND	0.086	0.09	ND	ND	Aklillu *et al.*, 1996, Gaedigk *et al.*, 2002
Zimbabweans (n=228)	0.47	0.13	0.02	0.04	ND	ND	ND	ND	0.34[b]	ND	ND	Dandara *et al.*, 2001, Masimirembwa *et al.*, 1996[b]
Ghanaians (n=386)	0.437	0.109	0.07	0.006	0	0	0	0.031	0.277	0.016	ND	Griese *et al.*, 1999
Nigerians												
Gabonese(n=154)	0.32	0.44		0.07					0.238			Panserat *et al.*, 1999
Venda (n=152)	0.50	0.178	0.033	0.046	ND	ND	ND	ND	0.24	ND	ND	Dandara *et al.*, 2001
***Caucasian (n=98)**	**0.735**	ND	**0.174**	**0.071**	**0**	**0**	**0**	**0.010**	**0**	ND	ND	
***Black (n=142)**	**0.472**	ND	**0.021**	**0.22**	**0**	**0**	**0**	**0.042**	**0.232**	ND	ND	
***Coloured (n=144)**	**0.480**	ND	**0.035**	**0.20**	**0**	**0.014**	**0**	**0.111**	**0.132**	ND	ND	

ND = not detected; n = number of subjects
* Results from this study

Caucasian group

In the Caucasian group a PM frequency of about 4 % and a EM frequency of 96 % was observed (Table 10; Figure 17). No intermediate metabolisers (IM) or ultra-rapid metabolisers (UM) were found in this group. Alleles CYP2D6*6, *7, *8, *14 and *17 were not detected among the Caucasians. The second most frequent CYP2D6 allele in the Caucasian group was CYP2D6*4 (0.17) (Tables 8 & 9). The other allele detected in a very high frequency was CYP2D6*1, by default. CYP2D6*5 was also found to occur in a higher frequency than expected, 0.07, for Caucasians, but this could be due to the small sample size used (n=49). One subject was genotyped as CYP2D6*3/*4. Although *3 occur at a very low frequency of about 1 %, the occurrence of this allele in our Caucasian group could be expected.

Black group

Seventy percent of the Black group was classified as extensive metabolisers using the genotype data, while 20 % was classified as intermediate, 8.5 % as poor and 1.4 % as ultra-rapid metabolisers (Table 10; Figure 18). The two most common alleles detected were CYP2D6*5 (0.22) and CYP2D6*17 (0.23) [Table 9; Figure 17]. Four subjects were found to have no functional CYP2D6 gene (CYP2D6*5/*5) and were subsequently classified as PMs. Griese and co-workers (2001) also found a CYP2D6*5/*5 subject when they performed genotyping among the Aborigines. This was the only subject classified as a PM in their study. The high occurrence of IMs is most probably due to the high prevalence of CYP2D6*17 in this group. Dandara and co-workers (2001) also found the IM status to be common among the Bantu populations they studied and attributed it to the high occurrence of CYP2D6*17 in these populations. In our study three subjects were even found to be homozygous for *17. CYP2D6*17 ($p < 0.0001$; 95 % CI: -0.2968; -0.1559) was also found in association with alleles *10, *14, *5 and *4 (Table 9). CYP2D6*14 is a rare allele of which the occurrence in this population needs further investigation. The occurrence of allele CYP2D6*10 (0.042) among the Black subjects compared well with results obtained for other Black African populations,

as well as the African Americans [Table 11] (Akklillu *et al.*, 1996; Gaedigk *et al.*, 2002; Masimirembwa *et al.*, 1996; Wennerholm *et al.*, 2001). Two subjects were found to have duplications of the wild type *CYP2D6* gene and were classified as UMs. In a third subject that showed a duplication, it was CYP2D6*4 that was duplicated. Since the other allele for this subject was identified as *17, the subject was subsequently classified as an IM.

Cardiovascular studies done on South African blacks also showed an ethnic variation in the response to beta-blockers between Caucasians and Blacks (Joubert *et al.*, 1988; Venter *et al.*, 1985). A higher dose of propranolol was needed in the Black population to produce similar degrees of inhibition of exercise-induced tachycardia than in the Caucasians. Metoprolol was another drug of which the metabolism was impaired in the Blacks and dosage adjustments should be considered (Joubert *et al.*, 1989; Swynghedauw, 2001). The incidence of hypertension was also found to be higher in Blacks than in Caucasians (Venter *et al.*, 1985). Although these studies were not concerned with CYP2D6 enzyme activity, it clearly shows the ethnic differences between the Black and Caucasian population groups in South Africa.

Coloured group

The most interesting results were observed for the Coloured group, in that alleles CYP2D6*5 (0.20), *10 (0.11) and *17 (0.13) were found to be the most common alleles (Table 9; Figure 17). Although CYP2D6*17 was found at a high frequency it was not as high as that obtained for the Black group in this study. Teh and co-workers (2001) also observed CYP2D6*17 among Malaysians. The high prevalence of allele CYP2D6*10 was also very interesting for this group and it was found to be statistically significantly different from the Caucasian group ($p = 0.0026$; 95 % CI: -0.1534; -0.0398) [Table 9]. The high occurrences of alleles CYP2D6*10 and *17 could be due to the mixed origin of this group, indicative of Oriental and Black origins, since CYP2D6*10 was found to be Oriental specific (Table 9). These two alleles were also observed among the Aborigines (Griese *et al.* 2001). The frequencies of PMs, IMs, EMs and UMs for the Coloured group

were, 4 %, 26 %, 67 % and 2.7 % respectively (Table 10; Figure 18). The high percentage of predicted intermediate metabolisers classified for this group is due to the high frequencies of alleles CYP2D6*10 and *17 identified for the Coloureds. We also observed the duplication of CYP2D6*4 in one subject and a CYP2D6*17 duplication in another subject; these need further investigation.

The difference in frequency of CYP2D6*17 in the Black and Coloured groups compared to the Caucasian group, proved to be statistically significant, $p < 0.0001$ (95 % CI: -0.2968; -0.1559) and $p = 0.0002$ (95 % CI: -0.1841; -0.0702), respectively. The high prevalence of CYP2D6*10 in the Coloured group was also found to be significantly different from the Black group with a p value of 0.0289 (95 % CI: -0.1296; -0.0061) [Table 9].

We also detected CYP2D6*14 in the Coloured and Black populations, but at very low frequencies of 0.0139 and 0.0141, respectively. CYP2D6*14 is a rare allele, although Ji and co-workers (2002) have also observed this allele among the Mainland Chinese.

The significantly higher than previously reported frequencies obtained for CYP2D6*5 in this study confirms results from a previous pilot study performed on Black hospitalised patients. We did confirm the XL PCR results using two different PCR approaches, however, we do not have an explanation for this high frequency and this definitely warrants further investigation.

Phenotypes were estimated from the genotype data obtained for each population. Since no phenotyping was performed, these extrapolated phenotypes are not absolutely accurate and should be seen as such. The high occurrence of IMs in the Black and Coloured groups was found to be statistically significantly higher than the Caucasian group with p values of 0.0002 (95 % CI: -0.3248; -0.1232) for the Coloured and $p = 0.0009$ (95 % CI: -2820; -0.0905) for the Black groups (Table 10; Figure 18).

Since one of the many benefits of pharmacogenetics is the possibility of a more informed selection of medication we suggest that the occurrence of CYP2D6

polymorphisms in the different ethnic groups in South Africa is very important and needs to be investigated thoroughly. The Coloured group especially needs a lot of attention in this regard due to their unique genetic makeup. Although it would be impossible to abolish all adverse reactions by genotyping for CYP2D6 variants, a reduction in the occurrence of adverse events and hospitalisation would already be a major step forward.

We were unable to detect any new mutations among the three populations as no phenotyping of the subjects was performed. Future research involving the Coloured population comparing phenotyping and genotyping data would be of considerable value and benefit for this population. Also, the inclusion of more alleles in a future study would ensure a more accurate prediction of CYP2D6 enzymatic status among the Coloured population of Southern Africa.

3.3 **DNA Sequencing** (Figures 19 - 21)

We sequenced four DNA samples chosen randomly from the Coloured group genotyped in this study for confirmation of mutations detected by genotyping. After amplification of the CYP2D6 gene from the genomic DNA, the ds PCR product was cleaned and sequenced. Ninety-five percent homology with the *CYP2D6* gene sequence, deposited in Genbank (reference number M33388.1) was obtained when the sequence data for the Coloured samples were subjected to a Blast search. No problems were encountered with the sequencing methods and the data obtained needed minimal editing. It was therefore decided to only sequence in the forward direction.

Single nucleotide polymorphisms (SNPs) were clearly visible from the sequence data (Figures 20 & 21) and it was possible to distinguish between a mutation and wild type. From the sequence data obtained for each of the four samples, it was possible to identify the characteristic SNP (Table 7) for each haplotype. The sequence data for subject 94C confirmed the presence of CYP2D6*4 (G1846A) [Figure 19C] and CYP2D6*10 (C100T) [Figures 19A & 20] mutations. This subject

was thus correctly identified as CYP2D6*4/*10 with the genotyping techniques used in this study.

A base substitution (G31A) in exon 1 was also observed from the sequence data for subject 94C (Figures 19A & 21). This mutation is characteristic of a duplication of CYP2D6*35x2 (CYP Allele Nomenclature committee web site), however, since we did not screen for this allele and the duplication PCR was negative, we could not propose a change in the genotype for this subject.

Three SNPs namely C1023T, G1638C and C2850T were detected by sequencing in subject 73C (Figure 17). Since we did not obtain results (no amplification) for this subject using the CYP2D6*17 allele-specific PCR, we were unable to adjust the genotype. This clearly indicated the problems we encountered applying the method used by Masimirembwa *et al.* (1996) to our Coloured population. We did minor $MgCl_2$ adjustments to adapt the method for our laboratory and excellent results were obtained for the Caucasian and Black populations. The problems could have been due to extended storage of the *CYP2D6* gene template and/or to as yet unknown polymorphisms affecting the upstream primer binding site. The downstream primer binding site is present in the sequence and is identical to the consensus sequence. The CYP2D6*17 allele-specific PCR thus needs more optimisation in our laboratory in order to make it reliable for all population groups.

The SNP (G1661C) in exon 3 was observed in three subjects, namely 95C, 94C and 73C (Figure 19C). This SNP forms part of many different haplotypes for CYP2D6, of which we tested for CYP2D6*4, *8 and *10. For CYP2D6*4, G1661C should be together with G1846A, for CYP2D6*8 with G1758T and for CYP2D6*10 together with C100T (Table 7). It could be that this SNP groups together with some of the haplotypes not determined in this study.

These results clearly shows the complexity of the Coloured subjects and is a definite indication of further research into the occurrence of CYP2D6 poplymorphisms in the Southern African population groups, especially the Coloureds.

A. CYP2D6 Exon 1

```
                +1                                                  G31A
M33388          ATGGGGCTAGAAGCACTGGTGCCCCTGGCCGTGATAGTGGCCATCTTCCTGCTCCTGGT
100C            ATGGGGTTANAANCACTGGGGGCCCTGGGCCNGATAATGGCCCTTCTTCTGCTCCTGGT
95C             ATGGGGCTAGAAGCACTGGTGCCCCTGGCCGTGATAGTGGCCATCTTCCTGCTCCTGGT
94C             ATGGGGCTAGAAGCACTGGTGCCCCTGGCCRTGATAGTGGCCATCTTCCTGCTCCTGGT
73C             ATGGGGCTAGAAGCACTGGTGCCCCTGGCCGTGATAGTGGCCATCTTCCTGCTCCTGGT
                ****** ** ** ****** * ****** *  **** ***** *  * **********

                          C73T G77A  C82T                    C100T
M33388          GGACCTGATGCACCGGCGCCAACGCTGGGCTGCACGCTACCCACCAGGCCCCCTGCCA
100C            GGACCTGATGCACCGGCCCCAACCTTTGGTTGAACGTTACCCCCCGGGCCCCCTGGTC
95C             GGACCTGATGCACCGGCGCCAACGCTGGGCTGCACGCTACCCACCAGGCCCCCTGCCA
94C             GGACCTGATGCACCGGCGCCAACGCTGGGCTGCACGCTACYCACCAGGCCCCCTGCCA
73C             GGACCTGATGCACCGGCGCCAACGCTGGGCTGCACGCTACCCACCAGGCCCCCTGCCA
                **************** *****  * ** ** *** *** * ** *********

M33388          CTGCCCGGGCTGGGCAACCTGCTGCATGTGGACTTCCAGAACACACCATACTGCTTCGAC
100C            TTGGCCGGGTTGGGGACCCTCTTTC----------------------------------
95C             CTGCCCGGGCTGGGCAACCTGCTGCATGTGGACTTCCAGAACACACCATACTGCTTCGAC
94C             CTGCCCGGGCTGGGCAACCTGCTGCATGTGGACTTCCAGAACACACCATACTGCTTCGAC
73C             CTGCCCGGGCTGGGCAACCTGCTGCATGTGGACTTCCAGAACACACCATACTGCTTCGAC
                 ** ***** **** * ***  * *

M33388          CAG
100C            ---
95C             CAG
94C             CAG
73C             CAG
```

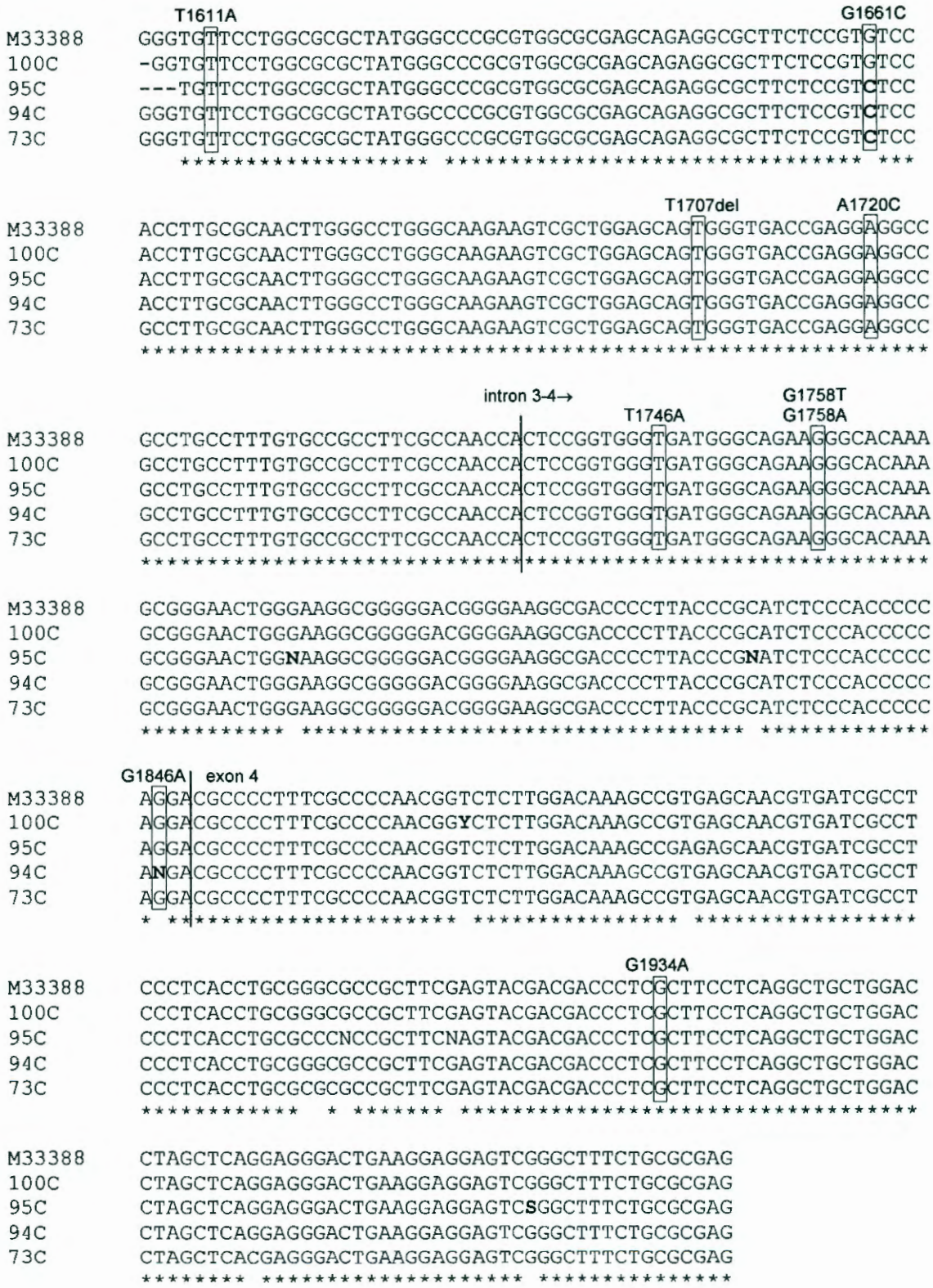
B. CYP2D6 Exon 2

```
M33388          TTGCGGCGCCGCTTCGGGGACGTGTTCAGCCTGCAGCTGGCCTGGACGCCGGTGGTCGTG
100C            TTGCGGCGCCGCTTCGGGGACGTGTTCAGCCTGCAGCTGGCCTGGACGCCGGTGGTCGTG
95C             TTGCGGCGCCGCTTCGGGGACGTGTTCAGCCTGCAGCTGGCCTGGACGCCGGTGGTCGTG
94C             TTGCGGCGCCGCTTCGGGGACGTGTTCAGCCTGCAGCTGGCCTGGACGCCGGTGGTCGTG
73C             TTGCGGCGCCGCTTCGGGGACGTGTTCAGCCTGCAGCTGGCCTGGACGCCGGTGGTCGTG
                ************************************************************

                                      C972T C974A      A984G          C997G
M33388          CTCAATGGGCTGGCGGCCGTGCGCGAGGCGCTGGTGACCCACGGCGAGGACACCGCCGA
100C            CTCAATGGGCTGGCGGCCGTGCGCGAGGCGCTGGTGACCCACGGCGAGGACACCGCCGA
95C             CTCAATGGGCTGGCGGCCGTGCGCGAGGCGCTGGTGACCCACGGCGAGGACACCGCCGA
94C             CTCAATGGGCTGGCGGCCGTGCGCGAGGCGATGGTGACCCACGGCGAGGACACCGCCGA
73C             CTCAATGGGCTGGCGGCCGTGCGCGAGGCGCTGGTGACCCACGGCGAGGACACCGCCGA
                ****************************** ******************************

                                C1023T                C1039T
M33388          CCGCCCGCCTGTGCCCATCACCCAGATCCTGGGTTTCGGGGCCGCGTTCCCAAG
100C            CCGCCCGCCTGTGCCCATCACCCAGATCCTGGGTTTCGGGGCCGCGTTCCCAAG
95C             CCGCCCGCCTGTGCCCATCATCCAGATCCTGGGTTTCGGGGCCGCGTTCCCAAG
94C             CCGCCCGCCTGTGCCCATCACCCAGATCCTGGGTTTCGGGGCCGCGTTCCCAAG
73C             CCGCCCGCCTGTGCCCATCATCCAGATCCTGGGTTTCGGGGCCGCGTTCCCAAG
                ******************* ***********************************
```

C. CYP2D6 Exons 3-4



D. CYP2D6 Exon 5

```
                                                                    A2549del
M33388    GTGCTGAATGCTGTCCCCGTCCTCCTGCATATCCCAGCGCTGGCTGGCAAGGTCCTACGC
100C      ------------------------------------------------------------
95C       ------------------------------------------------------------
94C       ------------------------------------------------------------
73C       ------------------------------------------------------------
                                                                    A2549del
M33388    TTCCAAAAGGCTTTCCTGACCCAGCTGGATGAGCTGCTAACTGAGCACAGGATGACCTGG
100C      ----------------TGACCCAGCTGGATGAGCTGCTAACTGAGCACAGGATGACCTGG
95C       ----------------------TGCTGACTGGTTGGAACTCGANNCANNNGATGACCTGG
94C       ----------------TGACCCAGCTGGATGAGCTGCTAACTGAGCACAGGATGACCTGG
73C       ----------------------CAGCTGGATGAGCTGCTAACTGAGCACAGGATGACCTGG
                                  ***  **    *    *    **   **********

M33388    GACCCAGCCCAGCCCCCCCGAGACCTGACTGAGGCCTTCCTGGCAGAGATGGAGAAG
100C      GACCCAGCCCAGCCCCCCCGAGACCTGACTGAGGCCTTCCTGGCAGAGATGGAGAAG
95C       TNCCNAGCCCAGCCCCCCCGAGACCTGACTGAGGCCTTCNTGGCAGAGATGGAGAAG
94C       GACCCAGCCCAGCCCCCCCGAGACCTGACTGAGGCCTTCCTGGCAGAGATGGAGAAG
73C       GACCCAGCCCAGCCCCCCCGAGACCTGACTGAGGCCTTCCTGGCAGAGATGGAGAAG
            ** ********************************** ****************
```

E. CYP2D6 exon 6

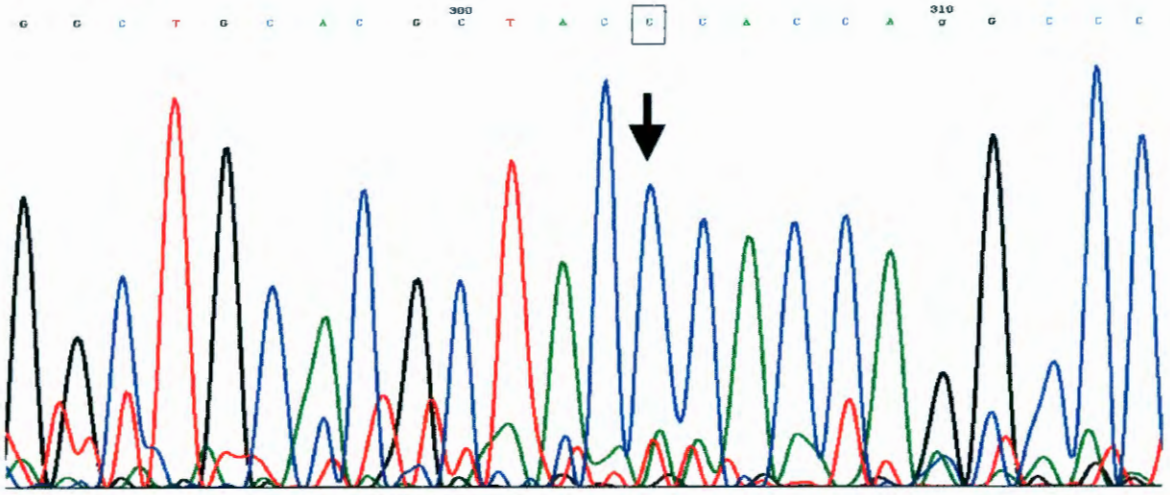
```
                                                          C2850T
M33388    GCCAAGGGGAACCCTGAGAGCAGCTTCAATGATGAGAACCTGCCGCATAGTGGTGGCTGAC
100C      GCCAAGGGGAACCCTGAGAGCAGCTTCAATGATGAGAACCTGCCGCATAGTGGTGGCTGAC
95C       GCCAAGGKGAACCCTGAGAKCAGCTTCAACGATGAGAACCTGCCGCATAGTGGTGGTTGAC
94C       GCCAAGGGGAACCCTGAGAGCAGCTTCAATGATGAGAACCTGCCCATAGTGGTGGCTGAC
73C       GCCAAGGGGAACCCTGAGAGCAGCTTCAATGATGAGAACCTGTGCATAGTGGTGGCTGAC
          ******* *********** ********* ************  *********** ****

M33388    CTGTTCTCTGCCGGGATGGTGACCACCTCGACCACGCTGGCCTGGGGCCTCCTGCTCAT
100C      CTGTTCTCTGCCGGGATGGTGACCACCTCGACCACGCTGGCCTGGGGCCTCCTGCTCAT
95C       CTGTTCTCTGCCGGGATGGTGACCNCTTCGACTACGCTGGCCTGGGGCCTCNTGCTCAT
94C       CTGTTCTCTGCCGGGATGGTGACCACCTCGACCACGCTGGCCTGGGGCCTCCTGCTCAT
73C       CTGTTCTCTGCCGGGATGGTGACCACCTCGACCACGCTGGCCTGGGGCCTCCTGCTCAT
          ************************ * ***** ****************** *******

                A2935C
M33388    GATCCTACATCCGGATGTGCAGC
100C      GATCCTACATCCGGATGTGCAGY
95C       GATCCTACATCCGGATGTGCATC
94C       GAYCCTACATCCGGATGTGCAGC
73C       GATCCTACATCCGGATGTGCAGC
          ** ******************
```

Figure 19. *Nucleotide sequences of exons 1-6 determined for the CYP2D6 gene in four Southern African Coloured subjects. GenBank sequence M33388.1 was use as reference sequence. Blocks depict the SNPs searched for in this study, as well as a few (G31A) that were not detected in this study. The SNPs occurring together with the main SNP in these exons are also shown (See Haplotype Table 7). Subjects were genotyped as follows: 100C-CYP2D6*1/*1, 95C-CYP2D6*1/*5, 94C-CYP2D6*4/*10 and 73C-CYP2D6*1/*1.*

A. wt (95C)



B. variant (94C)

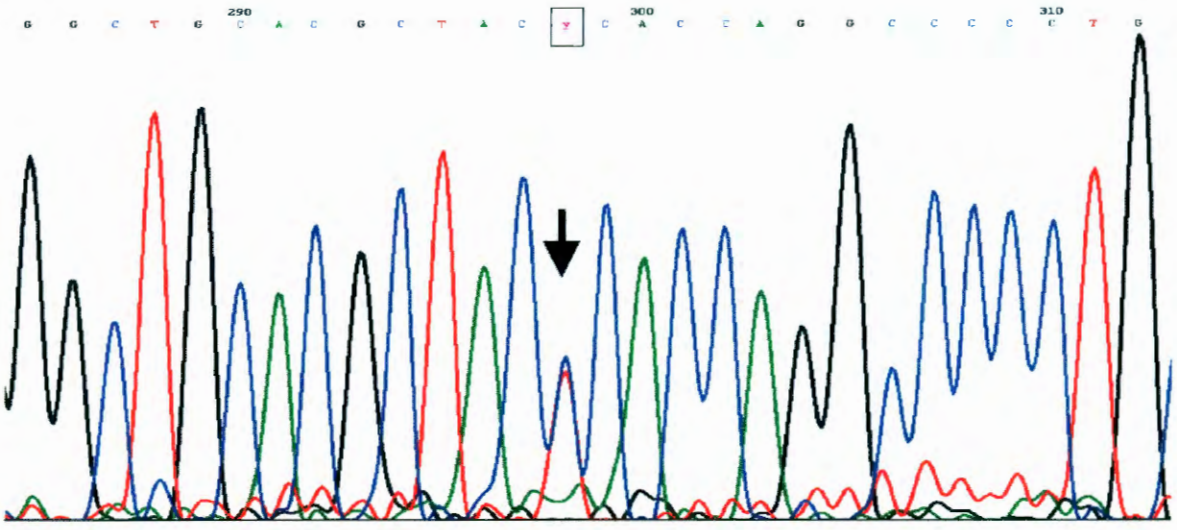
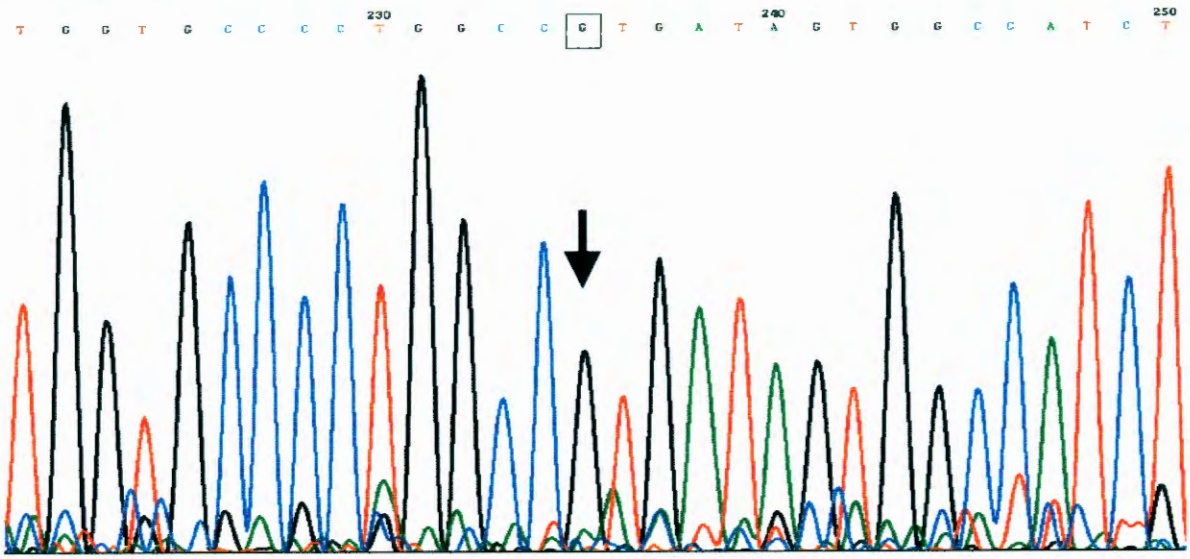


Figure 20. *Nucleotide sequence of a Southern African Coloured subject depicting the genetic polymorphism C100T in exon 1 of the CYP2D6 gene. Arrows and boxes indicate the substituted nucleotides. Sequencing was performed on the ds CYP2D6 gene.*

- ***A.*** *wild type sequence of a Coloured subject showing the C in position 100 of exon 1.*
- ***B.*** *Heterozygote sequence of subject CYP2D6*4/*10, with a Y in position 100.*

A. wt (95C)



B. variant (94C)

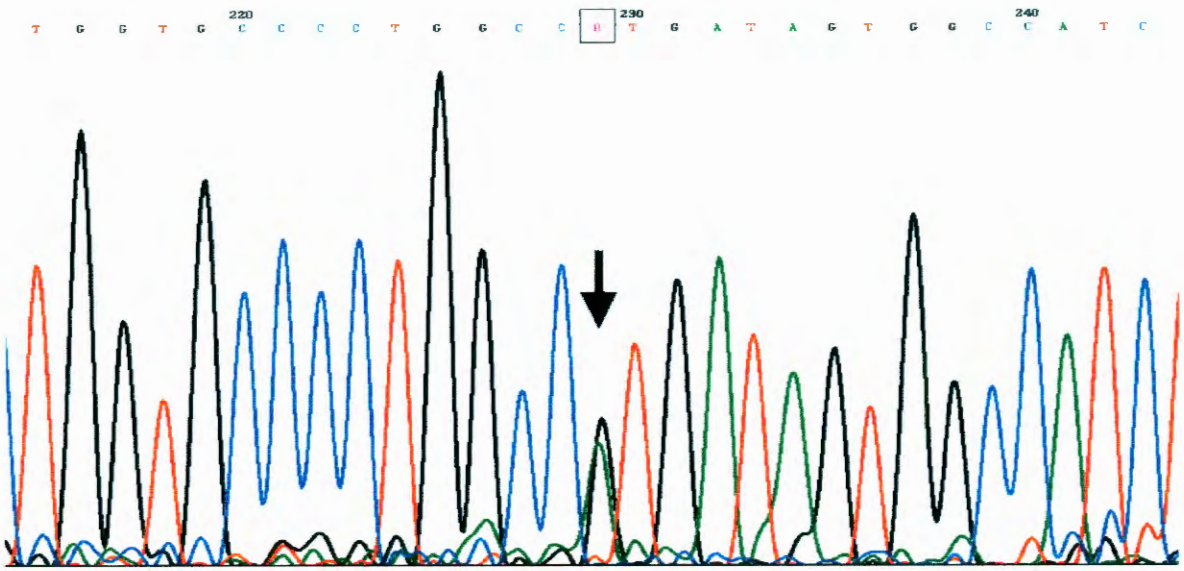
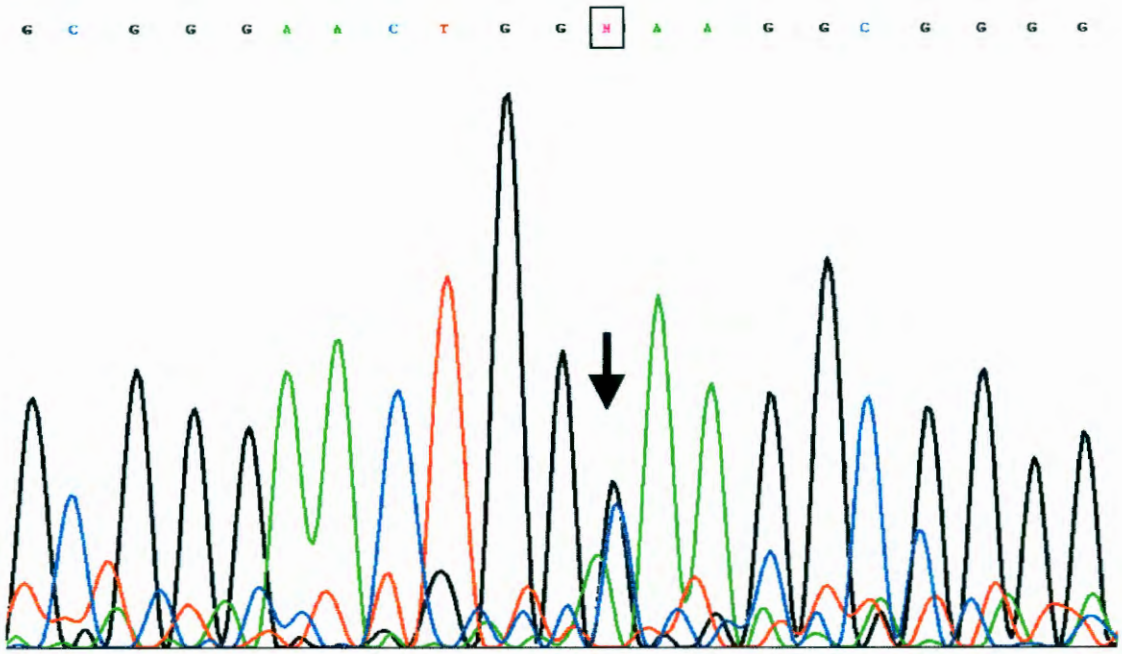


Figure 21. *Nucleotide sequence of part of exon 1 of the CYP2D6 gene of a Southern African Coloured subject depicting the SNP (G31A), shown as R. R depicts the equal strength for either a G (wt) or A (*35x2) at position 31.*

A. wild type sequence of a Coloured subject showing the G at position 31, exon 1

B. Heterozygote sequence of Coloured subject (CYP2D6*4/*10) with a R at position 31, exon 1.

A.



B.

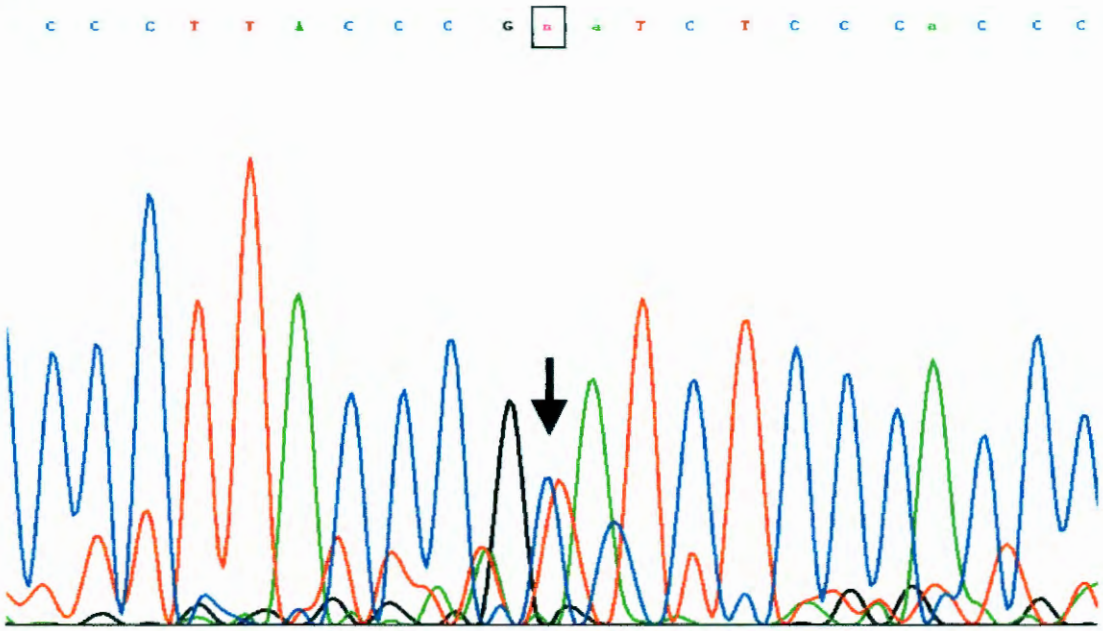


Figure 22. *Sequence from subject 95C depicting possible polymorphisms. See Intron 3-4, Figure 19C. This is an example of but a few possible polymorphisms observed with sequencing in this subject, but not genotyped for.*

- ***A.*** *N represents a possible C or G at position 1778 (IUB code = S)*
- ***B.*** *N represents a possible C or T at position 1813, (IUB code = Y)*

3.4. Conclusions

This study was a first attempt at characterising a part of the South African population for polymorphisms of the CYP2D6 gene. The population was chosen by convenience, by being the most prevalent groups available in this part of the country. In broad terms our results confirm the current understanding of the genetic origin of the different ethnic groups making up the South African population. The fact that the sequence results confirmed those obtained by allele-specific PCR is a vote of confidence for the methods chosen for doing this study.

The unknown polymorphisms found in the sequenced samples should be further investigated to firstly establish that they are real by sequencing the reverse strands of the relevant exons. Then one can design experiments to elucidate their effect on enzyme activity. This can be done by sequencing more samples, by phenotyping subjects showing these polymorphisms and/or by expressing these variant genes in recombinant systems.

We are confident that our results can be extrapolated to the vast majority of the South African population, except for a number of small minority groups, eg the local Indian population. The fact that both the Black and Coloured groups showed significant percentages of predicted intermediate metabolisers, is cause for further research on these groups to find out what the actual distribution of enzyme activity is in these subjects. Only then will it be possible to establish the real clinical significance of their intermediate metaboliser status. If it turns out that a significant proportion of the IMs is closer to PMs than EMs, genotyping of patients destined for drug therapy where CYP2D6 is involved, becomes imperative.

When genotyping is offered, it is only fair to include all patients, after all, the genetic history of no one can be established conclusively just by their self-defined ethnic group. By using the following protocol, the vast majority of poor/intermediate metabolisers in the broad South African population can be identified:

1. Test for *5 by XL PCR
2. Use the resultant fragment(s) to test for *4, *10 and *17

Using this approach rather than offering all-out testing for all patients seems to be the ethical and cost-effective option.

Chapter 4

Summary

Debrisoquine 4-hydroxylase, also known as CYP2D6, is a cytochrome P450 enzyme responsible for the metabolism of many commonly used drugs such as antispychotics, beta-blockers, opiates and tricyclic antidepressants. Polymorphic expression of CYP2D6 is observed in the interethnic and inter-individual variability in patients undergoing treatment with these drugs. Two distinct phenotypes have been described for this enzyme in Caucasian populations, namely extensive and poor metabolisers. The poor metaboliser phenotype behaves as an autosomal recessive trait occurring in about 7% of Caucasians. Major discordance has been observed when comparing Caucasian data with data from other ethnic groups. Accurate prediction of a patient's genotype could be important for many clinically used drugs as poor metabolisers are at a high risk for adverse drug reactions, onset of toxicity and even therapeutic failure. Early or preventative therapy guided by genotyping could significantly enhance the clinical outcome for these patients.

In this study we determined the frequency of CYP2D6 polymorphisms in three Southern African populations, namely Caucasian (control), Black and Coloureds. Our results for the Caucasian group largely confirm the findings of other studies in the sense that 96 % of subjects are extensive metabolisers (normal). However, based on their genotypes, only 67 % of Coloured and 69 % of Black subjects are predicted to be extensive metabolisers. A high prevalence of intermediate (IM) metabolisers was observed for both the Black (24 %) and Coloured (26 %) groups. CYP2D6*17 occurred at a high frequency in the Black (0.2324) and Coloured (0.1301) goups, while CYP2D6*10 also occurred at a high frequency in the Coloured (0.1301) group. Both these groups show reduced-activity alleles unique to them, not found in the Caucasian subjects. If confirmed by phenotyping subjects on a relevant treatment, these findings may have implications for treatment of patients with drugs metabolised by CYP2D6.

Keywords: pharmacogenetics, ethnicity, genotyping, sequencing, drug metabolising enzymes

Chapter 5

Opsomming

Debrisoquine 4-hidroksilase, ook bekend as CYP2D6, is 'n sitochroom P450 ensiem, verantwoordelik vir die metabolisme van baie algemeen gebruikte middels soos anti-psigotika, beta-blokkers, opiate en trisikliese antidepressante. Die polimorfiese uitdrukking van CYP2D6 word in pasiënte tydens behandeling waargeneem as interetniese en interindividuele variasie. Twee definitiewe fenotipes is vir hierdie ensiem beskryf in blanke populasies, nl. normale en swak metaboliseerders. Die swak metaboliseerder fenotipe is 'n autosomale resessiewe eienskap wat in omtrent 7 % blankes voorkom. Groot teenstrydighede is waargeneem wanneer blanke data met ander etniese groepe vergelyk word. Akkurate voorspelling van 'n pasiënt se genotipe kan baie belangrik wees vir 'n wye reeks geneesmiddels omdat swak metaboliseerders 'n hoë risiko vir nadelige geneesmiddel reaksies, ontwikkeling van toksisiteit en selfs die mislukking van terapie het. Die ondersteuning van vroeë of voorkomende terapie deur genotipering kan die kliniese uitkoms van hierdie pasiënte baie verhoog.

In hierdie studie het ons die frekwensie van die CYP2D6 polimorfismes in drie Suid Afrikaanse populasies, naamlik Blank, Swart en Kleurling, bepaal. Die resultate vergelyk baie goed met die bevindings vir ander Blanke bevolkingsgroepe en 96 % van proefpersone het normaal getoets. Net 67 % Kleurlinge en 69 % Swartes is as normaal geklassifiseer na aanleiding van hul genotipes. Die voorkoms van intermediêre metaboliseerders was baie hoog vir beide die Swart (24 %) en Kleurling (26 %) groepe. Ons het ook bevind dat CYP2D6*17 in 'n baie hoë frekwensie in die Swartes (0.2324) en Kleurlinge (0.1301) voor kom, terwyl CYP2D6*10 ook in Kleurlinge (0.1301) in 'n hoë frekwensie voorkom. In beide hierdie groepe is allele met verlaagde aktiwiteit gekry wat nie onder die Blankes voor kom nie. Bevestiging van hierdie resultate met fenotipering kan groot implikasies hê vir pasiente op behandeling met geneesmiddels wat deur CYP2D6 gemetaboliseer word.

Chapter 6

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