From
the Department of Medical Laboratory Sciences & Technology,
Division of Clinical Pharmacology,
Karolinska Institutet at Huddinge University Hospital,
Stockholm, Sweden

Cytochrome P450 2C9, 2C19 and 2D6 genetic polymorphisms
Evaluation of genotyping as a tool for individualised treatment

by

Maria Gabriella Scordo M.D.

Stockholm 2003
"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 impotent 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

Abstract ................................................................................................................................. 4
Table of contents .................................................................................................................. 5
List of original papers .......................................................................................................... 6
Abbreviations ...................................................................................................................... 7
Glossary ............................................................................................................................... 8
1. Introduction .................................................................................................................... 9
  1.1 Drug Metabolism .......................................................................................................... 9
  1.2 Drug oxidation ............................................................................................................ 9
  1.3 Cytochrome P450 ........................................................................................................ 10
  1.4 Interindividual variability in drug oxidation ............................................................. 11
  1.5 Genetic polymorphism .............................................................................................. 12
    1.5.1 CYP2D6 .............................................................................................................. 13
    1.5.2 CYP2C9 .............................................................................................................. 14
    1.5.3 CYP2C19 ............................................................................................................. 15
    1.5.4 CYP1A2 & CYP3A4 ............................................................................................ 16
  1.6 Therapeutic index and dose titration ......................................................................... 19
  1.7 Tools to individualise drug dosage ............................................................................ 19
    1.7.1 Therapeutic Drug Monitoring ............................................................................ 19
    1.7.2 Genotyping/Phenotyping .................................................................................... 20
  1.8 Risperidone metabolism ............................................................................................ 21
  1.9 Warfarin metabolism ............................................................................................... 21
  1.10 Fluoxetine metabolism ............................................................................................ 22
  1.11 Phenytoin metabolism ............................................................................................. 23
  1.12 Genotype and side-effects ....................................................................................... 23
2. Aims .................................................................................................................................. 25
3. Methods .......................................................................................................................... 26
  3.1 Patients and study design ........................................................................................ 26
  3.2 Genotyping methods ............................................................................................... 29
  3.3 Drug assays ................................................................................................................ 30
  3.4 Pharmacokinetic calculations ................................................................................ 31
  3.5 Statistical analysis .................................................................................................... 31
4. Results ............................................................................................................................ 33
5. Discussion ....................................................................................................................... 39
6. Concluding remarks ...................................................................................................... 45
7. Future perspectives ........................................................................................................ 47
8. Acknowledgements ........................................................................................................ 48
9. References ...................................................................................................................... 50

5
List of original papers

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


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*


Copyrights of the above papers are owned by Mosby and Springer-Verlag, that kindly supplied the reprinting permissions.
**Abbreviations:**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>C/D</td>
<td>concentration normalized for dose</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CL</td>
<td>clearance</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>CYP</td>
<td>cytochrome P450</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EM</td>
<td>extensive metaboliser</td>
</tr>
<tr>
<td>EPS</td>
<td>extrapyramidal side effects</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>INR</td>
<td>international normalized ratio</td>
</tr>
<tr>
<td>MR</td>
<td>metabolic ratio</td>
</tr>
<tr>
<td>NS</td>
<td>non statistically significant</td>
</tr>
<tr>
<td>PM</td>
<td>poor metaboliser</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>RFLP</td>
<td>restriction fragment length polymorphism</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>TDM</td>
<td>therapeutic drug monitoring</td>
</tr>
<tr>
<td>UM</td>
<td>ultrarapid metaboliser</td>
</tr>
<tr>
<td>WT</td>
<td>wild type</td>
</tr>
</tbody>
</table>
**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 enalaprillat. Metabolism can alter the pharmacological properties of a compound qualitatively: for example, salicylic acid shares with its parent drug aspirin the anti-inflammatory activity, 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 conjugation 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,
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.

![Diagram of NADPH-cytochrome P-450 reductase and cytochrome P-450 reactions](http://web.vet.cornell.edu/public/pharmacokinetics/sites/DT/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 protoporphyrin 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 polymorphism 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 acetylaters 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 antidepressants revealed marked variability in steady-state plasma concentrations of imipramine, desmethylinimipramine 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 played 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 UM. 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)

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 desmethyliamphetamine 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 UM (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 Arg144Cys mutation that displays reduced affinity for P450 oxidoeductase (Ingelman-Sundberg et al., 1999). The CYP2C9*3 allele, characterised by a A→T substitution at the codon 1016, yields an enzyme with an Ile235Leu 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 demonstrated 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 mephentoin 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-mephentoin 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 (Brüsen 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. However, 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.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Substrates</th>
<th>Inhibitors</th>
<th>Inducers</th>
<th>Polymorphism</th>
<th>Probe-drug</th>
</tr>
</thead>
</table>
| 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 | Sulfinpyrazone  
         | Fluconazole  
         | Miconazole  
         | 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: thicidazine, perphenazine, zuclopenthixol, haloperidol, risperidone, olanzapine, sertindole | Quinidine  
         | Thioridazine  
         | Perphenazine  
         | Fluoxetine  
         | Paroxetine  
         | Cimetidine  
         | Ritonavir | None known  
         | Yes  
         | Debrisoquine  
         | Dextromethorphan  
         | Sparteine  
         | Metoprolol  
         | Desmethylmepiramine |
| 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, ciprofloxacin, tamoxifen, amiodarone, quinidine, methadone, ethynylestradiol, levonorgestrel, statins | Ketoconazole  
         | Itraconazole  
         | Fluconazole  
         | Erythromycin  
         | TroTacan  
         | 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 of 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 (Leyesen 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 (Fjordside 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 (buccolingual-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<sub>SS,a</sub>) 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).

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 trihexphenidyl) 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 (dysarthria, 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 XbaI (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 AvaII (CYP2C9*2) and with NsiI (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:

\[
CL_{po,u} = \frac{(D/2\tau)}{C_{ts} \times f_u}
\]

where D is the daily dose of racemic warfarin, \(\tau\) the dosing interval (i.e., 24 h) and \(C_{ts}\) 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 \(C_{ts}\). 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 \(C_{ts}\) 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 $CL_{\text{pa,0}}$ 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 \((p < 0.02)\).

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).

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>
<thead>
<tr>
<th>Allele frequencies</th>
<th>LD group</th>
<th>MD group</th>
<th>HD group</th>
<th>X² - test</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*1/*1</td>
<td>0.564**</td>
<td>0.797**</td>
<td>0.978**</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>CYP2C9*1/*2</td>
<td>0.495**</td>
<td>0.161**</td>
<td>0.073**</td>
<td>p&lt;0.005</td>
</tr>
<tr>
<td>CYP2C9*1/*3</td>
<td>0.325**</td>
<td>0.064**</td>
<td>0.0**</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>CYP2C19*1/*1</td>
<td>0.678**</td>
<td>0.922**</td>
<td>0.812**</td>
<td>NS</td>
</tr>
<tr>
<td>CYP2C19*1/*2</td>
<td>0.122**</td>
<td>0.078**</td>
<td>0.188**</td>
<td>NS</td>
</tr>
</tbody>
</table>

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

| Genotypes $|$ | X² - test |
|-------------|----------|
| CYP2C9*1/*1 | 11 (29.7)** | 20 (62.5)** | 23 (95.8)** | p<0.001 |
| CYP2C9*1/*2 | 6 (16.2)** | 6 (35.0) | 1 (4.2) | NS |
| CYP2C9*1/*3 | 13 (35.1)** | 3 (9.4)** | 0** | 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)** | 1 (2.7) | 0** | NS |

$|$ Percentages in brackets

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

Warfarin maintenance dose, plasma S:R warfarin ratio and S-warfarin CLfree correlated significantly with CYP2C9 (Tab.5; Fig.5). The S-warfarin CLfree was significantly related to the CYP2C9 genotype groups (p<0.0001), although most patients (72%) with no mutated alleles showed an S-warfarin CLfree in the same range as those carrying mutated alleles (58–777 mil/min). No relationship was found between S-warfarin CLfree and CYP2C19 genotype or between R-warfarin CLfree 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 (mean ± SD, mg), plasma S/R warfarin ratios (mean ± SD) and clearances (mean ± SD, ml/min) in patients with different CYP2C9 and CYP2C19 genotypes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Weekly warfarin doses</th>
<th>ANOVA</th>
<th>Plasma S/R warfarin ratios</th>
<th>ANOVA</th>
<th>Cl_{min} S</th>
<th>ANOVA</th>
<th>Cl_{min} R</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*1/*1</td>
<td>39.14 ± 15.47</td>
<td></td>
<td>0.44 ± 0.11</td>
<td></td>
<td>660 ± 332</td>
<td></td>
<td>217 ± 116</td>
<td></td>
</tr>
<tr>
<td>CYP2C9*1/*2</td>
<td>27.68 ± 11.25</td>
<td>P &lt; 0.0001</td>
<td>0.61 ± 0.13</td>
<td></td>
<td>380 ± 173</td>
<td>NS</td>
<td>214 ± 83</td>
<td>NS</td>
</tr>
<tr>
<td>CYP2C9*1/*3</td>
<td>20.55 ± 7.14</td>
<td></td>
<td>0.71 ± 0.25</td>
<td></td>
<td>345 ± 185</td>
<td>207 ± 60</td>
<td>238 ± 57</td>
<td>NS</td>
</tr>
<tr>
<td>CYP2C9*2/*2</td>
<td>20.83 ± 4.42</td>
<td></td>
<td>1.14 ± 0.08</td>
<td></td>
<td>213 ± 43</td>
<td>NS</td>
<td>207 ± 96</td>
<td>NS</td>
</tr>
<tr>
<td>CYP2C9*2/*3</td>
<td>18.13 ± 9.44</td>
<td></td>
<td>1.42 ± 0.21</td>
<td></td>
<td>155 ± 60</td>
<td>NS</td>
<td>146 ± 37</td>
<td>NS</td>
</tr>
<tr>
<td>CYP2C9*3/*3</td>
<td>8.75 ± 3.54</td>
<td></td>
<td>2.37 ± 0.53</td>
<td></td>
<td>61 ± 4</td>
<td>NS</td>
<td>61 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>CYP2C19*1/*1</td>
<td>31.20 ± 14.93</td>
<td>NS</td>
<td>0.65 ± 0.42</td>
<td>NS</td>
<td>503 ± 321</td>
<td>NS</td>
<td>241 ± 101</td>
<td>NS</td>
</tr>
<tr>
<td>CYP2C19*1/*2</td>
<td>35.33 ± 18.23</td>
<td></td>
<td>0.49 ± 0.14</td>
<td></td>
<td>520 ± 299</td>
<td>NS</td>
<td>225 ± 114</td>
<td>NS</td>
</tr>
<tr>
<td>CYP2C19*2/*2</td>
<td>35.00 ± 26.52</td>
<td></td>
<td>0.35 ± 0.09</td>
<td></td>
<td>979 ± 273</td>
<td>NS</td>
<td>304 ± 200</td>
<td>NS</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UM (n=5)</td>
<td>6.35 (1.00-49.9)</td>
<td>4.95 (1.60-11.0)</td>
<td>18.0 (3.40-51.6)</td>
<td>8.45 (1.70-16.6)</td>
<td>29.9 (6.6-113)</td>
<td>1.75 (1.00-13.1)</td>
<td>2.4 (1.7-3.1)</td>
</tr>
<tr>
<td>Homozygous EM (n=59)</td>
<td>7.60</td>
<td>2.70</td>
<td>13.5</td>
<td>6.10</td>
<td>26.6 (3.2-39.6)</td>
<td>1.70 (1.00-13.1)</td>
<td>2.5 (1.7-3.1)</td>
</tr>
<tr>
<td>Heterozygous EM (n=26)</td>
<td>13.3</td>
<td>3.05</td>
<td></td>
<td></td>
<td>4.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM (n=1)</td>
<td>11.8</td>
<td>5.30</td>
<td>4.05</td>
<td>1.0</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p=0.005</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CYP2C9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1 (n=40)</td>
<td>6.70 (0.8-27.6)</td>
<td>2.55</td>
<td>11.9</td>
<td>5.8</td>
<td>25.3 (0.8-10.3)</td>
<td>2.04 (0.8-10.3)</td>
<td>2.38 (1.0-9.04)</td>
</tr>
<tr>
<td>/1<em>2 or /2</em>2</td>
<td>8.40 (0.8-49.6)</td>
<td>3.65</td>
<td>13.5</td>
<td>6.1</td>
<td>28.7 (0.8-10.3)</td>
<td>1.64 (0.8-10.3)</td>
<td>2.27 (0.8-10.3)</td>
</tr>
<tr>
<td>/1*3 (n=19)</td>
<td>5.7 (3.0-6.5)</td>
<td>4.75</td>
<td>12.4</td>
<td>7.8</td>
<td>40.8 (0.8-10.3)</td>
<td>0.88 (0.8-10.3)</td>
<td>2.77 (0.8-10.3)</td>
</tr>
<tr>
<td>/6 (n=8)</td>
<td>5.2 (2.5-6.8)</td>
<td>4.75</td>
<td>12.4</td>
<td>7.8</td>
<td>40.8 (0.8-10.3)</td>
<td>0.88 (0.8-10.3)</td>
<td>2.77 (0.8-10.3)</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>p=0.005</td>
<td>NS</td>
<td>p=0.005</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CYP2C19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1 (n=57)</td>
<td>9.90 (0.8-47.2)</td>
<td>2.90</td>
<td>12.5</td>
<td>4.90</td>
<td>26.6 (0.8-16.3)</td>
<td>1.2 (0.8-16.3)</td>
<td>2.14 (0.2-36.0)</td>
</tr>
<tr>
<td>/1*2 (n=23)</td>
<td>10.3 (0.8-49.6)</td>
<td>3.70</td>
<td>15.2</td>
<td>6.40</td>
<td>30.1 (0.8-20.0)</td>
<td>1.78 (0.8-20.0)</td>
<td>2.58 (0.8-20.0)</td>
</tr>
<tr>
<td>/2/*2 (n=5)</td>
<td>10.0 (0.8-49.6)</td>
<td>5.05</td>
<td>11.5</td>
<td>4.55</td>
<td>24.0 (0.8-20.0)</td>
<td>1.51 (0.8-20.0)</td>
<td>3.91 (0.8-20.0)</td>
</tr>
<tr>
<td>p</td>
<td>4.15 (0.8-12.2)</td>
<td>4.55</td>
<td>17.5 (0.8-30.4)</td>
<td>4.15</td>
<td>2.22 (0.8-20.0)</td>
<td>1.51 (0.8-20.0)</td>
<td>2.72 (0.8-20.0)</td>
</tr>
</tbody>
</table>
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.

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 life 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).
5. Discussion

Study 1

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 (Holfford, 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\textsubscript{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\textsubscript{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 polymorphisms 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, Ohnori 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 single 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 among patients with persistent 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 optimize 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 deficient 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.
8. Acknowledgements

I wish to express my sincere gratitude to the many people who have in different ways contributed to this thesis, and particularly to:
Professors Marja-Liisa Dahl and Folke Sjöqvist, my fantastic supervisors, for always being enormously supportive and innovative. Marja-Liisa for being always at my side when I needed most, with her contagious enthusiasm and her excellent judgement in research. Folke for his intellectual guidance, constant support and great enthusiasm
Professor Achille Patrizio Caputi and Associate professor Edoardo Spina, my mentors, for trusting me and introducing me to clinical pharmacology, for teaching excellence in research and being always supportive
Professor Leif Bertilsson, for sharing his invaluable scientific knowledge and for being such a unique and nice person
Associate professor Gunnel Tybring, for the fruitful collaboration, the interesting discussions, the great fun we have shared and for being such a warm, generous friend
Professor Anders Rane, current head of the Department, for providing and supporting an excellent research environment
Professor Lars Gustafsson, associate professors Lars Stähle, Ulf Bergman and Erik Eliasson for the valuable, constructive discussions
Professor Gunnar Alvan, for generously sharing knowledge during my experience at the Drug Information Center
Professors Magnus Ingelman-Sundberg and Inger Johansson for the fruitful collaborative work in the Division of Molecular Toxicology at Karolinska Institute
Biomedical analysts Lilleba Bohman, Ulla Pettersson, Margareta Lind and Birgitta Ask and laboratory engineer Margareta Rais for skilled support in the laboratory work and for the fun we had: thank you for making me feeling that working in the lab can be so nice!
Biomedical analysts Jolanta Widén and Maria Aravoglou and research nurse Christina Alm for taking me under their wings since I arrived, and for being such delightful persons and friends
Professors Emilio Perucca and Dr. Giuliana Gatti for the great collaboration and the sharp suggestions and comments
Professor Roberto Padrini for the valuable collaboration
Ümit Yasar, Rajaa Mirghani, Aurelijia Jucaite, Han-Jing Xie and Birgit Eiermann for friendship and great support: to be your friend is a great privilege
My colleagues and PhD student fellows Agneta, Magnus, Ylva, Ayman, Annika, Anita, Elin, Anna, Mia, Niklas, Mike, Jaran, Helena, Jenny, Jonathan
All other friends presently and before at the Department: Jolanta Gulbinovic, Kazuo Mihara, Karin Lindén, Natasha Borg, Karin Herrlin, Roberto Canaparo, Kari Laine, Sebastian Härtter, Monique Walkenkamp, Hyung-Keun Roh, Juan Antonio Carrillo, Maria Luisa Bernal, Chiaki Kawanishi for friendship, fruitful scientific discussions and great fun
Secretaries Margit Ekström, Yvonne Sjölin, Susan Molander, Elisabeth Angell, Jeanette Grunstein for administrative assistance
My co-authors, especially Angela Avenoso, Gabriella Facciola, Norio Yasui-Furokori, Eleni Akllu for fruitful collaboration
My family and friends for always supporting (and bearing) me with the warmest, endless enthusiasm, and for reminding me that there is a great world outside the lab

Grants
This work was supported by grants from Italian Ministero dell’Università e Ricerca Scientifica, the Societá Italiana di Farmacologia (SIF), Eli Lilly, Co. (Indianapolis, U.S.A.), European Union (Biomed 2 grant, BMH4-CT96-0291)
9. References


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.
Dahl ML, Sjöqvist F. Pharmacogenetic methods as a complement to therapeutic monitoring of antidepressants and neuroleptics. Ther Drug Monit 2000; 22: 114-7


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


Hammer W, Sjoqvist F. Plasma levels of mono-methylated tricyclic antidepressants during treatment with imipramine-like compounds. Life Sci 1967; 6: 1895-903


Heim M, Meyer UA (1990) Genotyping of poor metabolizers of debrisoquine by allele-specific
PCR amplification. Lancet 336: 529-532


Hughes HB, Bielh 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


Johansson I, Oscarson M, Yue QY, Bertilsson L, Sjöqvist F, Ingelman-Sundberg M. Genetic analysis of the Chinese cytochrome P450D2 locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. Mol Pharmacol 1994; 46: 452-9


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


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


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.


Richens A, Dunlop A. Serum-phenytoin levels in management of epilepsy. Lancet 1975; 2:247-8
Sachse C, Brockmoller J, Bauer S, Roots I. Functional significance of a C→A polymorphism in
Scordo MG, Spina E. Cytochrome P450 polymorphisms and response to antipsychotic therapy. Pharmacogenomics 2002; 3: 201-18
Tarsy D, Baldessarini RJ, Tarazi FI. Effects of newer antipsychotics on extrapyramidal function. CNS Drugs 2002; 16: 23-45


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