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Review

An overview on the allelic variant of CYP2D6 genotype

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The paper gives an overview on the allelic variant of CYP2D6 genotype. The gene CYP2D6*3 encodes a member of the cytochrome P_{450} super family of enzymes. The cytochrome P_{450} proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. The protein localizes to the endoplasmic reticulum and is known to metabolize as many as 20% of commonly prescribed drugs. Its substrates include debrisoquine, an adrenergic-blocking drug; sparteine and propafenone, both anti-arrythmic drugs; and amitryptiline, an anti-depressant. The emerging application of pharmacogenomics in the clinical trials requires careful comparison with the traditional genotypic methodologies particularly in the drug metabolism area.

Key words: CYP2D6 gene, PCR, CYP2D6*3, allelic variants.

INTRODUCTION

The *Homo sapiens* genome is stored on the 23 chromosome pairs in which twenty-two of these are autosomal chromosome pairs, while the remaining pairs are sex-determining. The haploid human genome occupies a total of just over 3 billion DNA base pairs and has a data size of approximately 750 megabytes. The Human Genome Project produced a reference sequence of the euchromatic human genome, which is used worldwide in biomedical sciences and to study the significance of genetic polymorphisms in human population. It can be defined as the occurrence together in the same population of more than one allele or genetic marker at the same locus with the least frequent allele or marker occurring more frequently than can be accounted for by mutation alone. The gene CYP2D6 is highly polymorphic in the population and certain alleles result in the poor metabolizer phenotype, characterized by a decreased ability to metabolize the enzyme's substrates. Polymorphisms in these genes will lead to inter individual variations with different patterns of susceptibility to the effects of carcinogenic substances and drugs and to detect the allelic variants of CYP2D6*3 genotype through polymerase chain reaction (PCR) (Steen et al*.,* 1995; Johansson et al*.,* 1996). One limitation, however, is the unaffordability of some of the current diagnostic methods. PCR is an affordable molecular diagnostic technology and is already in use in low income regions. The development of a strategy for *CYP2D6* genotyping covering the most commonly described alleles could therefore promote the use of these technologies in the developing world. The gene is located near two cytochrome P450 pseudogenes on the chromosome 22 q13.1. Cytochrome P450 monooxygenases, often referred simply to as CYP enzymes, catalyze oxygen insertion into many different kinds of substrates, including natural steroids, fatty acids and foreign compounds, in a step of their metabolism. The enzyme for drug metabolism, toxification as well as detoxification of xenobiotic (non-biological) compounds is prevalent in the environment (Nebert, 1997; Kohler et al*.,* 1997). It is expressed in both the gastrointestinal tract and hepatic microsomes, and the activity may vary between 6- and 30-fold among individuals due to genetic and non genetic factors such as drug therapy (Griese et al*.,* 1998; McElroy et al*.,* 2000; Nei et al., 2001). The general use of CYP2D6 genotyping may be of help to increase the use of drug therapy and hence, of global health (Daar et

Abbreviations: PCR, Polymerase chain reaction; **RFLP,** restriction fragment length polymorphism; **SNPs,** single nucleotide polymorphisms; **BBB,** blood brain barrier; **EM,** extensive metabolism; **PM,** poor metabolism.

al., 2002; Daar et al., 2005).

INTRODUCTION TO CYP2D6 GENE

The CYP2D6 gene encodes a member of the cytochrome P450 super family of enzymes. The gene is highly polymorphic in the population. Cytochrome P450 2D6 (CYP2D6) is of great importance for frequently identified alleles in a given clinical situation, independent variation in drug efficacy and toxicity. It has been recognized for many decades that individual differences in response to pharmacologic treatment, exhibited as drug toxicity or a lack of therapeutic effect, are often caused by genetic differences that result in altered rates of biotransformation (metabolism). Notable examples include nerve damage among individuals homozygous for some variants of the N-acetyltransferase 2 gene ("slow acetylators") given isoniazid as an antituberculosis therapy, haemolytic anaemia among glucose 6-phosphate dehydrogenase-deficient patients given aminoquinoline antimalarial drugs, and varied rates of biotransformation of debrisoquine, an antihypertensive drug, due to genetic variation at the CYP2D6 locus. The CYP2D6 is of great importance for the metabolism of clinically used drug (Lerena et al., 1996; Weber and Myers, 1997; Dahl., 2002; Berecez et al., 2002; Lerena et al., 2002 and Berecez et al., 2003). Still certain alleles result in the poor metabolizer phenotype, characterized by a decreased ability to metabolize the enzyme's substrates. The detection of allelic variant of CYP2D6*3 genotype could be done through PCR-restriction fragment length polymorphism (RFLP) by the property of genetic marker at the same locus with the least frequent allele or marker occurring more frequently than can be accounted for by mutation alone. With the initial completion of the first draft of the human genome sequence (Lander et al., 2001; Venter et al., 2001), interest has dramatically increased in the role of genetics as a determinant of health. In particular, the *CYP2D6* allele frequency varies between populations and geographical areas (Bradford, 2002). Progress in incorporating genetics into public health research has been steady over the last several years, relying mainly on the tools of genetic and molecular epidemiology. The recent abundance of epidemiologic research examining associations between polymorphic genes that code for enzymes involved in xenobiotic biotransformation and disease occasionally generated has interesting findings. Much of the impetus for this area of research has come from pharmacogenetics, which is concerned primarily with the study of genetics (Molden et al., 2002)

THE PROCESS OF BIOTRANSFORMATION

The enzymatic alteration of foreign or xenobiotic compounds is conventionally divided into two phases. Phase I enzymes modify existing functional groups (e.g., -OH, -SH) to xenobiotics and are catalyzed primarily by the cytochrome P450 enzymes (CYPs). These interme-diates are then conjugated with endogenous ligands during phase II, increasing the hydrophilic nature of the compound, facilitating excretion (Raunio et al., 1995). Table 1 shows the reactions and enzymes involved in metabolism of xenobiotics. These transporters facilitate the excretion of xenobiotics into bile or blood, and thus form what has been called phase III biotransformation.

THE ROLE OF SINGLE NUCLEOTIDE POLYMORPHISM

About 90% of all DNA sequence variations occur as single nucleotide polymorphisms (SNPs)--that is, single-base-pair substitution. SNPs represent a natural genetic variability at high density in the human genome. SNPs are considered to be the major genetic source to phenotypic variability that differentiates individuals within a given species. The frequency, stability, and relatively even distribution of SNPs in the genome make them particularly valuable as genetic markers. Where particular, SNP variants are close to a susceptibility gene allele, they tend to be inherited together over many generations. SNPs may occur in non-coding regions (SNPs) as well as in coding regions (cSNPs). There are estimated to be three or four SNPs in the average gene and roughly 120,000 common coding-region SNPs, of which approximately 40% are expected to be functional (Cargill et al., 1999). Functional polymorphism in XMEs is Point mutations in coding regions of genes resulting in amino acid substitutions, which may alter catalytic activity, enzyme stability, and/or substrate specificity. One of the most extensively studied genetic polymorphisms known to influence drug metabolism and response is the debrisoquine type (CYP2D6) oxidation polymorphism. CYP2D6 is the only one functional gene in the CYP2D subfamily, present in human genome (Nelson et al., 1996). The discovery of CYP2D6 polymorphism created new interest in the role of pharmacogenetics in clinical pharmacology. More than 60 allelic variants have been reported for CYP2D6 (Streetman et al., 2000). Of these, CYP2D6 mostly affects the activity of the expressed protein. CYP2D6 represents 1-5% of the total P450 (Pelkonen and Breimer, 1994; Shimada et al., 1994; Pelkonen et al., 1998), and 7-8 % of the Caucasian population are polymorphic (PM) for this enzyme (Heim and Meyer, 1992).

OVERVIEW ON CYP

Cytochrome P450 is a very large and diverse superfamily of hemoproteins found in all domains of life. Cytochrome P450 uses a plethora of both exogenous and endogenous

Table 1. Reactions and enzymes involved in metabolism of xenobiotics.

Reaction	Enzymes
Phase 1	
Oxidation	Cytochrome P450, peroxidases, flavin
Epoxidation	Monooxygenases, monoamine oxidases, alcohol dehydrogenases, aldehyde dehydrogenases
Cytochrome P450	Cytochrome P450, NADPH-cytochrome P450,
Reduction	Reductases, quinone, reductases
Dealkylation	Cytochrome P450
Hydrolysis	Carboxyl esterases, amidases
Dehalogenation	Cytochrome P450
Phase 2	
Conjugation with glucuronic acid	UDP-glucuronyl transferase
Conjugation with sulphates	Sulphotransferases
Conjugation with glutathione acetylation	Glutathione S-transferases, N-acetyl transferases
Conjugation with amino acids	Transferases
Methylation	Methyl transferases
Hydralation	Epoxide hydrolase

(Source http:// www. Wikipedia.org).

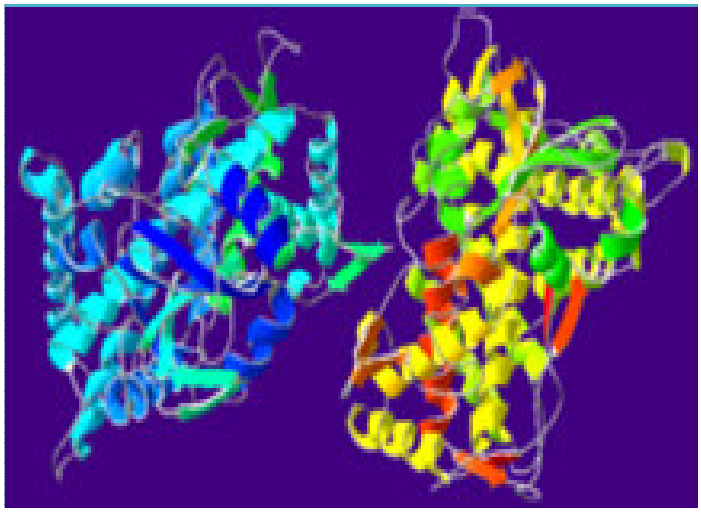

Figure 1. 3D structure of CYP.

compounds as substrates in enzymatic reactions. Usually, they form part of multicomponent electron transfer chains, called P450-containing systems. The most common reaction catalysed by cytochrome P450 is a monooxygenase reaction. CYP enzymes have been identified from all lineages of life, including mammals, birds, fish, insects, worms, sea squirts, sea urchins, plants, fungi, slime molds, bacteria and archaea. More than 7700 distinct CYP sequences are known. The name cytochrome P450 is derived from the fact that these are colored ('chrome') cellular ('cyto') proteins, with a "pigment at 450 nm", so named for the characteristic. Soret peak is formed by absorbance of light at wavelengths near 450 nm when the heme iron is reduced (often with sodium dithionite) and complexed to carbon monoxide. The active site of cytochrome P450 contains a heme iron center. The iron is tethered to the P450 protein via a thiolate ligand derived from a cysteine residue. This cysteine and several flanking residues (RXCXG) are highly conserved in known CYPs because of the vast variety of reactions catalyzed by CYPs; the activities and properties of the many CYPs differ in many aspects and its 3D structure is shown in Figure 1.

MECHANISM OF CYP450 ENZYME COMPLEX

The P450 catalytic cycle shows the steps involved when a substrate binds to the enzyme in the normal state of a P450 with the iron in its ferric [Fe^{3+}] state. When the substrate binds to the enzyme, the enzyme is reduced to the ferrous [Fe^{2+}] state by the addition of an electron from NADPH cytochrome P450 reductase. The bound substrate facilitates this process. Molecular oxygen binds and forms a $Fe^{2+}OOH$ complex with the addition of a proton and a second donation of an electron from either NADPH cytochrome P450 reductase or cytochrome b5. A second proton cleaves the $Fe^{2+}OOH$ complex to form water. An unstable $[FeO]^{3+}$ complex donates its oxygen to the substrate. The oxidised substrate is released and the enzyme returns to its initial state. The active site of cytochrome P450 contains a heme iron center. The iron is tethered to the P450 protein via a thiolate ligand derived from acysteine residue. This cysteine and several flanking residues are highly conserved in known CYPs enzyme complex (Daly et al., 1996).

Table 2. Characteristics of some of the most important xenobiotic metabolism enzymes in relation to genetic polymorphism.

Enzyme	Chromosome localization	Polymorphism	Function of polymorphic allele	Relation of polymorphism to the disease
CYP1A1	15q22-qter	Yes	Various inducibility	Bronchogenic carcinomas, tumors of the breast, mouth and pharynx.
CYP1A2	15q22-qter	Yes	Three various phenotypes	Tumors of liver, colon and urinary bladder.
CYP1B1	2p22-p21	Yes	Enzyme deficiency	Congenital glaucoma.
CYP2A6	19q13.1-13.2	Yes	Defective enzyme	Tumors of nasopharynx, effect on the metabolism of medicaments.
CYP2D6	22q13.1	Yes	Various phenotypes	Parkinson syndrome, tumors of the lungs, pharynx

THE ROLE OF CYP ENZYME COMPLEX

Cytochrome P450 mono-oxygenases (hydroxylases), often referred simply to as CYP enzymes, catalyze oxygen insertion into many different kinds of substrates, including natural steroids, fatty acids and foreign compounds, in a step of their metabolism. They serve as the enzymes for drug metabolism, toxification as well as detoxification of xenobiotic (non-biological) compounds prevalent in the environment. Several classes of xenobiotic compounds thus elicit characteristic biological effects *in vivo* due often to intermediate metabolites. These enzymes are expressed in both the gastro-intestinal tract and hepatic microsomes, and the activity may vary between 6- and 30-fold among individuals due to genetic and non genetic factors such as drug therapy (Pelkonen et al., 1994). Humans have 57 genes and more than 59 pseudogenes are divided among 18 families of cytochrome P450 genes and 43 subfamilies (Daly et al*.,* 1996).

SIGNIFICANCE OF CYTOCHROME P450 IN HUMANS

Human CYPs are primarily membrane-associated proteins, located either in the inner membrane of mitochondria or in the endoplasmic reticulum of cells. CYPs metabolise thousands of endogenous and exogenous compounds. Most CYPs can metabolize multiple substrates, and many can catalyze multiple reactions, which accounts for their central importance in metabolizing the extremely large number of endogenous and exogenous molecules. In the liver, these substrates include drugs and toxic compounds as well as metabolic products such as bilirubin (a breakdown product of hemoglobin). Cytochrome P450 enzymes are present in many other tissues of the body including the mucosa of the gastrointestinal tract, and play important roles in hormone synthesis and breakdown cholesterol synthesis, and vitamin D metabolism. Hepatic cytochromes P450 are the most widely studied of the P450 enzymes and the characteristics in relation to genetic polymorphism are shown in Table 2. Cytochrome P450s are also present in the blood brain barrier (BBB) in concentrations similar to those found in the liver. They strengthen the division between the blood and brain tissue by metabolising toxic compounds which may somehow cross the BBB. The Human Genome Project has identified more than 63 human genes (57 full genes and 5 pseudogenes) coding for the various cytochrome P450 enzymes (Heim and Meyer, 1992).

VARIOUS ASPECTS OF CYP2D6

Cytochrome P450 2D6 (CYP2D6), a member of the cytochrome P450 mixed-function oxidase system, is one of the most important enzymes involved in the metabolism of xenobiotics in the body. Its 3D structure is shown in Figure 2. Whilst CYP2D6 is involved in the oxidation of a wide range of substrates of all the CYPs, there is considerable variability in its expression in the liver. The gene is located near two cytochrome P450 pseudogenes on chromosome 22q13.1. Alternatively, spliced transcript variants encoding different isoforms have been found for this gene.

CYP2D6 shows the largest phenotypical variability amongst the CYPs, largely due to genetic polymorphism. The genotype accounts for the normal, reduced and non-existent CYP2D6 function in subjects. More than 50 human CYP Isoenzymes have been identified (Mc Leod and Danielson, 2002). The genetic basis for extensive and poor metaboliser variability is the CYP2D6 allele, located on chromosome 22 (Eichelbaum and Gross, 1987). Subjects who possess certain allelic variants will show normal, decreased or no CYP2D6 function depending on the allele. In CYP2D6, genetic polymorphism has been linked to three classes of phenotypes based on the extent of drug metabolism. Extensive metabolism (EM) of a drug is characteristic of the normal population; poor metabolism (PM) is associated with accumulation of specific drug substrates and is typically an autosomal recessive trait requiring mutation and/or deletion of both alleles for phenotypic expression; and ultra extensive metabolism (UEM) results in increased drug metabolism and is an autosomal dominant trait arising from gene

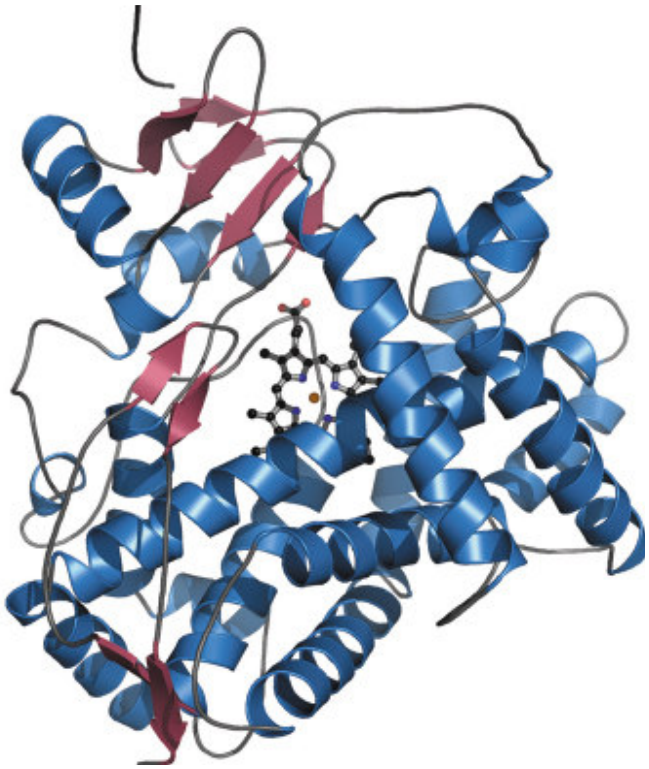

Figure 2. 3D Structure of CYP2D6.

amplification (Yachna, 2009).

Dextromethorphan (the cough suppressant part of Dimetapp DM) can be used as a "probe" to assess whether an individual is an extensive vs. normal vs. poor metabolizer with regards to CYP2D6. Notably, some drugs (e.g. quinidine) can change an extensive metabolizer into a poor metabolizer of dextromethorphan. CYP2D6 are bimodally distributed in the population allowing classification of individuals as either extensive (EMs) or poor (PMs) metabolizers. A patient's CYP2D6 phenotype is often clinically determined via the admini-stration of debrisoquine (a selective CYP2D6 substrate) and subse-quent plasma concentration assay of the debrisoquine metabolite (4-hydroxydebrisoquine).

Debrisoquine is a drug used for treating hypertension, and a wide variation has been observed in the hypoten-sive response. The P450-debrisoquine hydroxylase polymorphism in humans is one of the best-studied enzyme deficiencies, and it has been variously linked with different types of cancer, including those of the lung, bladder and breast. CYP2D6 converts debrisoquine (active) into 4-hydroxydebrisoquine (inactive). This polymorphism is characterized by the inability of certain individuals to metabolize specific drugs (e.g. debriso-quine, sparteine, and bufuralol); affected individuals, termed poor metabolizers (PMs), are known to be deficient in the minor liver enzyme P450-CYP2D6 which is responsible for the metabolism of these compounds. The PM phenotype is inherited in an autosomal recessive manner in 5-10% of the Caucasian population and is now associated with the inefficient metabolism of over 30 drugs with a wide range of clinical indications (Benny et al., 2001).

CYP2D6 AND CANCER SUSCEPTIBILITY

An association was observed between CYP2D6 gene and lung cancer (Caporaso et al., 1990) and oral cancer (Worrall et al., 1998). Bouchardy et al. (1996) evaluated CYP2D6 activity, determined by *in vivo* metabolic rate of dextromethorphan, in 128 cases of lung cancer and 157 controls. Increased CYP2D6 activity (EM phenotype) has been related to some malignant processes, like bladder cancer (Anwar et al., 1996). The data suggested that the increased metabolism of one or more agents in the diet or other environmental agents, mediated by CYP2D6, forms reactive intermediaries that influence the initiation or promotion of cancer in various tissues (Nebert, 1997). Meanwhile, reduced CYP2D6 activity (PM phenotype) has been related to greater risk of Parkinson's disease (Smith et al., 1992), leukemia and oral cancer (Worrall et al., 1998). The distinction was not clear between the EM and PM phenotypes and susceptibility to cancer. This could be explained by the fact that PM individuals, much less exposed than EMs to the metabolites of carcinogenic-genotoxic drugs, must be exposed longer to the toxic effects of non-metabolized drugs and numerous other factors still not identified. It was known that the toxic effects can contribute to carcinogenesis, for example, through a necrogenic response followed by compen-satory increased cell division (Butterworth et al., 1992). It has been found that polymorphism at loci that encode carcinogen-metabolizing enzyme such as cytochrome P450 catalyzing the detoxification of carcinogens may determine susceptibility to cervical cancer. Some studies showed no-association between CYP2D6 and cancer risks. London et al. (1997) found that the CYP2D6 genetic polymorphism is not the strong risk factor for lung cancer. Smith et al. (1992) and Goetz et al. (2007) stated that CYP2D6 genotype is not the causal factor for breast cancer.

DRUG METABOLISM IN CYP2D6

The basic purpose of drug metabolism in the body is to make drugs more water soluble and thus more readily excreted in the urine or bile. One common way of metabolizing drugs involves the alteration of functional groups on the parent molecule (e.g., oxidation) that is, the cytochrome P450 enzymes e.g., CYP2D6. Drug interactions involving the cytochrome P450 isoforms generally result from one of two processes, enzyme inhibition or enzyme induction. Enzyme inhibition usually involves competition with another drug for the enzyme binding site. This process usually begins with the first

dose of the inhibitor, and onset and offset of inhibition correlate with the half-lives of the drugs involved. Enzyme induction occurs when a drug stimulates the synthesis of more enzyme protein, enhancing the enzyme's metabolizing capacity (Shimada et al., 1994; Douglas et al., 1994).

CYP2D6 AND DRUG INTERACTIONS

Due to the high polymorphic character of CYP2D6, this enzyme is also the site of a number of drug interactions *in vivo*, which are of clinical significance. Substrates with a high affinity for the enzyme bind strongly to it and inhibit the metabolism of other compounds which have lower affinity. Consequently, drug interactions occur in extensive as well as poor metabolisers. By using this knowledge, pharmacokinetic interactions can be anticipated as follows: 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. This type of knowledge is also being used to decide which drugs to develop, because the inhibition of P450 enzyme is generally not the goal of treatment. The interaction of two substrates for CYP2D6 can result in a number of clinical responses. The first pass metabolism of the substrate may be inhibited or the rate of elimination may be prolonged such that higher plasma concentration and associated pharmaco-dynamic responses may occur. Inhibition of metabolism by CYP2D6 can also lead to a lack of therapeutic response when the pharmacological action is dependent on the active metabolite. Since CYP2D6 is not inducible by enzyme inducing drugs, drug interactions due to enzyme induction are very unlikely to occur (Broly et al., 1995; Johansson et al., 1996).

IMPLICATION FOR DRUG DEVELOPMENT

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

However, with respect to oxidation phenotype, the subjects may not be representative of the general population. If it were possible to predict that the metabolism of a drug co-segregates with a known polymorphism at the preclinical stage, the decision on whether or not to pursue development of the drug would be facilitated. Several *in vitro* approaches have been developed which allow a prediction to be made during preclinical testing if the metabolism of a new drug is subject to genetic polymorphism. Inhibitory monoclonal antibodies are available which determine cytochrome P450 substrate and product specificity. It is obviously also prudent to exclude potentially susceptible individuals from phase-1 dose escalation trials. This can prevent PM healthy subjects or patients being exposed to additional risk of toxicity during phase-1 and 2 developments. There is currently great interest in the pharmaceutical industries in pharmacogenetics and an increasing number of companies are genotyping their clinical trial populations. Moreover, the knowledge of genetic variability in drug response is becoming an increasingly important component of the drug registration process (Shimada et al., 1994; Daar and Singer, 2005).

REFERENCES

Anwar WA, Abdel Rahman SZ, EL ZeinRA, Mostafa HM (1996). Genetic polymorphism of GSTM1, CYP2E1 and CYP2D6 in Egyptian bladder cancer patients. *Carcinogenesis*, 17: 1923-1929.

Benny K, Abraham C, Adithan (2001). Genetic polymorphism of CYP2D6. Indian J. Pharmacol. 33: 147-169.

Berecez R, LLerena A, Rubia A, GomezJ, Kellermann M, Dorado P, Degrell I (2002). Relationship between risperidone and 9-hydroxy-risperidone plasma concentrations and CYP2D6 enzyme activity in psychiatric patients. Pharmacopsychiatry, 35: 231-234.

Berecez R, Rubia A, Dorado P, Fernandez-Salguero P, Dahl ML, LLerena A (2003). Thioridazine steady-state plasma concentrations are influenced by tobacco smoking and CYP2D6, but not by the *CYP2C9* genotype. Eur. J. Clin. Pharmacol. 59: 45-50.

Bouchardy C, Benhamou S, Dayer P (1996). The effect of tobacco on lung cancer risk depends on CYP2D6 activity. Cancer Res. 56: 251-253.

Bradford LD (2002). *CYP2D6* allele frequency in European Caucasians, Asians, Africans and their descendants. Pharmacogenomics, 3: 229-243.

Broly F, Marez D, Sabbagh N, Legrand M, Millecamps S, Guidice JMLO, Boone P, Meyer UA (1995). An efficient strategy for detection of known and new mutations of the *CYP2D6* gene using single strand conformation polymorphism analysis. Pharmacogenetics, 5: 373-384.

Butterworth BE, Popp JA, Conolly RB, Goldsworthy TL (1992). Chemically induced cell proliferation in carcinogenesis. In: Mechanisms of Carcinogenesis in Risk Identification. IARC Scientific Publication, 116: 279-305.

Caporaso N, DeBaun MR, Rothman N (1990). Lung cancer and CYP2D6 (the debrisoquine polymorphism): sources of heterogeneity in the proposed association. Pharmacogenetics, 5: 129-134.

Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K (1999). Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat. Genet. 22: 231-238.

Daar AS, Singer PA (2005). Pharmacogenetics and geographical ancestry: implications for drug development and global health. Nat. Rev. Genet. 6: 241-246.

Daar AS, Thorsteinsdottir H, Martin DK, Smith AC, Nast S, Singer PA (2002). Top ten biotechnologies for improving health in developing countries. Nat. Genet. 32: 229-232.

Dahl ML (2002). Cytochrome p450 phenotyping/genotyping in patients receiving antipsychotics: useful aid to prescribing? Clin. *Pharmacokinet*. 41: 453-470.

Daly AK, Brockmoller J, Broly F (1996). Nomenclature for human *CYP2D6* alleles. Pharmacogenetics, 6: 193-201.

Douglas AM, Atchison BA, Somogyi AA, Drummer OH (1994). Interpretation of a simple PCR analysis of *CYP2D6(A)* and *CYP2D6(B)* null alleles associated with the debrisoquine/sparteine genetic polymorphism. Pharmacogenetics, 4:154-158.

Eichelbaum M, Gross AS (1987). The genetic polymorphism of debrisoquine/sparteine metabolism-clinical aspects. Pharmacol. Ther.

46: 377-94.
Goetz MP, Kamal A, Ames MM (2007). Tamoxifen Pharmacogenomics: The Role of CYP2D6 as a Predictor of Drug Response. Pharmacogenetics, 83: 89-93.
Griese EU, Zanger UM, Brudermanns U (1998). Assessment of the predictive power of genotypes for the catalytic function of CYP2D6 in a German population. Pharmacogenetics, 8: 15-26.
Heim MH, Meyer UA (1992). Evolution of a highly polymorphic human cytochrome P450 gene cluster: CYP2D6. Genomics. 14: 49-58.
Johansson I, Lundqvist E, Dahl ML, Ingelman-Sundberg M (1996). PCR-based genotyping for duplicated and deleted *CYP2D6* genes. Pharmacogenetics, 6: 351-355.
Kohler D, Hartter S, Fuchs K, Sieghart W, Hiemke C (1997). *CYP2D6*genotype and phenotyping by determination of dextromethorphan and metabolites in serum of healthy controls and of patients under psychotropic medication.Pharmacogenetics, 7: 453-461.
Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC (2001). Initial sequencing and analysis of the human genome. Nature. 409: 860-921.
Llerena A, Dorado P, Berecz R, Gonzalez AP, Penas-LLedo EM (2002). Effect of *CYP2D6* and *CYP2C9* genotypes on fluoxetine and norfluoxetine plasma concentrations during steady-state conditions. Eur. J. Clin. Pharmacol. 59: 869-873.
LLerena A, Dorado P, Berecz R, Gonzalez AP, Penas-LLedo EM (2002). Effect of *CYP2D6* and *CYP2C9* genotypes on fluoxetine and norfluoxetine plasma concentrations during steady-state conditions. Eur. J. Clin. Pharmacol. 59: 869-873.
London SJ, Daly AK, Cooper J, Navidi WC, Carpenter CL, Idle JR (1997). Polymorphism of glutathione *S*-transferase M1 and lung cancer risk among African-Americans and Caucasians in Los Angeles County, California. J. Natl. Cancer Inst. 87: 1246-1253.
McElroy S, Richmond J, Lira M, Friedman D, Silber BM, Milos PM (2000). *AAPS* Pharmsci. 2: 1-11.
Molden E, Johansen GH, Bergan BS, Christensen H, Rugstad HE, Rootwelt H, Reubsaet L (2002). Pharmacokinetics of diltiazem and its metabolites in relation to *CYP2D6* genotype. Clin. Pharmacol. Ther. 72: 333-342.
Nebert DW (1997). Polymorphisms in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? Am. J. Hum. Genet. 60: 265-271.
Nei M, Xu P, Glazko G (2001). Estimation of divergence times from multiprotein sequences for a few mammalian species and several distantly related organisms. Proc. Natl. Acad. Sci. 98: 2497-502.
Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC, Nebert DW (1996). P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics, 6: 1-42.
Pelkonen O, Breimer DD (1994). Role of environmental factors in the pharmacokinetics of drugs: Considerations with respect to animal models, P-450 enzymes, and probe drugs. Handbook of Exp. Pharmacol. 110: 289-332.
Pelkonen O, Maenpaa J, Taavitsainen P, Rautio A, Raunio H (1998). Inhibition and induction of human cytochrome P450 (CYP) enzymes. Xenobiotica, 28: 1203-1253.
Raunio H, Pasanen M, Maenpaa J, Hakkola J, Pelkonen O (1995). Metabolism of xenobiotic compounds. In: Pacifici GM and Fracchia GN, (eds) Advances in drug metabolism in man. Office for the Official Publications of the European Communities, Luxembourg, pp. 234-287.
Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP (1994). Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J. Pharmacol. Exp. Ther. 270: 414-423.
Smith CA, Moss JE, Gough Alan C, Spurr Nigel K, Roland WC (1992). Molecular Genetic Analysis of the Cytochrome P450-Debrisoquine Hydroxylase Locus and Association with Cancer Susceptibility. Environ. Health Perspectives. 98: 107-112.
Steen VM, Andreassen OA, Daly AK, Tefre T, Borresen AL, Idle JR, Gulbrandsen AK (1995). Detection of the poor metabolizer-associated *CYP2D6*(D) gene deletion allele by long-PCR technology. Pharmacogenetics, 5: 215-223.
Streetman DS, Bertino JS, Natziger AN (2000). Phenotyping of drug-metabolizing enzymes in adults: a review of in vivo cytochrome P450 phenotyping probes. Pharmacogenetics. 10: 187-216.
Venter Craig J, Adams Mark D, Eugene W, Myers, Peter Li W (2001). The Sequence of the Human Genome *Science*, 291: 1304-1351.
Weber JL, Myers EW (1997). Selectivity in the inhibition of mammalian cytochromes P450 by chemical agents. Genome Res. 7: 401.
Worrall S, Corrigan M, High A (1998). Susceptibility and outcome in oral cancer: preliminary data showing an association with polymorphism in cytochrome P450 CYP2D6. Pharmacogenetics, 8: 433-439.