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Cytochrome P450 2C9, 2C19 and 2D6 genetic polymorphisms

Evaluation of genotyping as a tool for individualised treatment

by

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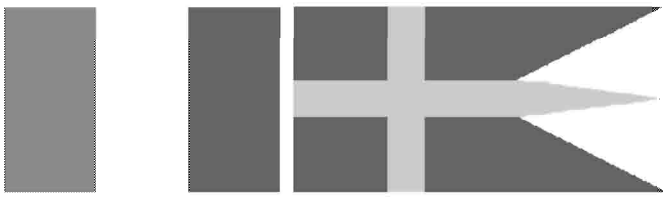

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"Ogni giorno è un giorno in più per amare,
un giorno in più per sognare,
un giorno in più per vivere."
Padre Pio da Pietralcina

To my family

Abstract

Recent advances in the understanding of drug metabolism and the molecular biology of CYPs have generated great expectations regarding the use of pharmacogenetic testing as a tool to individualize drug treatment. The individual pharmacogenetic constitution might in principle predict the response to drugs, hormones and toxins. Therefore, a simple phenotyping or genotyping test before treatment with a compound subject to polymorphic metabolism could be of value in selecting the dosage schedule, thus enhancing therapeutic efficacy and preventing toxicity. Many antipsychotics and antidepressants are mainly metabolised through CYP2D6. CYP2C9 and CYP2C19 are involved in the metabolism of warfarin, phenytoin and fluoxetine. All these three enzymes are highly polymorphic.

The general aims of this thesis were to elucidate the impact of polymorphic CYP isoenzymes on the metabolism of psychoactive drugs (risperidone, fluoxetine) and anticoagulants (warfarin), characterised by a narrow therapeutic range and the potential risk for drug-drug interactions, to investigate the role of CYP polymorphism as a risk factor for the appearance of psychoactive drug-induced side-effects, and to evaluate the potential usefulness of genotyping as a tool for individualising the therapy.

CYP2D6 genotype appears to partially explain the broad variability in the steady state plasma levels of risperidone and fluoxetine, but its usefulness in predicting clinical effects remains to be clarified. A significant relationship has been found in our studies between *CYP2C9* genotype and warfarin dose requirement and clearance, as well as between *CYP2C9* genotype and fluoxetine active moiety. Furthermore, our findings suggest that PMs of CYP2D6 are more prone to EPS during treatment with classical antipsychotics. Similarly, CYP2C9 and CYP2C19 genetically impaired metabolic activity seems to represent a predisposing factor for phenytoin toxicity.

CYP2D6 genotyping can today be recommended as a complement to the determination of plasma concentrations when aberrant metabolic capacity (poor or ultrarapid) is suspected, while its usefulness in predicting clinical effects must be further explored. Therapeutic drug monitoring still represents a better tool for individualization of dosage of CYP2D6 substrates. On the other hand *CYP2C9* genotype may be of value in choosing the right dose, and/or avoiding potentially harmful drugs in selected subgroups of patients with genetically impaired metabolic activity. The next step should involve prospective studies of impotant drugs to establish the clinical utility of genotype specific dosage-schedules.

Key words: drug metabolism, CYP2C9, CYP2C19, CYP2D6, genotyping, polymorphism, risperidone, warfarin, fluoxetine, phenytoin, extrapyramidal side-effects

Table of contents

List of original papers

This thesis is based on the following papers, referred to in the text by Roman numerals:

I. M.G. Scordo, E. Spina, G. Facciolà, A. Avenoso, I. Johansson, M.L. Dahl. CYP2D6 Genotype and steady-state plasma levels of risperidone and 9-hydroxyrisperidone. *Psychopharmacology* (1999); 147 (3): 300-305

II. M.G. Scordo, V. Pengo, E. Spina, M.L. Dahl, M. Gusella, R. Padrini. Influence of cytochrome P450s 2C9 and 2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. *Clinical Pharmacology and Therapeutics* (In press)

III. M.G. Scordo, E. Spina, M.L. Dahl, G. Gatti, E. Perucca. Influence of *CYP2C9, 2C19* and *2D6* genetic polymorphisms on plasma levels of the enantiomers of fluoxetine and norfluoxetine. *Manuscript*

IV. M.G. Scordo, E. Spina, P. Romeo, M.L. Dahl, L. Bertilsson, I. Johansson, F. Sjöqvist. *CYP2D6* genotype and antipsychotic-induced extrapyramidal side-effects in schizophrenic patients. *European Journal of Clinical Pharmacology* (2000); 56: 679-683

V. R. Brandolese, M.G. Scordo, E. Spina, M. Gusella, R. Padrini. Severe phenytoin intoxication in a homozygous CYP2C9*3 metabolizer. *Clinical Pharmacology and Therapeutics* (2001); 70: 391-394

Abbreviations:

ANOVA	analysis of variance
C/D	concentration normalized for dose
CI	confidence interval
CL	clearance
CV	coefficient of variation
CYP	cytochrome P450
DNA	deoxyribonucleic acid
EDTA	ethylendiaminetetraacetic acid
EM	extensive metaboliser
EPS	extrapyramidal side effects
HPLC	high-performance liquid chromatography
INR	international normalized ratio
MR	metabolic ratio
NS	non statistically significant
PM	poor metaboliser
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
SNP	single nucleotide polymorphism
SSRI	selective serotonin reuptake inhibitor
TDM	therapeutic drug monitoring
UM	ultrarapid metaboliser
WT	wild type

Glossary:

Allele	one of several alternative variants of a gene or DNA sequence at a specific chromosomal location (*locus*). Each individual possesses two alleles at each locus, one inherited from the father and one from the mother.
Enzyme	protein acting as a catalyst to induce chemical changes in other substances, itself remaining apparently unchanged by the process
Exon	portion of DNA that codes for messenger RNA and is therefore expressed, i.e. translated into protein
Gene	the functional unit of heredity that occupies a specific locus on a chromosome
Genotype	the genetic constitution of an individual, either overall or at one or more specific loci
Heterozygous	having two different alleles of the same gene
Homozygous	having two copies of the same gene
Intron	noncoding sequence of DNA that separates neighboring exons of a gene
Locus	part of a chromosome where genes are located
Metabolism	conversion to another chemical species
Mutation	permanent transmissible change in the genetic material
Pharmacogenetics	the study of genetically determined variations in response to drugs
Phenotype	observable character, produced by the sum of a genotype with a specific environment
Polymorphism	Mendelian traits existing in a population in at least two phenotypes, both of them present at a frequency >1%
Xenobiotics	chemical compounds foreign to a biological system, which can include naturally occurring compounds, drugs, environmental agents, carcinogens, etc.

1. Introduction

1.1 Drug metabolism

The clinical response to a therapeutic agent is related to the plasma concentration of the drug and/or its metabolites. It has been well established that the metabolism of a drug is often a major determinant of the duration and intensity of its clinical effects.

Metabolic processes are necessary to convert a lipophilic drug into one or more metabolites, which are more water soluble than the parent drug, facilitating urinary excretion. Drug metabolism may yield inactive metabolites, but this is not always true. In some cases, metabolites have pharmacological activity similar to that of the parent drug: well known examples are many benzodiazepines, whose long-lived active metabolites cause their effects to persist after the parent drug has been eliminated, or some antidepressants, such as imipramine and amitriptyline, whose antidepressant action is partially due to their metabolites desmethylimipramine and nortriptyline. In some cases, a drug, referred to as pro-drug, becomes pharmacologically active only after metabolism: an example is the angiotensin-converting enzyme inhibitor enalapril that exerts its pharmacological activity through its metabolite enalaprilat. Metabolism can alter the pharmacogical properties of a compound qualitatively: for example, salicylic acid shares with its parent drug aspirin the anti-inflammatory, but not the antiplatelet activity. There are also cases in which the metabolism yields toxic compounds: an example is the hepatotoxicity of paracetamol, caused by its metabolite N-acetyl-p-benzoquinone imine (Rang et al., 1999). Hence, variability in activity of drug metabolising enzymes can lead to interindividual differences in drug effects, one of the major problems in drug therapy (Sjöqvist et al., 1997).

1.2 Drug oxidation

A wide variety of biochemical reactions can take place during the metabolism of a drug. In *phase I* reactions, called functionalization reactions, polar groups are introduced into the drug molecule by oxidation, reduction or hydrolysis, while *phase II* reactions involve coniugation with different groups, such as glucuronic acid, sulfate, glycine and acetyl groups (Sjoqvist et al., 1997). Basically, *phase I* reactions place a functional group on a molecule, making it more hydrophilic or a better substrate for additional metabolic reactions. Oxidation is the most common metabolic pathway of drug detoxication in the body. Oxidative reactions are catalysed by the hepatic mono-oxygenase (mixed function oxidase) system (Rang et al., 1999). The activity of this enzyme system requires both a reducing agent (NADPH) and molecular oxygen. In a typical reaction, one molecule of oxygen is consumed (reduced) per substrate molecule, with

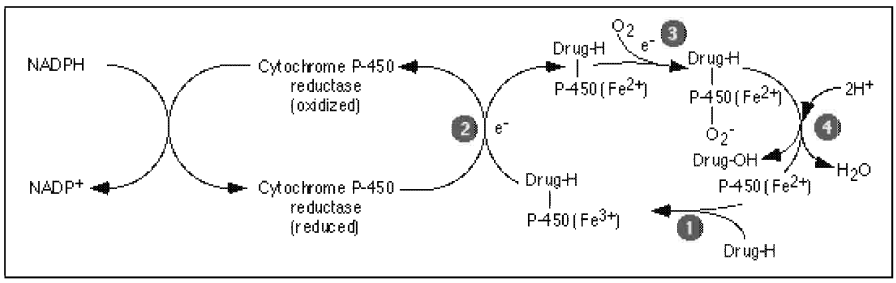
one oxygen atom appearing in the product and the other in the form of water (Box 1) (Rang et al., 1999). Two enzymes are important in this process:

1. *NADPH-cytochrome P-450 reductase*. One mole of this enzyme (molecular weight of 80,000) contains one mole each of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Because cytochrome c can serve as an electron acceptor, the enzyme is often referred to as NADPH-cytochrome c reductase.

2. *Cytochrome P-450*. The name cytochrome P-450 is derived from the spectral properties of this hemoprotein. In its reduced (ferrous) form, it binds carbon monoxide to give a ferrocarbonyl adduct that absorbs maximally in the visible region of the electromagnetic spectrum at 450 nm.

Box 1. Microsomal drug oxidations require cytochrome P-450, cytochrome P-450 reductase, NADPH, and molecular oxygen. The cycle involves four steps:

1. Oxidized (Fe3+) cytochrome P-450 combines with a drug substrate to form a binary complex;
2. NADPH donates an electron to the cytochrome P-450 reductase, which in turn reduces the oxidized cytochrome P-450-drug complex;
3. A second electron is introduced from NADPH *via* the same cytochrome P-450 reductase, which serves to reduce molecular oxygen and form an "activated oxygen"-cytochrome P-450-substrate complex;
4. This complex in turn transfers "activated" oxygen to the drug substrate to form the oxidized product. The potent oxidizing properties of this activated oxygen permit oxidation of a large number of substrates.



From http://web.vet.cornell.edu/public/pharmacokinetics/sitesDT/phase1.html

1.3 Cytochromes P450 (CYP)

The cytochrome P450 system consists of a superfamily of isoenzymes located in the membranes of the smooth endoplasmic reticulum, mainly in the liver, but also in extrahepatic tissues (e.g. intestinal mucosa, lung, kidney, brain, lymphocytes, placenta, etc.) (Gonzales, 1992; Guengerich, 1995). They are hemeproteins containing a single iron protoporfyrin IX prosthetic group. These enzymes are responsible for the oxidative metabolism of a number of drugs and other exogenous compounds, as well as many endogenous substrates, such as prostaglandins, fatty acids and steroids. The multiple CYP enzymes are classified into families, subfamilies and isoenzymes according to a systematic nomenclature based on similarities in their amino acid sequences (Nelson *et al.*, 1996). The first Arabic number designates the "family" (>40% sequence identity within family members), the capital letter that follows indicates the

"subfamily" (>60% sequence identity within subfamily members), while the second Arabic number designates individual isoenzymes. In humans, 18 families and 43 subfamilies of CYP codifying genes have so far been identified (http://drnelson.utmem.edu/P450lect.html). The major CYP enzymes involved in drug metabolism in humans belong to families 1, 2 and 3, the specific isoforms being CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (Daly *et al.*, 1993; Bertilsson *et al.*, 1995). Each CYP isoform is a specific gene product and possesses a characteristic spectrum of substrate specificity (Bertilsson *et al.*, 1992; Gonzales, 1992; Guengerich, 1995; Rendic & Di Carlo, 1997).

There are wide interindividual differences in the expression and activity of CYP isoenzymes. Such broad variability may be caused by genetic polymorphisms, environmental factors such as concomitant drug therapy, physiological status and pathological conditions. Genetic polymorphisms and inhibition/induction of drug metabolism appear to be of major importance for the occurrence of adverse effects or therapeutic failure in many cases. For some isoforms, the existence of a genetic polymorophism has been demonstrated, while other CYPs are mainly under influence of environmental factors.

1.4 Interindividual variability in drug oxidation

The individual differences in drug oxidation depend on the characteristics of the enzymes involved in the metabolic pathway. Thus, within the same population, steady-state plasma levels of drugs may reflect a 30-fold variation in the metabolism of one drug and only a 2-fold variation in the metabolism of another. Genetic factors that influence enzyme levels account for many of these differences. Succinylcholine, for example, is metabolized only half as rapidly in subjects with genetically determined deficiency in pseudocholinesterase as in subjects carrying the normal enzyme (Kalow & Genest, 1957). Clinical observations of inherited differences in drug effects began to be documented in the 1950´s, when it was discovered that the primaquine-induced hemolysis observed mostly in American black soldiers was due to a genetic deficiency in glucose-6-phosphate dehydrogenase (Carson et al., 1956). Later on, it was discovered that there was a distinction between genetically fast and slow acetylators of isoniazid (Hughes et al., 1954). The acetylation polymorphism is the first example of a genetic defect in drug metabolism to be fully elucidated (Price Evans et al., 1960). Polymorphic oxidation involving CYPs was first demonstrated in the late 70´s with the antihypertensive drug debrisoquine (Mahgoub et al., 1977), but had previously been suspected to occur for phenytoin (Kutt et al., 1964) and desmethylimipramine (Hammer & Sjoqvist, 1967).

Early studies from the 60´s of the kinetics of antidrepessants revealed marked variability in steady-state plasma concentrations of imipramine, desmethylimipramine and nortriptyline among patients receiving the same oral dose (Hammer & Sjoqvist, 1967; Moody et al., 1967). Twin studies clearly established that genetic factors were the most important determinants of the steady-state plasma concentrations of nortriptyline, but environmental factors (drug interactions) also plaied a role (Alexanderson et al., 1969). However, it was not until the 80´s that the genetic defect causing this variability was identified (Bertilsson et al., 1990). Since then, knowledge about the genetic defects that may cause interindividual variability in drug oxidation has rapidly increased (Bertilsson et al., 1995).

1.5 Genetic polymorphism

The inherited traits determining drug disposition may be either genetic polymorphisms, defined as Mendelian traits that exist in the population in at least two phenotypes neither of which is present with a frequency lower than 1%, or rare single gene defects (Kalow, 1991). Polygenic control of drug metabolism is also known to occur (Meyer, 1992).

A number of genes coding for CYP isoforms have variant alleles resulting from mutations. These include deletion of a whole gene, single point mutations (SNPs) within genes, deletions or insertions of fragments of DNA within genes, and multiple copies of genes. Although not all mutations are functionally important, they may lead to absent, deficient or enhanced enzyme activity (Meyer, 1994). Polymorphisms of drug metabolism divide a population into at least two phenotypes, extensive metabolizers (EM) and poor metabolizers (PM) and, eventually, ultrarapid metabolizers (UM) (Meyer, 1994; Bertilsson *et al.*, 1995; Eichelbaum & Evert, 1996). The clinical relevance of polymorphic metabolism depends on a number of factors, such as the pharmacological and toxic activity of the parent compound and/or its metabolite(s), the therapeutic index of the drug, the overall contribution of the polymorphic pathway to the total clearance of the drug and the possible saturation of the polymorphic enzyme. The clinical consequences of polymorphic genes for drug metabolizing enzymes, with respect to response to certain drugs, are most pronounced for subjects homozygous for detrimental alleles (PM), or for those with duplicate or amplified functional genes (UM) (Meyer, 1992; Bertilsson *et al.*, 1995). In the former case, the mutations may cause diminished first pass metabolism, increased bioavailability, impaired elimination and thus possibly an exaggerated response. If the enzyme activates the drug to an active metabolite, one might predict loss of therapeutic efficacy among the PMs. On the contrary, the UMs may not achieve therapeutic levels of the drug given at a standard dose and this might account for lack of therapeutic effect.

Furthermore, in cases where the metabolising enzyme is responsible for bioactivation of the drug, too high plasma levels of the active metabolite can be expected in UMs. Although most adverse reactions can be managed by appropriate dosage adjustment, a few are potentially life threatening. Therefore, the possibility of starting the therapy at the right dosage, according to the metabolic capacity of the patient, might prevent the appearance of some side effects.

The major CYP polymorphisms that have clinical implications are those related to the oxidation of drugs by CYP2D6, CYP2C9 and CYP2C19 (Goldstein, 2001; Bertilsson *et al.*, 2002)

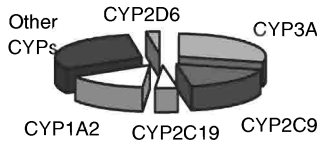


Figure 1. The proportions of human P450s in the liver (Rendic & Di Carlo, 1997)

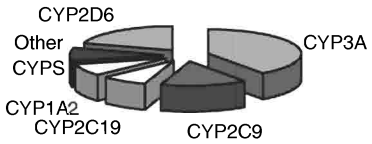


Figure 2. The proportions of drugs metabolized by CYPs (Rendic & Di Carlo, 1997)

1.5.1 CYP2D6

CYP2D6 represents an average of 2% of hepatic CYP content (Fig.1). The gene encoding its synthesis is located on the long arm of chromosome 22 (Brøsen & Gram, 1989; Eichelbaum & Gross, 1990; Meyer *et al.*, 1990). Although expressed at rather low levels compared with other human CYPs, this isoform plays an important role in drug metabolism, being partially or to a major extent responsible for the oxidative biotransformation of a variety of psychoactive and cardiovascular drugs (Fig.2; Tab.1). Quinidine, fluoxetine, paroxetine and various phenothiazines are potent inhibitors of this isoform (Tab.1). In contrast to all other CYPs involved in drug metabolism, CYP2D6 is not inducible.

The *CYP2D6* polymorphism was described first in the 70's for the drugs debrisoquine and sparteine (Mahgoub *et al.*, 1997; Tucker *et al.*, 1977; Eichelbaum *et al.*, 1979). Debrisoquine, sparteine, dextromethorphan and desmethylimipramine have been validated as probe drugs for CYP2D6. The metabolic ratio (MR) for these drugs is bimodally distributed; approximately 3 to 10% of Caucasians, but only 1 to 2% of Orientals, lack CYP2D6 activity and can thus be classified as PMs (Evans *et al.*, 1980; Alvan *et al.*, 1990; Bertilsson *et al.*, 1992; Bertilsson & Dahl, 1996). The phenotype PM is inherited as a recessive autosomal character (Evans *et al.*, 1980; Steiner *et al.*, 1985). Among the EMs, the catalytic activity varies largely. A subgroup of subjects with extremely high enzyme activity (low MR) can be identified among the EMs, and classified as UMs (Johansson *et al.*, 1993; Dahl *et al.*, 1995).

The *CYP2D6* gene is extremely polymorphic. To date, more than 70 allelic variants have been described (http://www.imm.ki.se/CYPalleles/cyp2d6.htm). Four major mutated alleles, *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5* and *CYP2D6*6*, account for 90-95% of the PM alleles in Caucasians. The most common allele associated with the PM phenotype is *CYP2D6*4*, characterised by a defective splicing between the intron 3 and the exon 4, leading to production of an inactive enzyme, with an allele frequency of ~ 21% in Caucasian populations (Ingelman-Sundberg *et al.*, 1999). *CYP2D6*4* is almost absent in Orientals; this accounts for the low incidence of PM in these populations (Bertilsson *et al.*, 1992; Johansson *et al.*, 1994). On the other hand, the high frequency (up to 50%) of the *CYP2D6*10* allele, causing decreased but not absent CYP2D6 activity, among Orientals, and its absence among Caucasians, explains the lower CYP2D6 activity found in Oriental EMs compared to Caucasians (Johansson *et al.*, 1994). The frequency of the *CYP2D6*5* allele, with deletion of the entire *CYP2D6* gene, is ~ 4-6%, very similar in different ethnic populations (Ingelman-Sundberg *et al.*, 1999). Other detrimental alleles frequently (~2-3%) found among Caucasians are *CYP2D6*3*, characterised by a single base deletion in exon 5, and *CYP2D6*6*, characterised by a single base deletion in exon 3, that cause a frameshift, with production of an inactive enzyme (Kagimoto *et al.*, 1990; Gaedick et *al.*, 1991; Broly *et al.*, 1991). Individuals heterozygous for the defect alleles have higher median MR than homozygous EM (Dahl *et al.*, 1993; Sachse *et al.*, 1997). Furthermore, alleles with duplication or multiduplication of a functional *CYP2D6* gene (**1* or **2*), causing increased CYP2D6 activity, have been described. The frequencies of subjects carrying extra copies of a functional gene vary among different Caucasian populations, between 1-2 % in Swedes (Dahl *et al.*, 1995) and up to 7-10 % in Spaniards (Agundez *et al.*, 1995; Bernal *et al.*, 1999) and southern Italians (Scordo et al., 1999; Scordo *et al.*, 2000).

1.5.2 CYP2C9

The human CYP2C subfamily consists of at least four isoforms, 2C8, 2C9, 2C18 and 2C19, whose genes are located together on chromosome 10. CYP2C9, the most abundant among human CYP2C isoforms, represents ~ 18% of total hepatic CYPs (Fig.1). This enzyme metabolizes a number of therapeutically important drugs, including most nonsteroidal anti-inflammatory drugs (NSAIDs), *S*-warfarin, phenytoin and losartan (Fig.2; Tab.1) (Miners & Birkett, 1998). Fluconazole, metronidazole and amiodarone are potent inhibitors of this enzyme, while barbiturates and phenytoin coadministration may enhance its activity by induction (Tab.1).

CYP2C9 is polymorphically expressed. *In vitro* studies have shown that even relatively conservative changes in the aminoacid sequence may significantly alter both enzymatic activity

and substrate specificity (Veronese *et al.*, 1993). To date, three different allelic variants (*CYP2C9*1*, *CYP2C9*2*, *CYP2C9*3*), coding for enzymes with different catalytic activity, have been well characterised (Miners & Birkett, 1998). The *CYP2C9*2* allele, characterised by a C➔T substitution at the codon 416, encodes an enzyme with an $Arg_{144}Cys$ mutation that displays reduced affinity for P450 oxidoreductase (Ingelman-Sundberg *et al.*, 1999). The *CYP2C9*3* allele, characterised by a A➔T substitution at the codon 1016, yields an enzyme with an $Ile_{359}Leu$ exchange that shows reduced affinity for many substrates (Ingelman-Sundberg *et al.*, 1999). The functional importance of *CYP2C9*2* and *CYP2C9*3* has been evaluated for many CYP2C9 substrates, both *in vitro* and *in vivo*. It has been shown that the **3* allele, in particular, appears to confer the largest reduction in metabolic activity *in vitro* while the **2* allele produces intermediate reduction in enzyme activity, as compared to **1* (Lee et al., 2002). Similarly, *in vivo* investigations demostrated that **3/*3* genotype is associated with clinically significant alterations in the pharmacokinetics of CYP2C9 substrates (Scordo et al., 2001; Lee et al., 2002, Scordo et al., In press; Yasar et al., 2002). The frequencies of the detrimental alleles *CYP2C9*2* and *CYP2C9*3* vary between 8–12% and 3–8%, respectively, among Caucasians, while they are lower in Orientals and black Africans (Miners & Birkett, 1998; Scordo *et al.*, 2001). Additional rare defect alleles have been described, but so far their impact on the enzyme activity *in vivo* is unclear (http://www.imm.ki.se/CYPalleles).

1.5.3 CYP2C19

CYP2C19 is a 490 amino acid protein, product of a gene that comprises 9 exons (Romkes *et al.*, 1991) and represents ~ 3% of total hepatic CYPs (Fig.1). The enzyme is responsible for the 4-hydroxylation of the *S*-enantiomer of the anticonvulsant mephenytoin and contributes to the clearance of diazepam, omeprazole, proguanil, citalopram, *R*-warfarin and many antidepressants (Fig.2; Tab.1) (Bertilsson et al., 1995). CYP2C19 also exhibits genetic polymorphism. About 3% of Caucasians have been found to be PMs of *S*-mephenytoin with very little variation noted between the studies (Alvan *et al.*, 1990; Bertilsson *et al.*, 1992; Wilkinson *et al.*, 1992). By contrast, several independent studies have shown a much higher incidence of PMs in Orientals, up to 18-23% in Japanese (Nakamura *et al.*, 1985; Jurima *et al.*, 1985); 15-17% in Chinese (Bertilsson *et al.*, 1992; Horai *et al.*, 1989); 12-16% in Koreans (Sohn *et al.*, 1992; Roh *et al.*, 1996). In black Africans PM frequencies vary between 4–7% (Goldstein, 2001). The PM condition is inherited as an autosomal recessive trait (Wilkinson *et al.*, 1992).

The best-characterized defect *CYP2C19* alleles responsible for the PM phenotype are *CYP2C19*2*, the most common among Caucasians and Orientals (de Morais et al., 1994a) and

*CYP2C19*3*, found at a frequency of about 12% among Oriental populations, but almost absent among Caucasians (de Morais et al., 1994b). *CYP2C19*2* differs from the wild-type gene by a single base pair change from G to A at base 681 in exon 5. The change alters the reading frame starting with amino acid 215 and creates an aberrant splice site by producing a premature stop codon 20 amino acids downstream. This results in a truncated, non-functional protein product that lacks the heme-binding region. This accounts for 75% of the defective alleles in Orientals (de Morais *et al.*, 1994a), and 93% in Caucasians (Chang *et al.*, 1995). The other well characterised detrimental allele (*CYP2C19*3*) has a G→A transition at base 636 in exon 4, creating a premature stop codon and truncated inactive enzyme. This mutation, discovered in Japanese PMs (de Morais *et al.*, 1994b), accounts for approximately 25% of all inactive forms in Orientals, being by converse extremely rare in non Oriental populations (Brøsen *et al.*, 1995). Therefore, the defective forms are still uncharacterised in 15% of the Caucasian PMs. Additional rare defect alleles have been described (http://www.imm.ki.se/CYPalleles), but their impact on the enzyme activity has to be further clarified.

1.5.4 CYP1A2 & CYP3A4

CYP1A2 accounts for approximately 13% of total CYPs expressed in human liver (Fig.1). There is increasing awareness of the importance of CYP1A2 in human hepatic drug metabolism. The enzyme catalyzes the metabolism of many clinically important drugs (Fig.2; Tab.1). Though CYP1A2 enzyme activity is not polymorphically distributed, it shows a wide, 40-fold, interindividual variability. Sex is one major variability factor, women showing a lower average activity than men (Relling *et al.*, 1992). The activity of this isoform is potently inhibited by fluvoxamine, and may be induced by polycyclic aromatic hydrocarbons, cigarette smoking and by rifampicin, phenytoin and, to a lesser extent, omeprazole (Tab.1). Recently, the existence of two polymorphisms, which seem to enhance the inducibility of CYP1A2 has been reported (Sachse et al. 1999; Nakajima et al., 1999). However, the impact of this enhanced CYP1A2 metabolic activity on the clinical response to drugs metabolised by this enzyme is still controversial.

The human CYP3A subfamily is composed of 3 isoforms, 3A4, 3A5 and 3A7, encoded by genes located on chromosome 7. CYP3A4 is the most abundant isoform in human liver, accounting for approximately 30% of total CYP liver content (Fig.1), and for the majority of CYPs in human small bowel (Shimada et al., 1994). This isoform catalyzes, at least partially, the biotransformation of a large number of structurally diverse drugs and endogenous compounds (Fig.2; Tab.1). CYP3A4 drug metabolizing activity has been reported to vary more than 20-fold

among individuals, but it has a unimodal distribution in the population and does not appear to be subject to genetic polymorphism. Wowever, the existence of an allelic variant, *CYP3A4*2*, associated with lower *in vitro* clearance of nifedipine has recently been demonstrated (Sata et al., 2000). The wide interindividual variability may be caused, at least partially, by ethnic or cultural differences, presumably related to an interaction between race and diet, and/or be a result of enzyme induction and inhibition. Among CYP3A4 inhibitors are ketoconazole, ritonavir, indinavir and saquinavir, some of the furanocoumarin dimers found in grapefruit juice, but also erythromycin, fluvoxamine and fluoxetine (Tab.1). The hepatic and possibly intestinal CYP3A4 isoform is induced by rifampicin, dexamethasone, phenobarbital, phenytoin and carbamazepine (Tab.1).

Table 1. Major cytochrome P450 isoforms involved in drug metabolism.

Enzymes	*Substrates*	*Inhibitors*	*Inducers*	*Polymorphism*	*Probe-drug*
CYP1A2	*Antidepressants*: amitriptyline, clomipramine, imipramine, fluvoxamine, mirtazapine *Antipsychotics*: haloperidol, clozapine, olanzapine *Methylxantines*: theophylline, caffeine *Miscellaneous*: paracetamol, phenacetin, tacrine, *R*-warfarin	Fluvoxamine Ciprofloxacin	Smoking Rifampicin Barbiturates Phenytoin Carbamazepine	Yes/?	Caffeine
CYP2C9	*NSAIDs*: diclofenac, ibuprofen, naproxen, piroxicam *Antiepileptics*: phenytoin, phenobarbital, valproic acid *Miscellaneous*: *S*-warfarin, tolbutamide, losartan, torasemide, fluoxetine	Sulfaphenazole Fluconazole Miconazole Fluoxetine Fluvoxamine	Rifampicin Barbiturates Phenytoin Carbamazepine	Yes	Losartan Warfarin Tolbutamide Phenytoin
CYP2C19	*Antidepressants*: amitriptyline, clomipramine, imipramine, citalopram, moclobemide, fluoxetine *Miscellaneous*: phenytoin, diazepam, omeprazole, propranolol, proguanil, *S*-mephenytoin, *R*-warfarin	Omeprazole Ticlopidine Fluvoxamine	Rifampicin Barbiturates Phenytoin Carbamazepine	Yes	Omeprazole *S*-mephenytoin
CYP2D6	*Antidepressants*: amitriptyline clomipramine, imipramine, desipramine, nortriptyline, trazodone, fluoxetine, paroxetine, fluvoxamine, citalopram, venlafaxine, mianserin, mirtazapine, sertraline *Antipsychotics*: thioridazine, perphenazine, zuclopenthixol, haloperidol, risperidone, olanzapine, sertindole *Opiates*: codeine, destromethorphan, tramadol; oxycodone *β-blockers*: alprenolol, bufuralol, metoprolol, propanolol, timolol, pindolol *Antiarrhythmics*: encainide, flecainide, propafenone *Miscellaneous*: debrisoquine, sparteine, diltiazem	Quinidine Thioridazine Perphenazine Fluoxetine Paroxetine Cimetidine Ritonavir	*None known*	Yes	Debrisoquine Dextrometorphan Spartein Metoprolol Desmethylimipramine
CYP3A4	*Antidepressants*: amitriptyline clomipramine, imipramine, trazodone, sertraline, nefazodone, mirtazapine *Antipsychotics*: haloperidol, clozapine, risperidone, quetiapine, ziprasidone, sertindole *Benzodiazepines*: alprazolam, midazolam, triazolam *Ca-antagonists*: diltiazem, felodipine, nifedipine, verapamil *Immunosuppressants*: cyclosporin, tacrolimus *Miscellaneous*: cisapride, terfenadine, astemizole, carbamazepine, erythromycin, clarytromycin, tamoxifen, amiodarone, quinidine, methadone, ethynilestradiol, levonorgestrel, statins	Ketoconazole Itraconazole Fluconazole Erythromycin Troleandomycin Nefazodone Grapefruit juice	Rifampicin Barbiturates Phenytoin Carbamazepine	No/?	Cortisol Dapsone Erythromycin Quinine

Modified from Scordo & Spina (2002)

1.6 Therapeutic index and Dose titration

The success of any fixed dosing regimen is based on the patient's clinical response to the drug. Fixed dosing regimens are designed to generate plasma drug concentrations within a therapeutic range, i.e. to achieve the desired effect while avoiding toxicity. However, physiologic, pathologic and pharmacologic factors can profoundly alter the disposition of a drug so that therapeutic failure or adverse reactions occur. Recommended dosing regimens are sometimes designed to compensate for the effects of some of these factors. Unfortunately, the effects of many factors are unpredictable and cannot be anticipated in the individual patient. A careful dose titration is needed when starting a therapy with drugs characterised by serious toxicity coupled with a poorly defined or difficult to detect clinical endpoint, a steep dose-response curve for which a small increase in dose can result in a marked increase in desired or undesired response, a narrow therapeutic range, marked inter-individual pharmacokinetic variability which increases the variability in the relationship between dose and plasma drug concentrations, non-linear pharmacokinetics which may lead to rapid accumulation of drugs to toxic concentrations, and risk of drug interactions.

1.7 Tools to individualise drug dosage

Different strategies may be proposed to optimise the drug dosage. The first one is based upon monitoring of the plasma drug levels. The second one is based on evaluation of the metabolic capacity of an individual. The third one is based on adjustment of the dose according to the kidney function, in case of drugs that are eliminated unchanged.

1.7.1 Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) is a tool that can guide the clinician to provide effective and safe drug dosage in the individual patient. Monitoring can be used to confirm a plasma drug concentration, which is above or below the therapeutic range, thus minimizing the time that elapses before corrective measures can be implemented in the patient (Dahl & Sjöqvist, 2000). TDM is indicated in clinical situations in which an expected therapeutic effect of a drug has not been observed, or in cases where concentration dependent drug toxicity is suspected. In addition, TDM can be used to establish whether or not optimum therapeutic drug concentrations have been achieved for drugs characterized by a response that is difficult to detect. TDM is indicated when a drug is used chronically, and thus is more likely to induce toxicity or changes in pharmacokinetics, or in life-threatening situations in which a timely response is critical to the patient. Drugs for which TDM might not be indicated include those characterized by a wide therapeutic index, that are seldom toxic even if plasma levels are higher than recommended, or

those for which response can be easily monitored by clinical signs (Sjöqvist et al., 1997). Drug monitoring is also useful in identifying noncompliance as a cause of therapeutic failure or adverse reactions.

1.7.2 Genotyping/Phenotyping

The metabolic capacity of an individual may be determined by phenotyping and/or by genotyping tests. There are advantages and disadvantages in both approaches.

Phenotyping is based upon administration of a single dose of a probe drug, followed by the measurement of urinary or plasma concentrations of the parent compound and its major metabolite. Their ratio (metabolic ratio, MR) is used as a measure of enzyme activity. Phenotyping is straightforward but has the disadvantage that it usually involves an invasive procedure. Furthermore, if the drug is given to a poor metabolizer, the subject might actually experience side effects. Besides, the evaluation of a patient phenotype presents some ethical and technical problems, since the possible coadministration of other drugs might interact with the probe drug metabolism, and might increase the risk for unwanted side-effects.

Genotyping is performed by molecular biology techniques to detect the presence of allelic variants ofr the genes codifying for the polymorphic isoenzymes. Genotyping is not influenced by drug-drug or food-drug interactions, and there are no problems with compliance. Currently, it is relatively easy to perform genotyping with samples of genomic DNA, obtained from various sources (e.g., tissue, blood leukocytes, buccal swabs, fingernails and hair). Many different assay methods (e.g., PCR-restriction fragment length polymorphism (RFLP), allele specific PCR, fluorescent dye-based genotyping, mass spectrometry, and gene chip technology) are today available.

TDM is a useful tool to help physicians to optimize an already established therapy, but it does not provide any support in the choice of the starting dose, a problematic issue with drugs as warfarin or phenytoin, that can cause severe adverse reactions even after the first doses. Conversely, theoretically genotyping might help clinicians in selecting the right starting dose, thereby minimizing the risk of side-effects or therapeutic failure. This might be an advantage in patients receiving antipsychotics, antiepileptics, anticoagulants, some antidepressants or cardiovascular drugs, since these patients are at a much greater risk than patients treated with other kinds of agents because of the narrow drug therapeutic index. In fact, as a consequence of either genetically impaired or enhanced metabolism, or interactions, plasma levels of a given

substrate are more likely to reach toxic or subtherapeutic values if the substrate has a narrow therapeutic index.

1.8 Risperidone metabolism

Risperidone is an atypical antipsychotic drug, pharmacologically characterised by both a selective serotonin 5-HT2 and a dopamine D2 receptor antagonism (Leysen et al. 1988). The drug is claimed to be effective in the treatment of both positive and negative symptoms of schizophrenia and to have a lower potential for extrapyramidal side effects compared to classical antipsychotics (Chouinard & Arnott, 1993). Risperidone is predominantly metabolised in the liver and 9-hydroxylation is the major metabolic pathway, while N-dealkylation and 7-hydroxylation are minor routes of biotransformation in humans (Mannens et al., 1993). As 9-hydroxyrisperidone (9-OH-risperidone) is claimed to be equipotent to the parent compound in terms of dopamine-receptor affinity (van Beijsterveldt et al., 1994), the sum of the plasma concentrations of risperidone and 9-OH-risperidone is referred to as the "active moiety". Recent studies suggest that increase in plasma levels of the active moiety enhance the risk for risperidone-induced EPS (Tarsy et al., 2002; Yoshimura et al., 2001). *In vitro* studies in human liver microsomes indicated that the 9-hydroxylation is catalysed mainly by CYP2D6 but also by CYP3A4 (Fang et al., 1999). The major role of CYP2D6 was confirmed in a single dose study in healthy volunteers showing that the formation of 9-hydroxyrisperidone was strongly related to the CYP2D6 phenotype (Huang et al., 1993).

1.9 Warfarin metabolism

Warfarin is the most widely prescribed anticoagulant drug in North America and the majority of European countries (Takahaski & Echizen, 2001). Its anticoagulant effect is subject to wide interpatient variability (Hirsh et al., 1995; Takahaski & Echizen, 2001; Lee et al., 2002), and, despite careful dose titration based on evaluation of the international normalized ratio (INR), bleeding frequently complicates warfarin therapy, at a rate of 7.6 to 16.5 per 100 patients per year (Aithal et al., 1999). The risk of serious hemorrhage is 1.3-4.2 per 100 patients per year of exposure (Taube et al., 2000). Although major bleeding can occur at therapeutic levels, the risk rises with increasing intensity of anticoagulation. Therefore, identification of risk factors for the development of a high INR may identify patients at high risk of bleeding.

Both pharmacodynamic and pharmacokinetic factors may be responsible for variability in the anticoagulant response (Hirsh et al., 1995; Holford, 1986). Warfarin is a racemic mixture of two enantiomers, *S*- and *R*-warfarin, with different pharmacodynamic and pharmacokinetic

properties (Holford, 1986). *S*-warfarin is 3-5 times more potent than *R*-warfarin, has a shorter half-life compared to the R-enantiomer (25 h vs 40 h) and is mainly metabolized to 7-hydroxywarfarin by CYP2C9 (Chan et al., 1994; Kaminsky & Zhang, 1997). Conversely, the *R*-isomer is metabolized by multiple cytochromes, including CYP1A2, CYP3A4 and the polymorphic CYP2C19 (Kaminsky & Zhang, 1997). Therefore, genetic polymorphisms within the *CYP2C9* gene, which give rise to proteins with differing catalytic activity, may have a major effect on the clearance of the more potent enantiomer and thus the response to the orally administered drug. A relationship between *CYP2C9* genotype and *S*-warfarin clearance has been demonstrated in one homozygous (**3/*3*) and four heterozygous (**3/*1*) patients, who exhibited 90% and 66% reduction in warfarin clearance, as compared with patients with the **1/*1* genotype, respectively (Takahashi et al., 1998). It has also been reported that patients with low warfarin dose requirements have a greater prevalence of *CYP2C9*2* and **3* alleles (Aitha et al., 1999; Margaglione et al., 2000; Taube et al., 2000) and a higher risk of major hemorrhages than control patients (Margaglione et al., 2000; Taube et al., 2000). By contrast, no significant differences in warfarin clearance were observed between *CYP2C19* genotype groups (Takahashi et al., 1998a), although 8-hydroxylation of *R*-warfarin has been suggested to represent a marker for CYP2C19 activity (Winkers et al., 1996).

1.10 Fluoxetine metabolism

Fluoxetine is one of the most widely prescribed antidepressants of the selective serotonin reuptake inhibitor (SSRI) class. It is also approved or under investigation for the management of a variety of other psychiatric and medical conditions, in which serotonin plays a role (Gram, 1994; Stokes & Holtz, 1997). Fluoxetine mainly undergoes N-demethylation, leading to the formation of the active metabolite norfluoxetine (Gram, 1994). The drug is administered as a racemic mixture of two enantiomers (S- and R-), approximately equipotent in blocking serotonin reuptake (Wong et al., 1985). Conversely, the enantiomers of the main metabolite, which are present in the circulation at higher concentrations than those of the parent drug, show marked differences in pharmacological activity (Fuller et al., 1992; Wong et al., 1993). S-norfluoxetine is about 20 times as potent as the R-enantiomer as a serotonin reuptake inhibitor both *in vitro* and *in vivo* (Fuller et al., 1992; Wong et al., 1993).

Previous investigations conducted *in vitro* and *in vivo* after single and multiple doses suggest that CYP2D6 plays a major role in the metabolism of S-fluoxetine (Fjordside et al., 1999; Margolis et al., 2000; Eap et al., 2001), and, possibly, R-fluoxetine (Fjordside et al., 1999; Margolis et al., 2000) and S-norfluoxetine (Fjordisiide et al., 1999; Eap et al., 2001). However, *in*

vitro evidence suggests that other isoenzymes including CYP2C9, CYP2C19 and CYP3A4 may also contribute to fluoxetine metabolism (von Moltke et al., 1997; Margolis et al., 2000; Ring et al., 2001, Liu et al., 2001a). A recent single dose study in healthy Chinese volunteers seems to confirm the involvement of CYP219 in fluoxetine N-demethylation (Liu et al., 2001b)

1.11 Phenytoin metabolism

Phenytoin is an anticonvulsant drug effective against various forms of partial and generalised seizures. The drug is characterized by a wide pharmacokinetic variability and a narrow range of therapeutic serum concentrations (10-20 mg/L). A well-known variability factor is the saturability of phenytoin metabolism, which is responsible for a nonlinear relationship between doses and plasma concentrations (Richens & Dunlop, 1975). Therefore, the dose has to be adjusted carefully to obtain serum concentrations within the acceptable plasma concentration range. The major pathway of phenytoin elimination in man is via 4-hydroxylation to form 5-(4 p-hydroxyphenyl)-5-phenylhydantoin (HPPH) (Eadie, 1981). Several studies have shown that CYP2C9 is the primary enzyme responsible for the formation of HPPH and the rate-limiting step in phenytoin clearance (Hansen et al., 1979; Doecke et al., 1991; Hall et al., 1994; Odani et al., 1997). CYP2C19 contributes to the clearance of phenytoin to a minor extent (Mamiya et al., 1998).

1.12 Genotype and side-effects

The use of antipsychotic drugs in the treatment of schizophrenia is often associated with extrapyramidal side-effects (EPS) (Grohman et al., 1983; Tarsy, 1984). Four types of EPS are recognised: akathisia (a subjective feeling of restlessness, which may be objectively manifested as pacing, shifting from one foot to the other, or marching on the spot); parkinsonism (bradykinesia, tremor, and rigidity); dystonia (sustained abnormal muscular contractions, most often localized to the oral region, the eyes, neck, and back (retrocollis)); and dyskinesia (hyperkinetic, involuntary movements, most often in the oral region (buccolinguo-masticatory syndrome)). The latter is also referred to as tardive dyskinesia, since usually it requires exposure to antipsychotics for at least 6 months before it develops and may persist indefinitely. EPS occur in a majority of patients receiving traditional antipsychotics, while the risk appears to be lower with newer compounds (Casey, 1996). Dopamine receptor blockade in the basal ganglia is believed to be the underlying mechanism of EPS (Casey, 1995).

Many studies have aimed to identify potential risk factors to develop severe EPS, but the results have been controversial (Sandyk et al., 1991; Kopala, 1996; Steen et al., 1997). It has

been suggested (Fenton et al., 1997) that the high frequency of dyskinesia among schizophrenic patients might be related not only to the antipsychotic therapy, but also to the pathophysiology of the disease itself.

EPS appear to be related to the antipsychotic dosage, and, consequently, to the plasma concentrations of the drug, since lower doses produce fewer EPS than moderate to high doses (Casey, 1995). Therefore, every factor, which might contribute to high drug plasma levels, may increase the risk of EPS. EPS could thus be related to the patient's metabolic capacity, whose influence on drug plasma levels is well established. Many traditional antipsychotics are metabolised, at least partially, by CYP2D6 (Tab.1).

Interindividual differences in the elimination kinetics and in steady-state plasma concentrations of antipsychotics resulting from genetically determined variability in the expression of CYP2D6 may thus have important clinical implications (Bertilsson & Dahl, 1996). An association between the PM phenotype and acute antipsychotic-induced adverse effects has been documented in a few patients (Meyer et al., 1990; Spina et al., 1992). In schizophrenic patients with tardive dyskinesia, no overrepresentation of PM was found, but it was suggested that the CYP2D6 metabolic capacity might influence the severity of EPS (Arthur et al., 1995). The results of two other studies (Armstrong et al., 1997; Andreassen et al, 1997) indicate that the *CYP2D6* PM genotype may be a contributing factor to antipsychotic-induced EPS, including tardive dyskinesia.

While a number of studies have addressed the pharmacokinetic consequences of polymorphic metabolism, very few studies have been performed to evaluate the relationship between polymorphism and clinical response or dosing. Furthermore, evidence for the role of polymorphic enzymes in drug kinetics often comes from *in vitro* studies or single dose studies in volunteers. However, this may not reflect the situation at steady-state, because of different factors, such as saturation kinetics or pathological conditions. Therefore, clinical studies in patients at steady-state are needed to clarify the role of CYPs polymorphisms in clinical conditions.

2. Aims

General aim

The general aims of this thesis were to elucidate the impact of CYP polymorphisms on the steady-state plasma kinetics and treatment outcome, and to consider whether genotype analysis might improve their safety and efficacy.

Specific aims

- To evaluate the influence of *CYP2D6* genotype on steady-state plasma concentrations of risperidone and 9-OH-risperidone in schizophrenic patients.
- To determine the impact of *CYP2C9* and *CYP2C19* genetic polymorphisms on warfarin dose requirement and metabolic clearance.
- To elucidate the role of *CYP2C9*, *CYP2C19* and *CYP2D6* polymorphisms on the steady-state plasma concentrations of the enantiomers of fluoxetine and its main metabolite norfluoxetine.
- To investigate the possible association between the *CYP2D6* genotype and the appearance of antipsychotic-induced EPS.
- To describe the role of genetically impaired *CYP2C9* and *CYP2C19* activity as a risk factor for phenytoin toxicity.

3. Methods

3.1 Patients and Study design

Correlation between *CYP2D6* genotype and steady-state plasma levels of risperidone and 9-OH-risperidone. (Study I)

Thirty-seven Italian schizophrenic patients of Caucasian origin, 30 males and 7 females, receiving risperidone at a daily dose ranging from 4 to 8 mg/day (mean dose 5.9 ± 1.1 mg/day) given orally in two divided daily administrations, participated in the study. Their age ranged from 27 to 60 years (mean ± SD = 41 ± 9) and body weight from 53 to 87 kg (mean ± SD = 72 ± 8). Eighteen subjects were smokers (>10 cigarettes per day) and the remaining 19 were non-smokers. All patients fulfilled the DSM IV criteria for schizophrenia. No other medication was given except for benzodiazepines, in 11 subjects. Blood samples for the determination of plasma concentrations of risperidone and 9-OH-risperidone were drawn 4-6 weeks after initiation of risperidone treatment, at 8.00 a.m., before the antipsychotic morning dose, 12 hours after the bedtime dose. Plasma was collected after centrifugation and kept frozen at –20 °C until assayed. On the same occasion, a 10 ml blood sample was obtained from each subject, for the determination of the *CYP2D6* genotype, and kept frozen at –20 °C until assayed.

The protocol was approved by the Ethics Committee at the Azienda Sanitaria Locale 5, Messina, Italy, and all the patients gave their written informed consent to participate in the study.

Influence of *CYP2C9* and *CYP2C19* genetic polymorphisms on warfarin maintenance dose and metabolic clearance. (Study II)

The study population consisted of 93 Italian outpatients of Caucasian origin (57 males, 36 females), aged 22-84 years (mean age ± SD: 68 ± 11 years), on long-term warfarin anticoagulant therapy. All patients were treated for cardiovascular diseases requiring a target INR value between 2 and 3, such as atrial fibrillation (n = 46), valvular disease (n = 25), deep venous thrombosis (n = 19) or occlusive arterial disease (n = 6), some patients being treated for two concomitant pathologies. In all cases, warfarin was given once daily, at 8 p.m. Patients had to receive stable maintenance warfarin doses, with INR value variations ≤ 15% at the last three visits. Subjects who were taking drugs known to interfere with warfarin metabolism (such as non-steroidal anti-inflammatory drugs, sulfonamides, anti-epileptics, rifampin, amiodarone, etc.) were excluded. Patients were divided, according to their weekly maintenance dose, into three groups: low (LD: <26.25 mg/week, n=37), medium (MD: 26.25-43.75 mg/week, n=32) and high (HD: >43.75 mg/week, n=24). The dose range of the MD group was identified by considering a mean daily maintenance dose of 5 mg and a 25% coefficient of variation. Twelve to fourteen

hours after the last drug administration (i.e., at 8-10 a.m.), a 5-ml blood sample was drawn (with sodium citrate as anticoagulant), centrifuged and divided into two aliquots: one was used for INR determination, and the other for the assay of total and unbound plasma concentrations ($C_{ss,u}$) of S- and R-warfarin. A 5-ml blood sample was drawn on the same occasion and stored with EDTA for *CYP2C9* and *CYP2C19* genotyping. Samples were frozen at –30°C until analysed.

The protocol was approved by the Ethics Committee at the Azienda Ospedaliera di Padova, Italy, and all patients gave their informed consent to participate in the study.

***CYP2C9*, *2C19* and *2D6* genotypes and plasma levels of fluoxetine and norfluoxetine enantiomers. (Study III)**

Ninety-two Italian patients of Caucasian origin, mostly from a previous study (Jannuzzi et al., 2002), 30 males and 62 females, aged 18-76 years (mean±SD: 47±4), 28 smokers and 64 non-smokers, were included in the study. The subjects were in maintenance treatment with fluoxetine for the treatment of major depression or other depressive disorders, at dosages ranging between 10 and 60 mg/day (mean±SD: 26 ± 12 mg/day), since 5 or more weeks. The majority of patients (n=78, 85%) received fluoxetine in monotherapy, while the others received concomitant benzodiazepines. No patient was treated with drugs known to interfere with CYP2D6, CYP2C9 and CYP2C19. Blood samples (10 ml) for drug analysis were obtained from each patient at trough level, usually about 12 hours after the dose of fluoxetine taken in the evening. The blood was immediately transferred to a tube containing EDTA as anticoagulant and the plasma was separated within 3 hours and stored at -20° C until analysis (Jannuzzi et al., 2002). A 5 ml blood sample for *CYP2C9*, *CYP2C19* and *CYP2D6* genotyping was drawn on the same occasion and stored with EDTA at -20° C.

All patients gave their informed consent, and the study protocol was approved by the Ethics Committee of the Department of Internal Medicine and Therapeutics of the University of Pavia, Italy.

***CYP2D6* genotype and antipsychotic-induced extrapyramidal side effects in schizophrenic patients. (Study IV)**

Among schizophrenic patients resident in a long-term psychiatric unit of Messina, Italy, a total of 119 subjects, 99 males and 20 females, aged 25 – 75 years (mean age ± SD: 50 ± 12), were recruited in the study. In order to be included, patients had to be diagnosed as suffering from schizophrenic disorders according to the DSM IV criteria and treated with antipsychotic drugs, metabolised, at least partially, by CYP2D6. Patients treated with antipsychotic drugs not

metabolised primarily by CYP2D6, such as clozapine (18 patients) or substituted benzamides (12 patients) were excluded. Sixteen patients with concomitant organic disorders or receiving no antipsychotic medication were not considered for the study. The participating patients had been continuously treated with antipsychotic drugs from 5 up to 27 years (mean ± SD exposure time: 17.5 ± 5.5 years). All subjects, of European Caucasian origin, were otherwise in good physical health.

Patients underwent neurological examination to evaluate the presence of EPS and their medical records were reviewed to recognise the occurrence of previous antipsychotic-induced acute dystonic reactions. Parkinsonism was assessed by using the Simpson-Angus Scale (SAS), while tardive dyskinesia was rated by the Abnormal Involuntary Movement Scale (AIMS). Sixty-three patients (52.9%) were included in the group of patients with EPS, having actual extrapyramidal symptoms and/or a history of movement disorders, while 56 patients (47.1%), with no such problems at the time of the study or earlier as assessed by the medical history, constituted the control group (Tab.2).

Table 2. Characteristics of the patients with and without EPS. Age, antipsychotic intake and antipsychotic exposure are given as mean (with SD in parentheses).
* during the year before evaluation of EPS

	Control patients (n=56)	Patients with EPS (n=63)
Sex M / F	48 / 8	51 / 12
Age (years)	51 (12)	49 (12)
Diagnosis		
Paranoid schizophrenia	21	25
Disorganised schizophrenia	10	22
Undifferentiated schizophrenia	17	11
Catatonic schizophrenia	0	4
Residual schizophrenia	8	1
Antipsychotic intake (mg/day chlorpromazine equivalents)*	322 (105)	309 (112)
Antipsychotic exposure (years)	17.3 (5.4)	17.5 (5.5)
Pharmacotherapy		
Butyrophenones	19	32
Phenothiazines	32	29
Thioxanthenes	9	13
Anticholinergics	0	36

The two groups were homogenous with respect to the number of patients and to demographic and drug exposure characteristics, including drug dosage (Tab.2). Dose comparison between the antipsychotic compounds was made by converting antipsychotic doses to chlorpromazine equivalents, defined based upon the therapeutic effect of the drugs (Baldessarini, 1985).

In the group with EPS, acute dystonia was present in 23 patients, parkinsonism in 37 and tardive dyskinesia in 15. Twelve patients had experienced two or more different EPS. Thirty-six patients with EPS (but none in the control group) also received anticholinergic drugs (biperiden, orphenadrine or trihexiphenidyl) in order to treat parkinsonism or to prevent reappearance of acute dystonia.

The protocol was approved by the Ethics Committee at the Azienda Sanitaria Locale (ASL) n.5 Messina, Italy, and written informed consent to participate in the study was obtained from the patients or their relatives.

Genetically impaired *CYP2C9* activity and phenytoin intoxication. (Study V)

In this paper, we reported a severe phenytoin intoxication occurring in a 31-year-old woman (weight: 55 Kg; height: 159 cm) treated with oral phenytoin (100 mg 3 times a day) to prevent posttraumatic seizures after a severe head injury. On day 10 of phenytoin treatment, 3 hours after the morning dose, the patient manifested neurologic signs compatible with phenytoin intoxication (dysartria, nystagmus, dysmetria, left hemifacial dyskinesia and alteration in mental status). All symptoms and signs, except nystagmus, disappeared spontaneously within 30 minutes. A diagnostic procedure was immediately performed to exclude possible late consequences of the head trauma. Cerebral CT scan revealed no densitometric alterations in brain parenchyma and EEG showed diffuse, unspecific changes. An ECG recording was normal and standard laboratory tests were within the normal ranges. Phenytoin overdosing was then suspected and therapeutic drug monitoring was performed. Drug serum concentrations were monitored daily for 12 days after stopping drug treatment.

3.2 Genotyping methods

Genomic DNA was isolated from peripheral leukocytes by Qiagen Blood and Cell Culture DNA kit® (Qiagen, Hilden, Germany), according to the manufacturer's guidelines.

***CYP2D6* Genotype (Studies I, III & IV)**

The *CYP2D6*3* and *CYP2D6*4* alleles associated with the PM phenotype were determined by allele-specific PCR analysis, as described by Heim and Meyer (1990), and the *CYP2D6*6* as described by Saxena *et al.* (1994) in study I, and as described by Wennerholm *et al.* (1999) in studies III and IV. The *CYP2D6*5* allele was identified by RFLP assay with the restriction enzyme *Xba*I (Daly *et al.*, 1991), and by long-PCR analysis as described by Johansson *et al* (1996), in study I, and by long-PCR analysis, according to Wennerholm et al. (1999), in studies III and IV. All samples were further analysed by long-PCR for the duplicated/multiduplicated *CYP2D6* gene (Johansson *et al.*, 1996, in study I; Lundqvist *et al.*, 1999, in studies III and IV). Alleles where neither *CYP2D6*3, *4, *5, *6* nor the duplicated gene could be identified were classified as *CYP2D6*1* (wild type; wt) alleles.

***CYP2C9* Genotype (Studies II, III & V)**

Genotyping for *CYP2C9*2* and *CYP2C9*3* allelic variants of *CYP2C9* was performed by PCR followed by restriction enzyme analysis, as described by Yasar *et al.* (1999). Amplification products were digested with *Ava*II (*CYP2C9*2*) and with *Nsi*I (*CYP2C9*3*).

***CYP2C19* Genotype (Studies II, III & V)**

The CYP2C19*2 allele variant was identified by PCR followed by restriction enzyme analysis with *Sma*I, according to the method previously described by deMorais *et al.* (1994a), with minor modifications.

3.3 Drug assays

Study I

Steady-state plasma concentrations of risperidone and 9-OH-risperidone were measured by high-performance liquid chromatography, according to the method of Olesen and Linnet (1997), with slight modifications by the authors (Avenoso et al., 2000). The lowest limit of quantification was 2 nmol/L for both risperidone and its active metabolite. The inter-day coefficient of variation was less than 8.2% for risperidone and less than 6.5% for 9-OH-risperidone. Linearity was established in the range of 5–100 ng/ ml.

Study II

S- and *R*-warfarin plasma concentrations were determined by an HPLC method, modified after Henne *et al.* (1998). One ml of plasma was acidified with 0.2 ml 1N HCl, and racemic warfarin was extracted with diethylether (8 ml) for 15 min by means of a horizontal shaker. R-naproxene (1.6µg/ml) was used as internal standard. After centrifugation, the organic layer was evaporated in a bath at 50°C, under a gentle nitrogen stream, and the residue was reconstituted with 200 µl mobile phase. Fifty µl were then injected into a chiral column [(R,R) WHELK-01 (5 µm), Merck KGaA] connected with an HPLC pump (Waters, model 515). The mobile phase consisted of hexane/2-propanol/glacial acetic acid (75:25:0.5 v:v:v), pumped at a flow rate of 1 ml/min. The effluent was analysed with a UV detector (Waters, model 2487; λ = 313 nm), connected to a Hitachi-Merck integrator (model 7500). Recovery of the two isomers was similar and close to 100% (96.2 ± 5.1 %; n = 10). Within-day coefficients of variation at 0.1 µg/ml and 1 µg/ml were 7-8% and 4-5 % (n = 10), respectively. The lowest detection limits were 0.005 µg/ml for S-warfarin and 0.01 µg/ml for R-warfarin.

The unbound plasma fraction (f_u) of both enantiomers was determined by 4-h equilibrium dialysis at 37°C, as described by Banefield *et al.* (1983); water shift was calculated (about 13%) and corrected using Huang's equation (1983).

Study III

Plasma concentrations of the four enantiomers of fluoxetine and norfluoxetine were measured by a stereoselective HPLC assay, as described elsewhere (Gatti *et al.*, In press). As the daily dose of fluoxetine varied between 10 and 60 mg, the measured concentrations of the fluoxetine and norfluoxetine enantiomers were corrected for the dose (C/D; nmol/L/mg).

Study V

Therapeutic drug monitoring for phenytoin was performed by Immuno Assay, Roche Diagnostic Corporation.

3.4 Pharmacokinetic calculations

Study II

The oral unbound plasma clearance ($CL_{po,u}$) of both warfarin isomers was calculated according to the equation:

$CL_{po,u} = (D/2\tau)/ C_{ss} \times f_u$

where D is the daily dose of racemic warfarin, τ the dosing interval (i.e., 24 h) and C_{ss} the total concentration of S- or R-warfarin. For this formula to be valid, two assumptions must be verified: oral bioavailability of racemic warfarin must be complete, and warfarin plasma concentration measured 12-14 hours after dosing must be very close to the C_{ss}. Both these assumptions have been shown to be tenable on the basis of experimental data and theoretical considerations (Takahashi *et al.*, 1998b).

Since warfarin is a low extraction drug with no significant extra-hepatic elimination, its $CL_{po,u}$ reflects the intrinsic metabolic capacity of the liver (Wilkinson & Shaud, 1975).

The *S*- to *R*-warfarin C_{ss} plasma concentration ratio (*S:R*) was also calculated and considered as an index of the relative metabolic clearance of the *S*- vs *R*-enantiomer.

3.5 Statistical analysis

Study I

Data were analysed by Kruskal-Wallis non-parametric rank analysis of variance to establish an overall difference between the genotypes, then by Mann-Whitney U-test for two-group comparisons. A *p* value of 0.05 or less was regarded as significant.

Study II

All data passed the Kolmogorov-Smirnov normality test, and were therefore expressed as mean values ± SD. Differences between the 3 dose groups and between the 6 *CYP2C9* and 3 *CYP2C19* genotype groups were evaluated by one-way ANOVA, followed by the post hoc

Newman-Keuls test. When categorical data had to be compared, the chi-square test was used instead. Correlations between the $CL_{po,u}$ of *S*- and *R*-warfarin were assessed by means linear regression analysis. The significance level was set at $p<0.05$.

Study III

The Kruskal-Wallis test was used to compare fluoxetine daily dose, as well as plasma levels of individual enantiomers and the active moiety among the different genotype groups, followed by the Dunn´s Multiple comparison test for two-groups comparisons. Correlations between plasma concentrations of individual enantiomers and dosage, or between different enantiomers, were tested by using the non-parametric Spearman rank correlation coefficient (r_s). A *p* value of 0.05 or less was regarded as statistically significant.

Study IV

The Student *t*-test for unpaired data was used to compare differences in demographic and clinical characteristics of patients with and without EPS. The Chi-square test was used to compare the frequency of the mutated alleles relative to the appearance of EPS. A *p* value of 0.05 or less was regarded as significant.

4. Results

Study I

Based on the genotype analysis, three patients were classified as UM, 16 were homozygous EM, 15 heterozygous EM and three PM.

As shown in Table 3, there was a statistically significant difference (p<0.001) in steady-state plasma concentration-to-dose (C/D) ratios of risperidone among genotype groups, with statistically significant differences between PM and the other genotypes (p < 0.02).

Table 3. Steady-state plasma concentration-to-dose (C/D) ratios of risperidone, 9-OH-risperidone, risperidone/9-OH-risperidone ratio and the active moiety (sum of risperidone and 9-OH-risperidone) in the different CYP2D6 genotype groups. Results are expressed as median with range of values in parenthesis.

Genotypes	Risperidone[1] (nmol/L/mg)	9-OH-Risperidone (nmol/L/mg)	Risperidone/9-OH-risperidone ratio[1]	Active moyety (nmol/L/mg)
UM	0.6 [a, b]	27.3	0.03 [c, d]	28.5
(n=3)	(0.6-1.2)	(17.9-32.8)	(0.02-0.05)	(18.6-33.4)
Homozygous EM	1.1 [a, e]	31.5	0.04 [c, e]	35.5
(n=16)	(0.4-12.6)	(9.4-64.0)	(0.01-0.54)	(11.0-70.0)
Heterozygous EM	9.7[a]	31.2	0.23[c]	42.7
(n=15)	(0.6-17.6)	(17.0-51.1)	(0.04-0.66)	(17.6-60.0)
PM	17.4	23.0	0.79	40.4
(n=3)	(17.0-22.3)	(14.6-28.1)	(0.76-1.16)	(31.6-50.4)

1. Difference between genotype groups statistically significant, p<0.001, Kruskal-Wallis
a. Difference compared to PM, p<0.02, Mann-Whitney U-test
b. Difference compared to heterozygous EM, p<0.05, Mann-Whitney U-test
c. Difference compared to PM, p<0.01, Mann-Whitney U-test
d. Difference compared to heterozygous EM, p<0.02, Mann-Whitney U-test
e. Difference compared to heterozygous EM, p<0.01, Mann-Whitney U-test

The C/D of 9-OH-risperidone also varied widely but was not related to the genotype (Tab.3). The risperidone/9-OH-risperidone ratio was strongly associated with the *CYP2D6* genotype, with the highest ratios in PM (Tab.3; Fig.3). Heterozygous EM also had significantly higher ratios than homozygous EM or UM (Tab.3; Fig.3). No significant differences were found in the C/D of the sum of the plasma concentrations of risperidone and 9-OH-risperidone between the genotype groups (Tab.3; Fig.4).

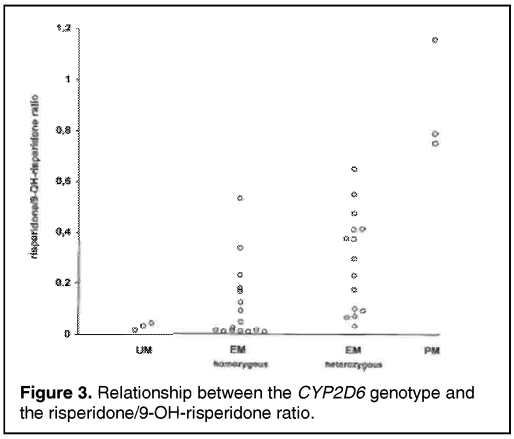


Figure 3. Relationship between the *CYP2D6* genotype and the risperidone/9-OH-risperidone ratio.

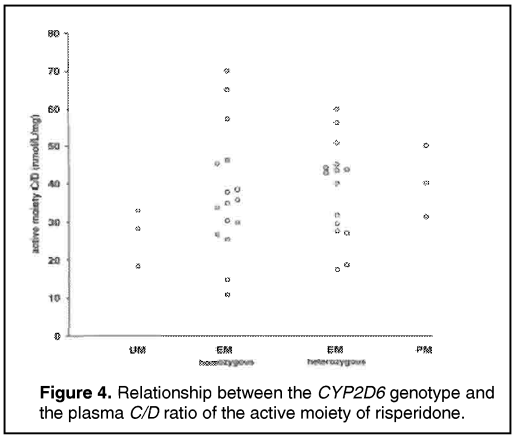


Figure 4. Relationship between the *CYP2D6* genotype and the plasma *C/D* ratio of the active moiety of risperidone.

In conclusion, the steady-state plasma concentrations of risperidone and the risperidone/9-OH-risperidone ratio were highly dependent on the *CYP2D6* genotype. However, there was lack of relationship between the genotype and the sum of risperidone and 9-OH-risperidone, suggesting that the *CYP2D6* polymorphism may be of limited importance for the

clinical outcome of the treatment, as as risperidone and 9-OH-risperidone are considered to have similar pharmacological activity.

Study II

Fifty-four patients carried no *CYP2C9* mutated alleles (**1/*1*), 31 carried one (**1/*2*, n=15; and **1/*3*, n= 16) and 8 two mutated alleles (**2/*2*, n=2, **3/*3*, n=2, and **2/*3*, n=4). Two subjects were homozygous and 19 were heterozygous for the *CYP2C19*2* allele variant. There was a statistically significant difference in the *CYP2C9* allele distribution between the dose groups, with the highest frequencies of *CYP2C9* mutated alleles in the LD group and the lowest in the HD group (Tab.4). On the other hand, no statistically significant difference was found in *CYP2C19* allele frequencies between the three groups (Tab.4).

Table 4. Allele and genotype distributions among the patients grouped according to warfarin dose requirement.

Allele frequencies	LD group (n=37)	MD group (n=32)	HD group (n=24)	X^2 - test
*CYP2C9*1*	0.554 [a,c]	0.797 [a,b]	0.979 [b,c]	$p<0.001$
*CYP2C9*2*	0.176 [a]	0.141 [b]	0.021 [a,b]	$p<0.005$
*CYP2C9*3*	0.270 [a,b]	0.062 [a]	0 [b]	$p<0.001$
*CYP2C19*1*	0.878	0.922	0.812	NS
*CYP2C19*2*	0.122	0.078	0.188	NS
Genotypes §				X^2 - test
*CYP2C9*1/*1*	11 (29.7) [a,c]	20 (62.5) [a,b]	23 (95.8) [b,c]	$p<0.001$
*CYP2C9*1/*2*	6 (16.2)	8 (25.0)	1 (4.2)	NS
*CYP2C9*1/*3*	13 (35.1) [a,b]	3 (9.4) [a]	0 [b]	$p<0.001$
*CYP2C9*2/*2*	2 (5.4)	0	0	NS
*CYP2C9*2/*3*	3 (8.2)	1 (3.1)	0	NS
*CYP2C9*3/*3*	2 (5.4)	0	0	NS
*CYP2C19*1/*1*	29 (78.4)	27 (84.4)	16 (66.7)	NS
*CYP2C19*1/*2*	7 (18.9)	5 (15.6)	7 (29.1)	NS
*CYP2C19*2/*2*	1 (2.7)	0	1 (4.2)	NS

§ Percentages in brackets
Values labeled with same letter, in each row, are significantly different.

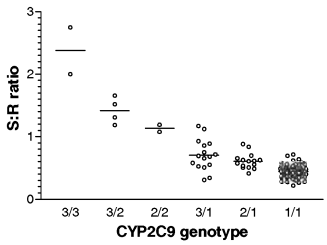


Figure 5. Relationship between *S:R* warfarin ratio and *CYP2C9* genotype

Warfarin maintenance dose, plasma *S:R* warfarin ratio and *S*-warfarin CL_{free} correlated significantly with *CYP2C9* (Tab.5; Fig.5). The *S*-warfarin Cl_{free} was significantly related to the *CYP2C9* genotype groups ($p<0.0001$), although most patients (72%) with no mutated alleles showed an *S*-warfarin Cl_{free} in the same range as those carrying mutated alleles (58-777 mil/min). No relationship was found between *S*-warfarin Cl_{free} and *CYP2C19* genotype or between *R*-warfarin Cl_{free} and either *CYP2C9* or *CYP2C19* genotype.

Our data confirm that there is a large inter-individual variability in warfarin dose requirements and pharmacokinetics. One major determinant for this appears to be *CYP2C9* polymorphism and, particularly, the **3* allelic variant. On the other hand, the *CYP2C19* genetic polymorphism is unlikely to represent a major source of variability.

Table 5. Weekly warfarin dose requirements (means ± SD, mg), plasma *S:R* warfarin ratios (mean ± SD) and clearances (mean ± SD, ml/min) in patients with different *CYP2C9* and *CYP2C19* genotypes.

Genotypes	Weekly warfarin doses	ANOVA	Plasma *S:R* warfarin ratios	ANOVA	CL_{free} S	ANOVA	CL_{free} R	ANOVA
							217 ± 116	
*CYP2C9*1/*1*	39.14 ± 15.47[a,b,c]		0.44 ± 0.11		660 ± 323[a,b]			
*CYP2C9*1/*2*	27.58 ± 11.25		0.61 ± 0.13*		380 ± 173		214 ± 83	
*CYP2C9*1/*3*	20.55 ± 7.14[a]	P < 0.0001	0.71 ± 0.25*	P < 0.0001	345 ± 185	P < 0.0001	207 ± 60	NS
*CYP2C9*2/*2*	20.63 ± 4.42		1.14 ± 0.08		213 ± 43		238 ± 57	
*CYP2C9*2/*3*	18.13 ± 9.44[b]		1.42 ± 0.21		155 ± 60[a]		207 ± 96	
*CYP2C9*3/*3*	8.75 ± 3.54[c]		2.37 ± 0.53		61 ± 4[b]		146 ± 37	
*CYP2C19*1/*1*	31.20 ± 14.93		0.65 ± 0.42		503 ± 321		241 ± 101	
*CYP2C19*1/*2*	35.33 ± 18.23	NS	0.49 ± 0.14	NS	520 ± 299	NS	225 ± 114	NS
*CYP2C19*2/*2*	35.00 ± 26.52		0.35 ± 0.09		979 ± 273		304 ± 200	

Values labeled with same letter, in each column, are significantly different.
The asterisks in the *S:R* warfarin ratio column indicate the only two values which are not significantly different following multiple comparisons.

Study III

The patients could be divided in four *CYP2D6* genotype groups: 59 homozygous EM, 26 heterozygous EM, 6 UM, and one PM. Fifty-five subjects were homozygous for *CYP2C9*1*, 25 carried the *CYP2C9*2* allele and 12 the *CYP2C9*3*. Two subjects were PM and 23 heterozygous EM for CYP2C19. Median (range) plasma concentrations corrected for the dose (C/D) were 9.9 (0.8-49.8) nmol/L/mg for *S*-fluoxetine, 3.0 (0.8-11) nmol/L/mg for *R*-fluoxetine, 13.5 (0.8-51.8) nmol/L/mg for *S*-norfluoxetine, and 5.5 (0.8-20) nmol/L/mg for *R*-norfluoxetine.

Table 6. Steady-state plasma concentration-to-dose (C/D) ratios of the enantiomers of fluoxetine and norfluoxetine in the different *CYP2D6* and *CYP2C19* genotype and CYP2C9 haplotype groups. Results are expressed as median with range of value in parenthesis (nmol/L per mg). (Kruskal-Wallis test)

Genotypes	S-fluoxetine	R-fluoxetine	S-norfluoxetine	R-norfluoxetine	Active Moiety	S-nor/S-flu ratio	S:R-norfluoxetine ratio
CYP2D6							
UM	6.35	4.95	18.0	8.45	28.9	1.75	2.4
(n=6)	(1.60-49.8)	(1.60-11.0)	(3.40-51.8)	(1.70-16.6)	(6.6-113)	(1.0-13.1)	(1.7-3.1)
Homozygous EM	7.80	2.70	13.5	6.10	26.6	1.73	2.35
(n=59)	(0.8-34.9)	(0.8-9.90)	(0.8-33.7)	(0.8-16.3)	(2.4-67.5)	(0.05-16.8)	(0.96-9-04)
Heterozygous EM	13.3	3.05	13.2	4.05	30.7	0.98	2.2
(n=26)	(0.8-47.2)	(0.8-7.90)	(0.8-33.2)	(0.8-20.0)	(2.5-70.8)	(0.22-4.71)	(0.62-5.54)
PM (n=1)	11.8	5.30	1.30	5.50	18.3	0.1	0.2
p	NS	NS	NS	NS	NS	$p<0.005$	NS
CYP2C9							
**1 /*1*	6.70	2.55	11.9	5.8	25.3	2.04	2.38
(n=40)	(0.8-27.8)	(0.8-7.4)	(0.8-33.7)	(0.8-10.3)	(2.4-51.3)	(0.05-13.1)	(1.0-9.04)
**1/*2* or **2/*2*	8.40	3.6	13.5	6.1	28.7	1.64	2.27
(n=19)	(0.8-49.8)	(0.8-11.0)	(0.8-51.8)	(0.8-16.6)	(2.4-113)	(0.56-16.8)	(0.96-6.30)
**1/*3*	17.8	4.75	12.4	7.8	40.8	0.88	2.77
(n=6)	(5.2-26.5)	(2.6-6.8)	(8.5-24.7)	(1.4-11.8)	(26.4-50.6)	(0.48-3.6)	(1.7-5.88)
p	NS	$p<0.05$	NS	NS	$p<0.05$	NS	NS
CYP2C19							
**1/*1*	9.90	2.90	12.5	4.90	26.6	1.2	2.14
(n=67)	(0.8-47.2)	(0.8-9.90)	(0.8-31.3)	(0.8-16.3)	(2.4-70.8)	(0.05-16.8)	(0.23-6.30)
**1/*2*	10.3	3.70	15.2	6.40	30.1	1.78	2.58
(n=23)	(0.8-49.8)	(0.8-11.0)	(0.8-51.8)	(0.8-20.0)	(2.4-113)	(0.48-13.1)	(1.0-9.04)
**2/*2*	10.0	2.50	11.5	3.45	24.0	1.51	3.91
(n=2)	(4.8-15.2)	(1.90-3.10)	(10.8-12.2)	(2.20-4.70)	(17.5-30.4)	(0.8-2.22)	(2.27-5.54)
p	NS	NS	NS	NS	NS	NS	NS

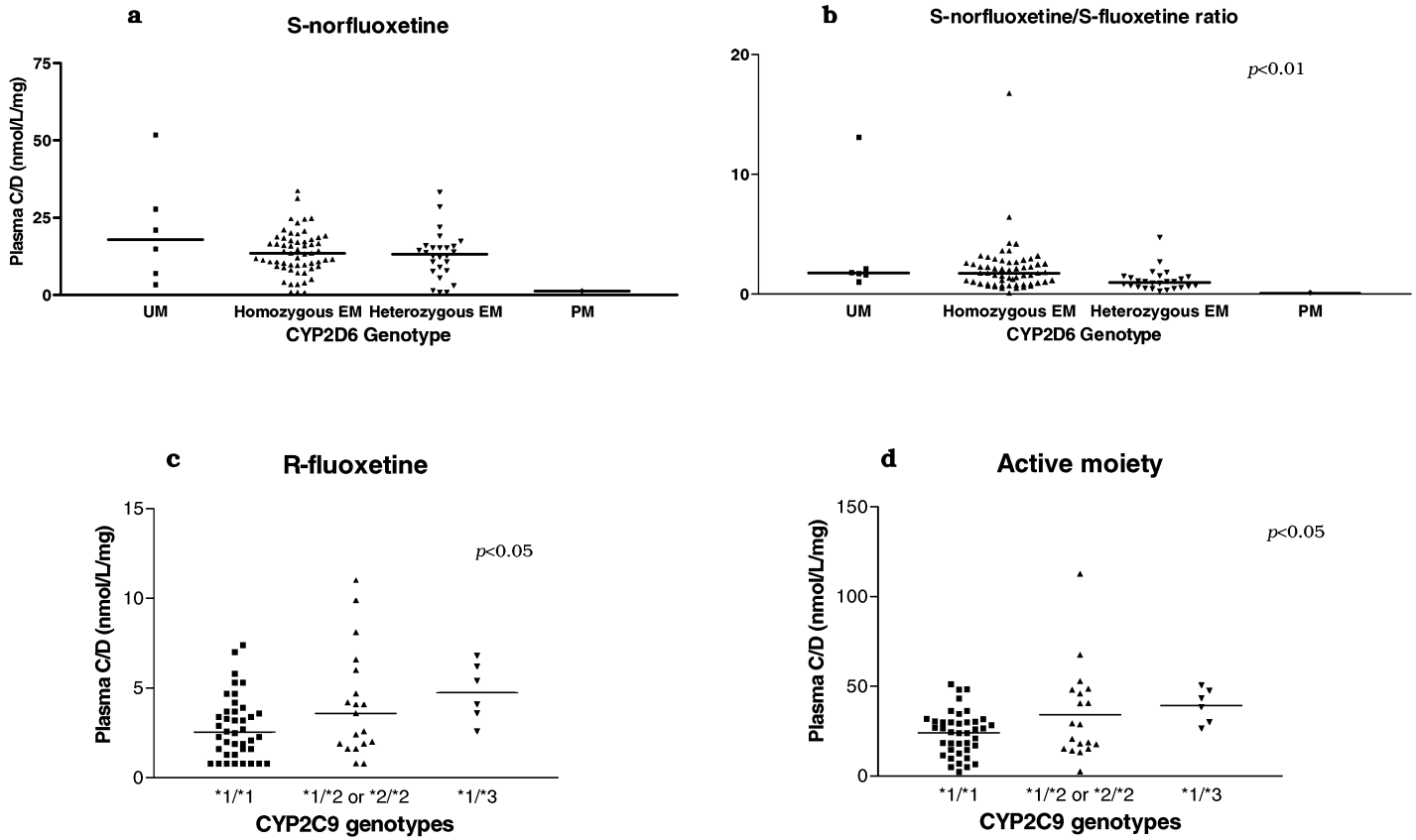


Figure 6. Relationship between *CYP2D6* genotype and concentration-to-dose (C/D) ratios of S- norfluoxetine (a); relationship between *CYP2D6* genotype and S-norfluoxetine/S-fluoxetine ratio (b); relationship between *CYP2C9* genotype and C/D of R-fluoxetine (c); relationship between *CYP2C9* genotype and active moiety of fluoxetine (d). Lines showing the median. Kruskal-Wallis test

There was no statistically significant relationship between the *CYP2D6* or *CYP2C19* genotypes and the enantiomer plasma levels or their active moiety, defined as the sum of S-fluoxetine, R-fluoxetine and S-norfluoxetine, except for the finding of very low levels of *S*-norfluoxetine in the only CYP2D6 PM (Tab.6; Fig.6a). A significant relationship ($p<0.01$) was found between *CYP2D6* genotype and S-norfluoxetine/S-fluoxetine ratio (Tab.6; Fig.6b). A statistically significant relationship ($p<0.05$) was also found between *CYP2C9* genotype (Fig.6c) and C/D of R-fluoxetine, as well as between *CYP2C9* genotype and active moiety (Tab.6; Fig.6d).

Our results suggest that the polymorphic CYP2D6 and CYP2C9 may contribute to the broad interindividual variability in the fluoxetine kinetics at steady-state.

Study IV

Sixty-five patients (54.6%) were homozygous for a functional *CYP2D6*1* allele, 44 (37.0%) were heterozygous for detrimental alleles, and 4 (3.4%), who carried two detrimental alleles, were classified as PM. In six patients (5.0%) duplication of a functional *CYP2D6* gene was found, and they were consequently classified as ultrarapid metabolizers (UM). The frequency of detrimental alleles was slightly higher in the group with EPS than in the control group (Table 7), but this difference was not statistically significant.

Table 7. *CYP2D6* genotype and allele frequencies in patients with EPS and control patients (%).

Genotype	Controls (n=56)	Patients with EPS (n=63)
**1/*2x2*	3 (5.4)	3 (4.8)
**1/*1*	32 (57.1)	33 (52.4)
**1/*3, *1/*4, *1/*5, *1/*6* or **2x2/*4*[a]	21 (37.5)	23 (36.5)
**4/*4* or **4/*5*	0	4 (6.3)
Alleles		
**1*	87/112 (77.7)	89/126 (70.6)
**3, *4, *5* or **6*	21/112 (18.7)	31/126 (24.6)
**2x2*	3/112 (2.7)	3/126 (2.4)
**2x2* or **4x2*	1/112 (0.9)	3/126 (2.4)

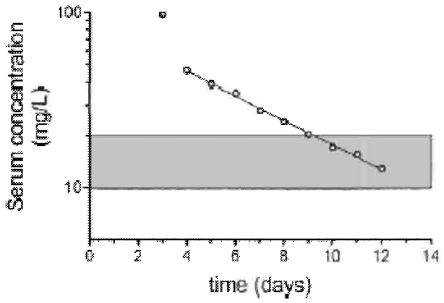
Homo- and heterozygous extensive metabolizers (EM) as well as UM were equally distributed between patients with and without EPS, whereas all the PM had a history of EPS (Tab.7). This suggests that the PM genotype may represent a predisposing factor for antipsychotic-induced EPS.

Study V

At the first determination, the phenytoin serum concentration was higher than the upper limit of the assay method (>100 mg/L) and remained so on the next day. Three days after the onset of symptoms and withdrawn of phenytoin, the phenytoin serum concentration fell into the assay range (97 mg/L) and could be reliably measured.

The time course of drug serum concentrations was monitored daily until they fell within the established therapeutic range (10-20 mg/L), namely for 12 days. Phenytoin concentrations decayed according to a one-compartment linear model with an elimination half live of 103 h (Fig. 7), i.e. about 5 times longer than the mean value generally quoted (22 hours) (Dollery, 1999). In the absence of any acquired predisposing factor for phenytoin toxicity, mutations in the CYPs responsible for phenytoin metabolism (CYP2C9 and CYP2C19) were suspected. Genotyping revealed that the patient was homozygous for the *CYP2C9*3* allele (*CYP2C9*3/*3*) and heterozygous for the *CYP2C19*2* allele (*CYP2C19*1/*2*).

Figure 7. Time course of phenytoin serum concentration decay after drug withdrawal. *Shaded area,* Therapeutic range (10- 20 mg/L). Concentrations on days 1 and 2 were above the upper detection limit of assay (100 mg/L).



5. Discussion

Study I

Most psychotropic drugs are metabolised, at least partially, through CYP2D6. Therefore, the genetically determined variability in its activity might affect the clinical outcome of antipsychotic therapy, by influencing the plasma levels of the drugs or their metabolites. This may be of particular importance for drugs with severe concentration dependent side effects. It has been shown that the likelihood for risperidone-induced EPS increases with increasing plasma riperidone and 9-OH-risperidone concentrations (Tarsy et al., 2002; Yoshimura et al., 2001). Since risperidone metabolism is, at least partially, catalysed by CYP2D6, polymorphic enzyme activity might influence clinical outcome. Our data show that the steady-state plasma concentrations of risperidone are highly dependent on the *CYP2D6* genotype. Among our patients, the PM reached the highest C/Ds of risperidone (Tab.3). Furthermore, the C/Ds of risperidone in the three UM patients were in the lower range of those seen in EM. The risperidone/9-OH-risperidone ratio was also strongly related to the CYP2D6 genotype, with no overlapping between PM and EM subjects and with significantly higher ratios in heterozygous EM than in homozygous EM or in UM (Tab.3; Fig.3). However, a considerable overlap was seen both in risperidone concentrations and the risperidone/9-OH-risperidone ratio between homo- and heterozygous EM (Fig.3). Thus, patients with either PM (no functional CYP2D6 genes) or UM (gene duplication/amplification) genotypes constituted two clear extremes with respect to risperidone metabolism, while a large interindividual variability and an overlapping was seen within the homo- and heterozygous EM genotypes. The sum of the plasma concentrations of risperidone and 9-OH-risperidone, corrected for the dose, did not differ significantly between subjects of different genotypes (Tab.3; Fig.4). These results are consistent with other studies, conducted in Caucasians and in Orientals (Olesen et al.1998; Roh et al., 2001).

Risperidone and 9-OH-risperidone are claimed to have similar pharmacological activity. Therefore, the lack of relationship between the genotype and the sum of risperidone and 9-OH-risperidone suggests that the *CYP2D6* polymorphism may be of limited importance for the clinical outcome of the treatment. However, the similarity in pharmacological activity has been inferred from studies of receptor occupancy, mainly conducted *in vitro* or in animal models, while clinical data are lacking (van Beijsterveldt et al., 1994; Nyberg et al., 1995). There are anecdotal reports suggesting that PMs might be more prone to side effects (Bork et al., 1999) and UMs to therapeutic failure (Gusey et al., 2000) as compared to EMs. Besides, subjects who lack CYP2D6 activity, depend almost completely on CYP3A4 for the metabolism of the drug. Therefore, they may be more susceptible to drug-drug interactions when CYP3A4 inhibitors or

inducers are co-administered (Spina et al., 2001a). Recent evidence indicates that certain inhibitors of CYP2D6, i.e. paroxetine and fluoxetine (Spina et al., 2001b; Spina et al., 2002), or inducers of CYP3A4, i.e. carbamazepine (Spina et al., 2000), may cause a significant increase or decrease in total plasma risperidone concentrations (risperidone plus 9-hydroxyrisperidone), possibly associated with clinically relevant effects. In this respect, while no clear-cut correlation has been found between plasma concentrations of risperidone and 9-hydroxyrisperidone and antipsychotic response, the plasma levels of the active moiety may correlate with the occurrence of extrapyramidal side effects (Spina et al., 2001c).

It has recently been shown that CYP2D6 plays a predominant role in the formation of (+)-9-OH-risperidone, the major metabolic pathway in clinical conditions, whereas enzymes of the CYP3A family catalyse the formation of the (-)-9-OH-metabolite (Yasui-Furukori et al., 2001). So far, no information about pharmacological activity of the two enantiomers is available, i.e. whether or not they have equal pharmacological activity. Therefore, it might be hypothesised that, if the (+)-9-OH-risperidone has different activity than its (-)-enantiomer, as shown for other drugs (for example norfluoxetine), the *CYP2D6* polymorphism might have an impact on clinical outcome.

Three of the 37 patients (8%) were found to carry a duplicated functional *CYP2D6* gene and were thus classified as UM. Although the number of subjects in the present study is small, the results indicate that the prevalence of this allelic variant in the South-Italian (Sicilian) population is similar to that among Spaniards (Agundez et al. 1995). This is consistent with the more frequent distribution of *CYP2D6* gene duplication in the Mediterranean area compared to Northern Europe. The low levels of risperidone and the low risperidone/9-OH-risperidone ratio among subjects carrying gene duplication compared to other EM are also consistent with the association between this genotype and extremely high CYP2D6 activity, in the Italian population.

Study II

Recent clinical studies suggest that patients with low warfarin dose requirement have a greater prevalence of *CYP2C9*2* and **3* alleles and a higher risk of major haemorrhages than control patients (Steward et al., 1997; Aithal et al, 1999; Margaglione et al., 2000). Maintenance warfarin daily doses (adjusted to get a target INR value) are highly variable among patients because of several factors (polymorphic metabolism, hereditary resistance, pharmacokinetic/dynamic interactions, dietary vitamin K intake, etc.). Among pharmacokinetic factors influencing warfarin response, liver metabolism plays a pivotal role, since nearly 100%

of the dose is eliminated by biotransformation (Holford, 1986). Therefore, unbound oral clearance of S- and R-warfarin may be related to the activity of CYP2C9 and possibly CYP2C19, respectively.

A major question that needs to be answered is whether *CYP2C9* genotyping is clinically useful to identify patients with poor warfarin metabolism. Our data confirm that there is large inter-individual variability in warfarin dose requirements and pharmacokinetics. One major determinant for this appears to be *CYP2C9* polymorphism and, particularly, the **3* allele variant. By contrast, *CYP2C19* genetic polymorphism is unlikely to represent a major source of variability. Confirming previous *in vivo* and *in vitro* finding (Lee et al, 2002; Yasar et al., 2002), *CYP2C9*2* variant was found to cause less pronounced reduction in S-warfarin Cl_{free} compared to *CYP2C9*3*. Evaluation of the *CYP2C9* genotype before starting warfarin therapy might help physicians to choose the right initial dose, thereby lowering the risk of bleeding complications.

A clinical benefit from genotyping may be predicted for the group of patients who carry two defect *CYP2C9* alleles (**3/*3*, **3/*2*, or **2/*2* genotypes) and have low $Cl_{free.}$ This condition, found at a frequency of 8.6% among our patients, is present on average at a frequency of 4-6% among various Caucasian populations. These patients may also be identified by an *S:R* enantiomeric ratio of warfarin >1. These findings also suggest that warfarin could be a useful probe-drug to phenotype subjects for CYP2C9, and that the warfarin *S:R* ratio may be a good index of CYP2C9 activity. Conversely, the broad variability in warfarin dose requirement and metabolism and the great overlapping seen between subjects carrying one or no detrimental alleles, suggests that *CYP2C9* genotyping is unlikely to result in clinical benefit in these genotype groups (Tab.5).

Study III

Many CNS-active drugs are metabolised through several polymorphic enzymes. An important example is the antidepressant fluoxetine. Previous investigations conducted *in vitro* and *in vivo* after single and multiple doses suggest that CYP2D6 plays a major role in the metabolism of fluoxetine (Fjordside et al., 1999; Margolis et al., 2000; Eap et al., 2001). The role of CYP2D6 in the stereoselective metabolism of S-fluoxetine to S-norfluoxetine is also suggested by our findings of very low levels of S-norfluoxetine and very low S-norfluoxetine/S-fluoxetine and S:R-norfluoxetine ratios in the only *CYP2D6* PM (Tab.6; Fig.6a). Unfortunately, the small number of CYP2D6 PMs found among our patients did not allow statistical evaluations.

Apart from CYP2D6, other isozymes such as CYP2C9, CYP2C19 and CYP3A4 have also been reported to catalyze fluoxetine metabolism (Stevens & Wrighton, 1993; von Moltke et al., 1997; Margolis et al., 2000; Liu et al., 2001b). Their role might become more important during chronic dosing when the contribution of CYP2D6 is diminished due to its saturation kinetics and the inhibitory action of fluoxetine and norfluoxetine enantiomers on the latter enzyme (Ring et al., 2001).

While in our patients no relationship was observed between *CYP2C19* genotypes and plasma levels of fluoxetine enantiomers, a significant relationship was found between *CYP2C9* genotype and C/D of R-fluoxetine (Tab.6; Fig.6c). These results suggest a role for CYP2C9 in fluoxetine metabolism, confirming previous *in vitro* findings (von Moltke et al., 1997). As seen for other CYP2C9 substrates, such as warfarin (Scordo et al., In press) and losartan (Yasar et al., 2002), the *CYP2C9* allelic variants evaluated have a different impact on fluoxetine metabolism. The findings of higher levels of both S- and R-fluoxetine among subjects carrying *CYP2C9*3* compared to the other genotypes, suggest that the *CYP2C9*3* allele is associated with an impaired enzymatic activity, compared to *CYP2C9*1* and **2*.

Because R-fluoxetine, S-fluoxetine and S-norfluoxetine are much more potent than R-norfluoxetine as inhibitors of serotonin reuptake, we also explored correlations between genotypes and the plasma levels of the active moiety, defined as the sum of the concentrations of the three active enantiomers. *CYP2D6* and *CYP2C19* genotypes did not show a significant association with the active moiety (Tab.6). Therefore, genetic polymorphims affecting the activity of CYP2D6 or CYP2C19 are not likely to play a major role in the therapeutic outcome. Conversely, the active moiety significantly correlated with the *CYP2C9* genotype, subjects carrying the *CYP2C9*3* having the highest levels (Tab.6; Fig.6d). This suggests that genetically determined interindividual differences in CYP2C9 activity might theoretically influence clinical response to fluoxetine therapy.

Study IV

The use of antipsychotic drugs in the treatment of schizophrenia is associated with extrapyramidal side effects (EPS). They occur in a majority of patients receiving traditional antipsychotics, while the risk for EPS appears to be lower with the newer compounds. EPS appear to be related to the antipsychotic dosage, and, consequently, to the plasma concentration of the drug. Therefore, interindividual differences in the elimination kinetics and in steady state plasma concentrations of antipsychotics resulting from genetically determined variability in the expression of CYP2D6 may have important clinical implications.

Several studies have investigated the possible association between *CYP2D6* genotype and movement disorders induced by antipsychotic drugs (Scordo & Spina, 2002). The results of studies evaluating the relationship between CYP2D6 activity and parkinsonism and/or tardive dyskinesia indicated that, although *CYP2D6* genotype is unlikely to be a determinant of susceptibility to acute dystonic reactions, it may represent a contributing factor for the occurrence and severity of other antipsychotic-induced movement disorders, including tardive dyskinesia. Consistent with these findings, in our study, all the four PM subjects found among the 119 schizophrenic patients recruited, had developed EPS (parkinsonism or tardive dyskinesia) while treated with different classical antipsychotics. The results of a more recent study addressing the association between *CYP2D6* genotype, antipsychotic exposure and Abnormal Involuntary Movement Scale (AIMS) score in a group of 31 schizophrenic patients, suggest that patients carrying a *CYP2D6*3* or **4* allele may have a higher risk for developing antipsychotic induced abnormal movements (Ellingrod et al., 2000). Consistently, Ohmori and coll. (1998), in a retrospective investigation, found a significant association between the detrimental *CYP2D6*10* allele and the total AIMS score and a moderate association with tardive dyskinesia in 100 Japanese schizophrenics treated with several antipsychotics. Some, but not all studies have shown an overrepresentation or a tendency towards such, of mutated *CYP2D6* alleles in patients experiencing these side-effects (Scordo & Spina, 2002). Consistently, in a recent study (Jaanson et al., 2002) a tendency to higher risk of parkinsonism or TD has been found in patients carrying at least one mutated *CYP2D6* allele. These results are consistent with the findings that in most studies PM subjects were more prone to develop EPS than EMs. As the PM condition may contribute to the susceptibility to antipsychotic-induced EPS, knowledge of genotype could allow physicians to give lower initial doses of antipsychotics to the PM, thereby reducing the risk of EPS.

Study V

Among CNS-active drugs metabolised through polymorphic enzymes, an important example is the anticonvulsant phenytoin, metabolised by CYP2C9 and CYP2C19. There is evidence that CYP2C9 mutations have a greater influence on phenytoin metabolism than CYP2C19 mutations. Odani et al (1997) reported that the mean phenytoin maximum rate of metabolism (Vmax) in patients heterozygous for *CYP2C19*2* is slightly but not significantly lower than in patients homozygous for *CYP2C19*1*; conversely, patients heterozygous for *CYP2C9*3* allele have a significantly lower Vmax than subjects with homozygous for *CYP2C9*1*. Similar results were reported by Mamiya et al. (1998), who found greater

impairment of phenytoin hydroxylation in patients carrying *CYP2C9* mutated alleles than in those carrying *CYP2C19* mutated alleles. Kidd et al. (1999) reported that the oral clearance of phenytoin in a *CYP2C9*3* homozygous subject was 21% of the mean value of subjects homozygous for *CYP2C9*1*. Another, less severe case of phenytoin intoxication has recently been reported by Ninomiya et al. (2000) in a patient with epilepsy who complained of diplopia and ataxia while receiving a low maintenance dose (187.5 mg/d; phenytoin serum concentration, 32.6 mg/L). He was heterozygous for both *CYP2C9*3(*1/*3)* and *CYP2C19*3(*1/*3)*, and his Vmax and Km values were 18.4% lower and 3 times higher, respectively, than those found in patients with normal *CYP2C9/19* alleles. In view of the markedly reduced metabolic activity of CYP2C*3 in comparison with the wild-type enzyme (about one fifth) and of the minor role of CYP2C19 in phenytoin metabolism, it is likely that the *CYP2C9* genotype of our patient was largely responsible for the intoxication with phenytoin. Our data and evidence in the literature indicate that homozygosity for mutated *CYP2C9* alleles may cause marked impairment of phenytoin metabolism and clinically relevant adverse drug reactions. Recently, Keb et al., (2001) have shown that the number of *CYP2C9* mutated alleles is a major determinant of phenytoin plasma levels in volunteers after sigle doses, and suggested that it has some predictive value also in patients at steady-state.

6. Concluding remarks

Recent advances in the understanding of drug metabolism and the molecular biology of CYPs have generated great expectations for the use of pharmacogenetic testing as a tool to individualize drug treatment. In principle, a simple phenotyping or genotyping test before treatment with a compound undergoing polymorphic metabolism could be of value in selecting the starting dose to enhance therapeutic efficacy and to prevent toxicity (Bertilsson et al., 1993; Kirchheiner et al., 2001).

However, although the role of polymorphic CYPs in the metabolism of many drugs has been assessed by several *in vitro* and *in vivo* studies, no clear relationship has been demonstrated between polymorphic CYPs activity and therapeutic outcome. Theoretically, when treated with standard doses of substrates of polymorphic CYPs, PMs should be more prone to develop concentration-dependent adverse effects, while UMs should be at higher risk of therapeutic failure. In accordance with this, our studies suggest that PMs of CYP2D6 are more prone to EPS during treatment with classical antipsychotics. Similarly, genetically impaired CYP2C9 and CYP2C19 metabolic activity seems to be a predisposing factor for phenytoin toxicity.

Whether pheno- or genotyping can be used prospectively to predict an optimal dose range and to improve the therapeutic outcome and minimise side-effects remains to be evaluated. In a recent study, Chou *et al.* (2000) found a trend towards an increasing number of adverse effects in patients treated with psychoactive drugs primarily metabolized by CYP2D6 (tricyclic antidepressants and antipsychotics), moving from UM to PM genotypes. Furthermore the cost for treating patients with extremely low or high CYP2D6 activity (i.e. PM and UM) was fairly higher (on average 4000-6000 dollars/year) than the costs of treating patients with homozygous or heterozygous EM genotypes, and the total duration of hospital stay also tended to be longer for patients in the PM group.

CYP2D6 genotype appears to partially explain the broad variability in the steady state plasma levels of risperidone and fluoxetine, but its usefulness in predicting optimal dosage and clinical effects remains low. On the other hand, the significant relationship found in our studies between *CYP2C9* genotype and warfarin dose requirement and clearance, as well as between *CYP2C9* genotype and fluoxetine active moiety, suggest that knowledge of this genotype before starting therapy might help physicians in choosing the right dose, and/or avoiding potentially harmful drugs in selected subgroups of patients carrying defect alleles.

At present, pheno- and genotyping appear to be only of relatively limited value to optimize the dosage regimen of psychotropic drugs and cannot replace therapeutic drug monitoring. Besides, many patients are treated concomitantly with more than one drug, being

thus at high risk for pharmacokinetic interactions. Furthermore, patients often show a poor compliance with the therapy. Therefore, TDM still represents a better tool for individualization of dosage, compared to evaluation of polymorphic enzyme activity by geno- or phenotyping. *CYP2D6* genotyping can today be recommended as a complement to plasma concentration determination when aberrant metabolic capacity (poor or ultrarapid) of CYP2D6 substrates is suspected (Dahl & Sjöqvist, 2000). Kawanishi et al. (personal comm.) have found an increased incidence of subjects carrying *CYP2D6* gene duplication amonr patients with persistant mood disorders. These results suggest that *CYP2D6* gene duplication may represent a risk factor for antidepressant drug-resistance, probably because of ultrarapid drug metabolism (Kawanishi et al., personal comm.).

On the other hand, *CYP2C9* genotyping might be useful in order to optimise the therapy with drugs metabolised by this enzyme such as warfarin and phenytoin, both having a very narrow therapeutic index.

The primary goal of therapy-optimising tools is to provide relevant information to clinicians, so that they may more efficiently and effectively use drugs to manage diseases in patients. TDM has proved to be a useful tool to evaluate and, if needed, to correct an already established therapy, but it does not provide any support to clinicians in choosing the starting dose or the most effective and safe therapy for an individual patient.

Conversely, knowledge of the patient´s genotype before starting a therapy might theoretically result in great help for the clinicians in choosing the right starting dose and in avoiding potentially harmful drugs for a specific patient, thus minimising the risk of adverse events or therapeutic failure. Clinical research on CYP2C9 genotyping is beginning to converge to provide this insight. There appears to be sufficient evidence to proceed with the prospective evaluation of genotype and phenotype- guided drug dosage design in patients requiring warfarin and phenytoin.

7. Future perspectives

Pharmacogenomics has great potential to improve the appropriate and effective use of drugs. Besides the practical application of genomic knowledge to develop better drugs with fewer side effects, pharmacogenomics has the potential to narrow a drug's target audience and thus reduce costly adverse effects. Variation in patient response to therapy is a significant therapeutic problem. Genetic differences contribute to broad interpatient variability. Polymorphisms have been identified in different CYPs that may determine whether an individual will fail to respond to a drug or suffer an exacerbated clinical response. However, although there is extensive *in vitro* and *in vivo* evidence on the involvement of polymorphic CYPs in the metabolism of many drugs, there is still lack of evidence of significant relationships between CYP activity or genotype and therapeutic response. Therapeutic drug monitoring is still a first-choice tool for individualization of dosage, but genotyping can be of additional value to identify genetically determined deficent or ultrarapid metabolism as a cause of high or low plasma levels of drugs metabolised by polymorphic enzymes.

Over the next decade predetermination of a patient CYP profile might become possible in the physician's office. The next step should involve prospective studies to establish the predictive power of genotyping for preventing toxicity and determining the specific dosage recommendations.

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9. References

Agúndez JAG, Ledesma MC, Ladero JM, Benitez J (1995) Prevalence of CYP2D6 gene duplication and its repercussion on the oxidative phenotype in a white population. Clin Pharmacol Ther 57: 265-269

Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphism in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet 1999: 353, 717-719

Alexanderson B, Evans DA, Sjoqvist F. Steady-state plasma levels of nortriptyline in twins: influence of genetic factors and drug therapy. Br Med J 1969; 4: 764-8

Alván G, Bechtel P, Iselius L, Gundert-Remy U (1990) Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. Eur J Clin Pharmacol 39: 533-537

Andreassen OA, MacEwan T, Gulbrandsen AK, McCreadie RG, Steen VM (1997) Non-functional CYP2D6 alleles and risk for neuroleptic-induced movement disorders in schizophrenic patients. Psychopharmacology 131: 174-179

Armstrong M, Daly AK, Blennerhassett R, Ferrier N, Idle J (1997) Antipsychotic drug-induced movement disorders in schizophrenic patients in relation to CYP2D6 genotype. Br J Psychiatry 170: 23-26

Arthur H, Dahl ML, Siwers B, Sjöqvist F (1995) Polymorphic drug metabolism in schizophrenic patients with tardive dyskinesia. J Clin Psychopharmacol 15: 211-216

Avenoso A, Facciola G, Salemi M, Spina E (2000) Determination of risperidone and its major metabolite 9-hydroxyrisperidone in human plasma by reversed-phase liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Sci Appl 746:173-81

Baldessarini RJ (1985) Chemotherapy in Psychiatry. Harvard University Press, Cambridge

Banefield C, O'Reilly R, Chan E, Rowland M. Phenylbutazone-warfarin interaction in man: further stereochemical and metabolic considerations. Br J Clin Pharmacol 1983; 16: 669-675.

Bernal ML, Sinues B, Johansson I, McLellan RA, Wennerholm A, Dahl ML, Ingelman-Sundberg M, Bertilsson L. Ten percent of North Spanish individuals carry duplicated or triplicated CYP2D6 genes associated with ultrarapid metabolism of debrisoquine. Pharmacogenetics 1999; 9: 657-60

Bertilsson L, Dahl ML (1996) Polymorphic drug oxidation: relevance to the treatment of psychiatric disorders. CNS Drugs 5: 200-223

Bertilsson L, Dahl ML, Dalen P, Al-Shurbaji A. Molecular genetics of CYP2D6: Clinical relevance with focus on psychotropic drugs. Br J Clin Pharmacol 2002; 53: 111-122

Bertilsson L, Dahl ML, Ingelman-Sundberg M, Johansson I, Sjoqvist F. Inter-individual and inter-ethnic differences in polymorphic drug oxidation. Implication for drug therapy with focus on psychoactive drugs. In: Pacifici GM, Fracchia GN eds. Advanced in Drug metabolism in man. Bruxelles: European Communities, 1995; 86-136

Bertilsson L, Dahl M-L, Sjöqvist F, Åberg-Wistedt A, Humble M, Johansson I, Lundqvist E, Ingelman-Sundberg M (1993) Molecular basis for rational megaprescribing in ultrarapid hydroxylators of debrisoquine. Lancet 341: 63

Bertilsson L, Eichelbaum M, Mellström B, Sawe J, Schulz HU, Sjöqvist F. Nortriptyline and antipyrine clearance in relation to debrisoquine hydroxylation in man. Life Sci 1980; 27:1673-7

Bertilsson L, Lou Y-Q, Du Y-L, Liu Y, Kuang T-Y, Liao X-M. Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylation of debrisoquine and S-mephenytoin. Clin. Pharmacol. Ther.; 51: 388-397, 1992.

Bork JA, Rogers T, Wedlund PJ, de Leon J (1999) A pilot study on risperidone metabolism: the role of cytochrome P450 2D6 and 3A. J Clin Psychiatry 60: 469-476

Broly F, Gaedigk A, Heim M, Eichelbaum M, Morike K, Meyer UA (1991) Debrisoquine/sparteine hydroxylation genotype and phenotype: analysis of common mutations and alleles of CYP2D6 in a European population. DNA Cell Biol 10: 545-558

Brøsen K, de Morais SMF, Meyer UA, Goldstein JA. A multifamily study on thr relationship between CYP2C19 genotype and S-mephenytoin oxidation phenotype. Pharmacogenetics; 5: 312-317, 1995.
Brøsen K, Gram L. Clinical significance of sparteine/debrisoquine oxidation polymorphism. Eur J Clin Pharmacol 1989; 36: 537-47
Carson PE, Flanagan CL, Iokes CE, Alving AS. Enzymatic deficiency in primaquine-sensitive erythrocytes. Science 1956; 124: 484-5
Casey DE (1995) Motor and mental aspects of extrapyramidal syndromes. Int Clin Psychopharmacol 10 (suppl 3): 105-114
Casey DE (1996) Extrapyramidal syndromes: epidemiology, pathophysiology and the diagnostic dilemma. CNS Drugs 10 (suppl 1): 1-12.
Chan E, McLachlan A, O'Reilly R, Rowland M. Stereochemical aspects of warfarin drug interactions. Use of a combined pharmacokinetic-pharmacodynamic model. Clin Pharmacol Ther 1994; 56: 286-94.
Chang M, Dahl M-L, Tybring G, Gotharson E, Bertilsson L. Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: comparison with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype. Pharmacogenetics; 5: 358-363, 1995.
Chouinard G, Arnott W (1993) Clinical review of risperidone. Can J Psychiatry 38 (suppl 3): 89-95
Chou WH, Yan FX, de Leon J, Barnhill J, Rogers T, Cronin M, Pho M, Xiao V, Ryder TB, Liu WW, Teiling C, Wedlund PJ. Extension of a pilot study: impact from the cytochrome P450 2D6 polymorphism on outcome and costs associated with severe mental illness. J Clin Psychopharmacol 2000; 20:246-51
Dahl ML, Iselius L, Alm C, Svensson JO, Lee D, Johansson I, Ingelman-Sundberg M, Sjoqvist F. Polymorphic 2-hydroxylation of desipramine. A population and family study. Eur J Clin Pharmacol 1993; 44: 445-50
Dahl ML, Johansson I, Bertilsson L, Ingelman-Sundberg M, Sjöqvist F (1995) Ultrarapid hydroxylation of debrisoquine in a Swedish population. Analysis of the molecular genetic basis. J Pharmacol Exp Ther 274: 516-520
Dahl ML, Johansson I, Porsmyr Palmertz M, Ingelman-Sundberg M, Sjöqvist F (1992) Analysis of the CYP2D6 gene in relation to debrisoquine and desipramine hydroxylation in a Swedish population. Clin Pharmacol Ther 51: 12-17
Dahl ML, Sjoqvist F. Pharmacogenetic methods as a complement to therapeutic monitoring of antidepressants and neuroleptics. Ther Drug Monit 2000; 22: 114-7
Daly AK, Armstrong M, Monkman SC, Idle JR (1991) Genetic and metabolic criteria for the assignment of debrisoquine 4-hydroxylation (cytochrome P4502D6) phenotypes. Pharmacogenetics 1: 33-41
Daly AK, Cholerton S, Gregory W, Idle JR. Metabolic polyporphisms. Pharmacol Ther 1993; 57: 129-160
de Morais SMF, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K, Goldstein JA. Identification of a new gentic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese. Mol Pharmacol 1994; 46: 594-598b
de Morais SMF, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA. The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. J. Biol. Chem.; 269: 15419-15422, 1994a.
Doecke CJ, Veronese ME, Pond SM, Miners JO, Birkett DJ, Sansom LN et al. Relationship between phenytoin and tolbutamide hydroxylations in human liver microsomes. Br J Clin Pharmacol 1991; 31:125-130.
Dollery C. Therapeutic drugs. 2nd ed. London: Churchill Livingstone; 1999. p. 110.
Eadie M. Metabolism of anticonvulsant drugs. Rev Drug Metab Drug Interact 1981; 3:317-417.

Eap CB, Bondolfi G, Zullino D, et al. Concentrations of the enantiomers of fluoxetine and norfluoxetine after multiple doses of fluoxetine in cytochrome P4502D6 poor and extensive metabolizers. J Clin Psychopharmacol 2001; 21: 330-4.
Eichelbaum M, Evert B. Influence of pharmacogenetics on drug disposition and response. Clin Exp Pharmacol Physiol 1996; 23: 983-5
Eichelbaum M, Gross AS (1990) The genetic polymorphism of debrisoquine/sparteine metabolism – clinical aspects. Pharmacol Ther 46: 377-394
Eichelbaum M, Spannbrucker N, Steincke B, Dengler JH (1979) Defective N-oxidation of sparteine in man: A new pharmacogenetic defect. Eur J Clin Pharmacol 16: 183-187
Ellingrod VL, Schultz SK, Arndt S. Association between cytochrome P4502D6 (CYP2D6) genotype, antipsychotic exposure, and abnormal involuntary movement scale (AIMS) score. Psychiatr. Genet. (2000) 10:9-11
Evans DAP, Mahgoub A, Sloan TP, Idle JR, Smith RL (1980) A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population. J Med Genet 17: 102-105
Fang J, Bourin M, Baker GB. Metabolism of risperidone to 9-hydroxyrisperidone by human cytochromes P450 2D6 and 3A4. Naunyn Schmiedebergs Arch Pharmacol 1999; 359: 147-51
Fenton WS, Blyler CR, Wyatt RJ, McGlashan TH (1997) Prevalence of spontaneous dyskinesia in schizophrenic and non-schizophrenic psychiatric patients. Br J Psychiatry 171: 265-268
Fjordside L, Jeppesen U, Eap CB, et al. The stereoselective metabolism of fluoxetine in poor and extensive metabolizers of sparteine. Pharmacogenetics 1999; 9: 55-60.
Fuller RW, Snoddy HD, Rushinski JH, et al. Comparison of norfluoxetine enantiomers as serotonin uptake inhibitors in vivo. Neuropharmacology 1992; 31: 997-1000.
Gaedigk A, Blum M, Gaedigk R, Eichelbaum M, Meyer UA. Deletion of the entire cytochrome P450 CYP2D6 gene as a cause of impaired drug metabolism in poor metabolizers of the debrisoquine/sparteine polymorphism. Am J Hum Genet 1991; 48: 943-950
Gatti G, Bonomi I, Marchiselli R, Fattore C, Spina E, Scordo G, Pacifici R, Perucca E. An Improved Stereoselective Assay for the Determination of Fluoxetine and Norfluoxetine Enantiomers in Human Plasma by High-Performance Liquid Chromatography. J Chromatogr B, In press
Goldstein JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. Br J Clin Pharmacol 2001; 52: 349-355.
Gonzales FJ. Human cytochrome P450: problems and prospects. Trends Pharmacol Sci 1992; 13: 346-352.
Gram LF. Fluoxetine. New Engl J Med 1994; 331: 1354-61.
Grohman R, Günther W, Rüther E (1983) Adverse effects of psychotrophic drugs. Clin Psychopharmacol 1: 378-397
Guengerich FP. Cytochromes P450 of human liver. Classification and activity profiles of the major enzymes. In: Pacifici GM, Fracchia GN, eds. Advanced in Drug Metabolism in Man. Bruxelles: European Communities, 1995: 179-231
Güsey C, Aamo T, Spigset O. Risperidone metabolism and the impact of being a cytochrome p450 2D6 ultrarapid metabolizer. J. Clin. Psychiatry. 61, 600-601 (2000).
Hall SD, Hamman MA, Rettie AE, Wienkers LC, Trager WF, Vandenbranden M et al. Relationships between the levels of cytochrome P4502C9 and its prototypic catalytic activities in human liver microsomes. Drug Metab Dispos 1994; 22:975-978.
Hammer W, Sjoqvist F. Plasma levels of monomethylated tricyclic antidepressants during treatment with imipramine-like compounds. Life Sci 1967; 6: 1895-903
Hansen JM, Kampmann JP, Siersbaek-Nielsen K, Lumholtz IB, Arroe M, Abildgaard U et al. The effect of different sulfonamides on phenytoin metabolism in man. Acta Med Scand Suppl 1979; 624:106-110.
Heim M, Meyer UA (1990) Genotyping of poor metabolizers of debrisoquine by allele-specific

PCR amplification. Lancet 336: 529-532
Henne KR, Gaedick A, Gupta G, Leeder JS, Rettie AE. Chiral phase analysis of warfarin enantiomers in patient plasma in relation to CYP2C9 genotype. J Chromatogr B 1998; 710: 143-148
Hirsh J, Dalen JE, Deykin D, Poller L, Bussey H. Oral anticoagulants: mechanism of action, clinical effectiveness and optimal therapeutic range. Chest 1995; 108 (suppl): 231-46.
Holford NHG. Clinical pharmacokinetics and pharmacodynamics of warfarin. Understanding the dose-effect relationship. Clin Pharmacokin 1986; 11: 483-504.
Horai Y, Nakano M, Ishizaki T, Ishikawa K, Zhou HH, Zhou BI, Liao CL, Zhang LM. Metoprolol and mephenytoin oxidation polymorphisms in Far Eastern Oriental subjects: Japanese versus mainland Chinese. Clin Pharmacol Ther 1989; 46: 198-207
Huang J. Errors in estimating the unbound fraction of drugs to the volume shift in equilibrium dialysis. J Pharm Sci 1983; 72: 1368-1369.
Huang ML, van Peer A, Woestenborghs R, De Coster R, Heykants J, Jansen AAJ, Zylicz Z, Visscher HW, Jonkman JHG (1993) Pharmacokinetics of the novel antipsychotic agent risperidone and the prolactin response in healthy subjects. Clin Pharm Ther 54: 257-268
Hughes HB, Biehl JP, Jones AP, Schmidt LH. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. Am Rev Tuberculosis 1954; 70: 266-273
Ingelman-Sundberg M, Oscarson M, Mc Lellan RA. Polymorphic Human cytochrome P450 enzymes: an opportunity for individualized drug treatment. TIPS 1999; 20:342-349
Jaanson P, Marandi T, Kiivet RA, Vasar V, Vaan S, Svensson JO, Dahl ML. Maintenance therapy with zuclopenthixol decanoate: associations between plasma concentrations, neurological side effects and CYP2D6 genotype. Psychopharmacology (Berl) 2002;162:67-73
Jannuzzi G, Gatti G, Magni P et al. Plasma concentrations of the enantiomers of fluoxetine and norfluoxetine: sources of variability and preliminary observations on relations with clinical response. Ther Drug Monit. 2002; 24: 616-27.
Johansson I, Lundqvist E, Bertilsson L, Dahl M-L, Sjöqvist F, Ingelman-Sundberg M (1993) Inherited amplification of an active gene in the cytochrome P450 2D-locus as a cause of ultrarapid metabolism of debrisoquine. Proc Natl Acad Sci USA 90: 11825-11829
Johansson I, Lundqvist E, Dahl M-L, Ingelman-Sundberg M (1996) PCR-based genotyping for duplicated and deleted CYP2D6 genes. Pharmacogenetics 6: 351-355
Johansson I, Oscarson M, Yue QY, Bertilsson L, Sjoqvist F, Ingelman-Sundberg M. Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. Mol Pharmacol 1994; 46: 452-9
Jurima M, Inaba T, Kadar D, Kalow W. Genetic polymorphism of mephenytoin p(4')-hydroxylation: difference between Orientals and Caucasians. Br. J. Clin. Pharmacol.; 19: 483-487, 1985.
Kagimoto M, Heim M, Kagimoto K, Zeugin T, Meyer UA. Multiple mutations of the human cytochrome P450IID6 gene (CYP2D6) in poor metabolizers of debrisoquine. Study of the functional significance of individual mutations by expression of chimeric genes. J Biol Chem 1990; 265: 17209-17214
Kalow W. Interethnic variation of drug metabolism. Trends Pharmacol Sci 1991;12:102-7
Kalow W, Genest K. A method for the detection of atypical forms of human serum cholinesterase. Can J Biochem Physiol 1957; 35:339-46
Kaminsky LS, Zhang ZY. Human P450 metabolism of warfarin. Pharmacol Ther 1997; 73: 67-74.
Kawanishi C, Lundgren S, Ågren H, Bertilsson L. Increased incidence of *CYP2D6* gene duplication in patient with persistent mood disorders: ultrarapid metabolism as a cause of nonresponse. *Personal Communication*

Kerb R, Aynacioglu AS, Brockmoller J, Schlagenhaufer R, Bauer S, Szekeres T, Hamwi A, Fritzer-Szekeres M, Baumgartner C, Ongen HZ, Guzelbey P, Roots I, Brinkmann U. The predictive value of MDR1, CYP2C9, and CYP2C19 polymorphisms for phenytoin plasma levels. Pharmacogenomics J 2001;1:204-10

Kidd RS, Straughn AB, Meyer MC, Blaisdell J, Goldstein JA, Dalton JT. Pharmacokinetics of chlorpheniramine, phenytoin, glipizide and nifedipine in an individual homozygous for the CYP2C9*3 allele. Pharmacogenetics. 1999 Feb;9(1):71-80.

Kirchheiner J, Brøsen K, Dahl ML, Gram LF, Kasper S, Roots I, Sjöqvist F, Spina E, Brockmöller J. CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: a first step towards subpopulation-specific dosages. Acta Psychiatr Scand 2001;104:173-92

Kopala LC (1996) Spontaneous and drug-induced movement disorders in schizophrenia. Acta Psychiatr Scand 94: 12-17

Kutt H, Wolk M, Scherman R, McDowell F. Insufficient parahydroxylation as a cause of diphenylidantoin toxicity. Neurology 1964; 14: 542-8

Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in vitro and human data. Pharmacogenetics 2002; 12: 251-63.

Leysen JE, Gommeren W, Eens A, de Chaffoy de Courcelles D, Stoof JC, Janssen PAJ (1988) Biochemical profile of risperidone, a new antipsychotic. J Pharmacol Exp Ther 247: 661-670

Liu ZQ, Cheng ZN, Huang SL, et al. Effect of the CYP2C19 oxidation polymorphism on fluoxetine metabolism in Chinese healthy subjects. Br J Clin Pharmacol 2001b; 52(1): 96-9

Liu ZQ, Shu Y, Huang SL, et al. Effects of CYP2C19 genotype and CYP2C9 on fluoxetine N-demethylation in human liver microsomes. Acta Pharmacol Sin 2001a; 22(1): 85-90

Lundmark J, Reis M and Bengtsson F. Serum concentrations of fluoxetine in the clinical treatment setting. Ther Drug Monit 2001; 23: 139-47.

Lundqvist E, Johansson I, Ingelman-Sundberg M (1999) Genetic mechanism for duplication and multiduplication of the CYP2D6 gene and methods for detection of duplicated CYP2D6 genes. Gene 226: 327-338

Mahgoub A, Idle JR, Dring LG, Lancaster R, Smith RL (1977) Polymorphic hydroxylation of debrisoquine in man. Lancet 2: 584-586

Mamiya K, Ieiri I, Shimamoto J, Yukawa E, Imai J, Ninomiya H, et al. The effect of genetic polymorphisms of CYP2C9 and CYP2C19 on phenytoin metabolism in Japanese adult patients with epilepsy: studies in stereoselective hydroxylation and population pharmacokinetics. Epilepsia 1998; 39: 1317-1323.

Mannens G, Huang ML, Meuldermans W, Hendrickx J, Woestenborghs R, Heykants J (1993) Absorption, metabolism and excretion of risperidone in humans. Drug Metab Dispos 21: 1134-1141

Margaglione M, Colaizzo D, D'Andera G, Brancaccio V, Ciampa A, Grandone E, et al. Genetic modulation of oral anticoagulation with warfarin. Thromb Haemost 2000; 84: 775-778.

Margolis JM, O'Donnell JP, Mankowski DC, et al. (R)-, (S)-, and racemic fluoxetine N-demethylation by human cytochrome P450 enzymes. Drug Metab Dispos 2000; 28: 1187-91.

Meyer JW, Woggon B, Baumann P, Meyer UA (1990) Clinical implications of slow sulphoxidation of thioridazine in a poor metabolizer of the debrisoquine type. Eur J Clin Pharmacol 39: 613-614

Meyer UA. Drugs in special patient group: Clinical importance of genetics in drug effects. In: Melmon KL, Morrelli HF, Hoffman BB, Nierenberg DW, eds. Clinical Pharmacology. Basic principles in therapeutics. New York: McGraw-Hill, Inc., 1992: 875-94

Meyer UA. The molecular basis of genetic polymorphisms of drug metabolism. J Pharm Pharmacol 1994; 46 (Suppl.1): 409-415.

Miners J, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. Br J Clin Pharmacol 1998; 45: 525-538
Moody JP, Tait AC, Todrick A. Plasma levels of imipramine and desmethylimipramine during therapy. Br J Psychiatry 1967; 113: 183-93
Nakajima M, Yokoi T, Mizutani M, Kinoshita M, Funayama M, Kamataki T. Genetic polymorphism in the 5'-flanking region of human CYP1A2 gene: effect on the CYP1A2 inducibility in humans. J Biochem 1999; 125: 803-8
Nakamura K, Goto F, Ray WA, Mc Allister CB, Jacqz E, Wilkinson GR, Branch RA. Interethnic differences in genetic polymorphism of debrisoquine and mephenytoin hydroxylation between Japanese and Caucasian populations. Clin. Pharmacol. Ther.; 38: 402-408, 1985.
Nelson DR, Koymans L, Kamataki T et al.. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics 1996; 6: 1-42.
Ninomiya H, Mamiya K, Matsuo S, Ieiri I, Higuchi S, Tashiro N. Genetic polymorphism of the CYP2C subfamily and excessive serum phenytoin concentration with central nervous system intoxication. Ther Drug Monit 2000; 22: 230-232.
Nyberg S, Dahl M-L, Halldin C (1995) A PET study of D2 and 5-HT2 receptor occupancy induced by risperidone in poor metabolizers of debrisoquin and risperidone. Psychopharmacology 119: 345-348
Odani A, Hashimoto Y, Otsiki Y, Uwai Y, Hattori H, Furusho K. Genetic polymorphism of the CYP2C subfamily and its effect on the pharmacokinetics of phenytoin in Japanese patients with epilepsy. Clin Pharmacol Ther 1997; 62: 287-292.
Ohmori O, Suzuki T, Kojima H et al. Tardive dyskinesia and debrisoquine 4-hydroxylase (CYP2D6) genotype in Japanese schizophrenics. Schizophr. Res. (1998) 32: 107-13
Olesen OV, Licht RW, Thomsen E, Bruun T, Viftrup JE, Linnet K (1998) Serum concentrations and side effects in psychiatric patients during risperidone therapy. Ther Drug Monit 20: 380-384
Olesen OV, Linnet K (1997) Simplified high-performance liquid chromatographic method for determination of risperidone and 9-hydroxyrisperidone in serum from patients comedicated with other psychotropic drugs. J Chromatogr B Biomed Appl 698: 209-216
Price Evans DA, Manley KA, McKusick VA. Genetic control of isoniazid metabolism in man. Br Med J 1960; 2: 485-491
Rang HP, Dale MM, Ritter JM. Pharmacology. Churchill Livingstone Eds 1999; 78-92
Relling MV, Lin JS, Ayers GD, Evans WE. Racial and gender differences in N-acetyltransferase, xanthine oxidase, and CYP1A2 activities. Clin Pharmacol Ther 1992; 52: 643-58
Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. Drug Metab. Rev. (1997) 29:413-580
Richens A, Dunlop A. Serum-phenytoin levels in management of epilepsy. Lancet 1975; 2:247-8
Ring BJ, Eckstein JA, Gillespie JS, et al. Identification of the human cytochromes p450 responsible for in vitro formation of R- and S-norfluoxetine. J Pharmacol Exp Ther 2001; 297: 1044-50.
Roh H-K, Dahl M-L, Tybring G, Yamada H, Cha Y-N, Bertilsson L. CYP2C19 genotype and phenotype determined by omeprazole in a Korean population. Pharmacogenetics; 6: 547-551, 1996.
Roh HK, Kim CE, Chung WG, Park CS, Svensson JO, Bertilsson L. Risperidone metabolism in relation to CYP2D6*10 allele in Korean schizophrenic patients. Eur J Clin Pharmacol 2001; 57: 671-5
Romkes M, Faletto MB, Blaisdell JA, Raucy JL, Goldstein JA. Cloning and expression of complementary DNAs for multiple members of the human cytochrome P450IIC subfamily. Biochemistry; 30: 3247-3255, 1991.
Sachse C, Brockmöller J, Bauer S, Roots I (1997) Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 60: 284-295
Sachse C, Brockmoller J, Bauer S, Roots I. Functional significance of a C→A polymorphism in

intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol 1999; 47: 445-9
Sandyk R, Kay SR (1991) Drug-induced parkinsonism: relationship to age at onset of schizophrenia. Funct Neurol 6: 151-157
Sata F, Sapone A, Elizondo G, Stocker P, Miller VP, Zheng W, Raunio H, Crespi CL, Gonzalez FJ. CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. Clin Pharmacol Ther 2000; 67: 48-56
Saxena R, Shaw GL, Relling MV, Frame JN, Moir DT, Evans WE, Caporaso N, Weiffenbach B (1994) Identification of a new variant CYP2D6 allele with a single base deletion in exon 3 and its association with the poor metabolizer phenotype. Hum Mol Genet 3: 923-926
Scordo MG, Aklillu E, Yasar U, Dahl ML, Spina E, Ingelman-Sundberg M. Genetic polymorphism of cytochrome P450 2C9 in a Caucasian and a black African population. Br J Clin Pharmacol 2001; 52: 447-450.
Scordo MG, Pengo V, Spina E, Dahl, ML, Gusella M, Padrini R. Influence of cytochrome P450s 2C9 and 2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. Clin Pharmacol Ther, In press
Scordo MG, Spina E. Cytochrome P450 polymorphisms and response to antipsychotic therapy. Pharmacogenomics 2002; 3: 201-18
Scordo MG, Spina E, Facciolá G, Avenoso A, Johansson I, Dahl ML (1999) Cytochrome P450 2D6 genotype and steady state plasma levels of risperidone and 9-hydroxy risperidone. Psychopharmacology 147: 300-305
Scordo MG, Spina E, Romeo P, Dahl ML, Bertilsson L, Johansson I, Sjoqvist F. CYP2D6 genotype and antipsychotic-induced extrapyramidal side effects in schizophrenic patients. Eur J Clin Pharmacol 2000; 56: 679-83
Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. 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 1994; 270: 414-23
Sjöqvist F, Borgå O, Dahl ML, Orme ML´E. Fundamental of Clinical Pharmacology. In: Speight TM & Holford NHG eds. Avery´s Drug Treatment. Adis International Limited (New Zeland), 1997; 1-73
Sohn DR, Kusaka M, Ishizaki T, Shin SG, Jang IJ, Shin JG, Chiba K. Incidence of S-mephenytoin hydroxylation deficiency in a Korean population and the interphenotypic differences in diazepam pharmacokinetics. Clin Pharmacol Ther 1992; 52: 160-9
Spina E, Ancione M, Di Rosa AE, Meduri M, Caputi AP (1992) Polymorphic debrisoquine oxidation and acute neuroleptic-induced adverse effects. Eur J Clin Pharmacol 42: 347-348
Spina E, Avenoso A, Facciola G, Salemi M, Scordo MG, Ancione M, Madia AG, Perucca E. Relationship between plasma risperidone and 9-hydroxyrisperidone concentrations and clinical response in patients with schizophrenia. Psychopharmacology 2001c; 153: 238-43
Spina E, Avenoso A, Facciola G, Salemi M, Scordo MG, Giacobello T, Madia AG, Perucca E. Plasma concentrations of risperidone and 9-hydroxyrisperidone: effect of comedication with carbamazepine or valproate. Ther Drug Monit 2000; 22: 481-5
Spina E, Avenoso A, Facciolà G, Scordo MG, Ancione M, Madia A. Plasma concentrations of risperidone and 9-hydroxyrisperidone during combined treatment with paroxetine. Ther. Drug Monit. 23, 223-227 (2001b).
Spina E, Avenoso A, Scordo MG, Ancione M, Madia A, Gatti G, Perucca E. Inhibition of risperidone metabolism by fluoxetine in patients with schizophrenia: a clinically relevant pharmacokinetic drug interaction. J Clin Psychopharmacol 2002; 22: 419-23
Spina E, Scordo MG, Avenoso A, Perucca E. Adverse drug interaction between risperidone and carbamazepine in a patient with chronic schizophrenia and deficient CYP2D6 activity. J. Clin. Psychopharmacol. 21, 108-109 (2001a).

Steen VM, Løvlie R, MacEwan T, McCreadie RG (1997) Dopamine D3-receptor gene variant and susceptibility to tardive dyskinesia in schizophrenic patients. Molecular Psychiatry 2: 139-145
Steiner E, Iselius L, Alván G, Lindsten J, Sjöqvist F (1985) A family study of genetic and environmental factors determining polymorphic hydroxylation of debrisoquin. Clin Pharmacol Ther 38: 394-401
Stevens JC and Wrighton SA. Interaction of the enantiomers of fluoxetine and norfluoxetine with human liver cytochromes P450. J Pharmacol Exp Ther 1993; 266: 964-71.
Steward DJ, Haining RL, Henne KR, Davis G, Rushmore TH, Trager WF, Rettie AE. Genetic association between sensitivity to warfarin and expression of CYP2C9*3. Pharmacogenetics 1997; 7: 361-367
Stokes P and Holtz A. Fluoxetine tenth anniversary update: The progress continues. Clin Ther 1997; 19: 1-86
Takahashi H, Echizen H. Pharmacogenetics of warfarin elimination and its clinical implications. Clin Pharmacokinet 2001; 40: 587-603.
Takahashi H, Kashima T, Nomizo Y, Muramoto N, Shimizu T, Nasu K, et al. Metabolism of warfarin enantiomers in Japanese patients with heart diseases having different CYP2C9 and CYP2C19 genotypes. Clin Pharmacol Ther 1998; 63: 519-528.
Takahashi H, Kashima T, Nomoto S, Iwade K, Tainaka H, Shimizu T, et al. Comparison between in-vitro and in-vivo metabolism of (S)-wafarin: catalytic activities of cDNA-expressed CYP2C9, its Leu359 variant and their mixture versus unbound clearance in patients with the corresponding CYP2C9 genotypes. Pharmacogenetics 1998; 8: 365-73.
Tarsy D (1984) Movement disorders with neuroleptic drug treatment. Psychiatric Clin North 3: 435-471
Tarsy D, Baldessarini RJ, Tarazi FI. Effects of newer antipsychotics on extrapyramidal function. CNS Drugs 2002; 16: 23-45
Taube J, Halsall D, Baglin T. Influence of cytochrome P-450 CYP2C9 polymorphisms on warfarin sensitivity and risk of over-anticoagulation in patients on long-term treatment. Bood 2000; 96; 1816-1819.
Tucker GT, Silas JH, Iyun AO, Lennard M, Smith AJ (1977) Polymorphic hydroxylation of debrisoquine. Lancet 2: 718
van Beijsterveldt LEC, Geerts RJF, Leysen JE, Megens AAHP, Van den Eynde HMJ, Meuldermans WEG, Heykants JJP (1994) The regional brain distribution of risperidone and its active metabolite 9-hydroxy-risperidone in the rat. Psychopharmacology 114: 53-62
Veronese ME, Doecke CJ, Mackenzie PI, McManus ME, Miners JO, Rees DL, Gasser R, Meyer UA, Birkett DJ. Site-directed mutation studies of human liver cytochrome P-450 isoenzymes in the CYP2C subfamily. Biochem J 1993: 289, 533-538.
von Moltke LL, Greenblatt D, Xian Duan S, et al. Human cytochromes mediating N-demethylation of fluoxetine in vitro. Psychopharmacology 1997; 132: 402-7.
Wennerholm A, Johansson I, Massele AY, Jande M, Alm C, Aden Abdi Y, Dahl ML, Ingelman-Sundberg M, Bertilsson L, Gustafsson LL (1999) Decreased capacity for debrisoquine metabolism among black Tanzanians: analyses of the CYP2D6 genotype and phenotype. Pharmacogenetics 9: 707-714
Wienkers LC, Wurden CJ, Storch E, Kunze KL, Rettie AE, Trager WF. Formation of (R)-8-hydroxywarfarin in human liver microsomes: a new metabolic marker for the (S)-mephenytoin hydroxylase, P4502C19. Drug Metab Dispos 1996; 24: 610-614.
Wilkinson GR, Guengerich FP, Branch RA. Genetic polymorphism of S-mephenytoin hydroxylation. Pharmacogenetics of drug metabolism; Pergamon Press, 657-685, 1992.
Wilkinson GR, Shand DG. A physiologic approach to hepatic drug clearance. Clin Pharmacol Ther 1975; 18: 377-390.

Wong DT, Bymaster FP, Reid LR, et al. Inhibition of serotonin uptake by optical isomers of fluoxetine. Drug Dev Res 1985; 6: 397-403.
Wong DT, Bymaster FP, Reid LR, et al. Norfluoxetine enantiomers as inhibitors of serotonin uptake in rat brain. Neuropsychopharmacology 1993; 8: 337-44.
Yasar U, Eliasson E, Dahl ML, Johansson I, Ingelman-Sundberg M, Sjoqvist F. Validation of methods for CYP2C9 genotyping: frequencies of mutant alleles in a Swedish population. Biochem Biophys Res Commun, 1999; 254: 628-31. Erratum in: Biochem Biophys Res Commun 1999; 258:227
Yasar U, Forslund-Bergengren C, Tybring G et al. Pharmacokinetics of losartan and its metabolite E-3174 in relation to the CYP2C9 genotype. Clin Pharmacol Ther. 2002; 71:89-98.
Yasui-Furukori N, Hidestrand M, Spina E, Facciola G, Scordo MG, Tybring G. Different enantioselective 9-hydroxylation of risperidone by the two human CYP2D6 and CYP3A4 enzymes. Drug Metab Dispos 2001; 29: 1263-8
Yoshimura R, Ueda N, Nakamura J. Possible relationship between combined plasma concentrations of risperidone plus 9-hydroxyrisperidone and extrapyramidal symptoms. Preliminary study. Neuropsychobiology 2001; 44: 129-33