

The Pharmacogenetics of *CYP2C9* and *CYP2C19*: Ethnic Variation and Clinical Significance

J. Rosemary and C. Adithan*

Pharmacogenomics Laboratory, Department of Pharmacology, JIPMER, Pondicherry – 605006, India

Abstract: *CYP2C9* and *CYP2C19* are important drug metabolizing enzymes and together metabolize about 18% of currently available drugs. Some of the important groups of drugs that are metabolized by them are antihypertensives, hypoglycemics, anticonvulsants, antiulcer drugs etc. Genes encoding these enzymes are polymorphically expressed. Thirty variant alleles for *CYP2C9* and 21 for *CYP2C19* have been reported. The frequencies of these polymorphic alleles show marked inter-ethnic variation. Several reports have been published showing the clinical importance of this polymorphism. This review summarizes the currently available important information on this topic.

INTRODUCTION

Cytochrome P450 (CYP) is a complex gene superfamily consisting of heme-containing enzymes that comprises of over 70 families [1]. In humans, more than 50 distinct families of cytochrome P450 enzymes have been identified [2]. The enzymes belonging to the families CYP1, CYP2 and CYP3 catalyze the oxidative biotransformation of exogenous compounds, including many drugs, pro-carcinogens, and alcohols. The other CYP450 enzymes are involved in the metabolism of endogenous compounds such as fatty acids, prostaglandins and steroids [3].

Of the CYP450 content in human liver, CYP3A4 is the most abundant (~28%), followed by the CYP2C family (18%), CYP1A2 (~12%), CYP2E1 (7%), CYP2A6 (4%), CYP2D6 (1.5%) and CYP2B6 (0.2%). Of these, CYP3A metabolizes more than half of the currently prescribed drugs (51%), followed by CYP2D6 (24%) and the CYP2C subfamily (~20%) [4].

The human CYP2C subfamily of enzymes consists of four members (*CYP2C8*, *CYP2C9*, *CYP2C18* and *CYP2C19*) [5]. Of the four enzymes, *CYP2C9* and *CYP2C19* are primarily concerned with xenobiotic metabolism [6].

The genes encoding *CYP2C9* and *CYP2C19* are polymorphically expressed [7], with 30 variant alleles for *CYP2C9* and 21 for *CYP2C19* [8,9]. Many of these variants, the most common being *2 and *3, are associated with decreased metabolism of the respective substrates [10,11].

The frequency of polymorphic alleles shows marked inter-ethnic variation. The frequencies of alleles *CYP2C9**2, *3 and *CYP2C19**2 and *3 have been studied in different global populations. In Caucasians, the frequency of *CYP2C9* mutant alleles is higher (*2: 12%, *3: 8.3%) [12] while that for *CYP2C19* mutant alleles is lower (*2: 15%, *3: 0%) [13] when compared to Chinese (*CYP2C9**2: 0%, *CYP2C9**3: 3.3%, *CYP2C19**2: 30%, *CYP2C19**3: 5%) [12,14]. The population of the island of Vanuatu in Eastern Melanesia has the highest frequency of predicted poor metabolizers (PMs)

for *CYP2C19* reported so far (>74%) [15]. While the Chinese have a relatively high frequency of *CYP2C19* PMs (~14%) [14], no PM genotypes for *CYP2C9* [12] have been identified. Altered activity of CYP450 is one of the main causes of inter-individual variability in oxidative metabolism of drugs [16].

This review summarizes the inter-ethnic distribution of *CYP2C9* and *CYP2C19* allelic variants, clinical implications and attempts at dose adjustment based on the genotype.

The *CYP2C* Gene

The genes encoding the CYP2C subfamily of enzymes are located on chromosome 10q24 [17].

The *CYP2C9* and *CYP2C19* genes are of particular interest because they exhibit marked genetic polymorphism. In fact, of the three CYP subfamilies involved in phase I drug metabolism (1, 2 and 3), the maximum amount of variants are seen in subfamily 2 [18]. *Genetic polymorphism* is defined as a mutation in the DNA sequence which is present in at least 1% of the population. The polymorphic alleles contain SNPs owing to deletions, insertions or base substitutions. These SNPs may result in a change in the amino acid sequence, premature stop codon or a splicing defect. The resulting enzyme may have increased, decreased or zero activity (Table 1). The differential metabolism brought about by these polymorphic enzymes results in four distinctive phenotypes: extensive (normal) metabolizers (EMs), poor metabolizers (PMs), intermediate metabolizers (IMs) and ultrarapid metabolizers (UMs).

CYP2C9 – ALLELIC VARIANTS

*CYP2C9**2, *3

Among the 30 variant alleles of *CYP2C9* that have been characterized, the most common are *CYP2C9**2, and *CYP2C9**3. *CYP2C9**2 is formed by a C430T substitution on exon 3 which leads to Arg¹⁴⁴Cys conversion resulting in the formation of an enzyme with decreased activity [19]. The *CYP2C9**3 allele is due to a C1075T on exon 7 of *CYP2C9*. This results in an altered protein with an Ile³⁵⁹Leu substitution, which exhibits further reduced enzyme activity than the *CYP2C9**2 variant [20,21].

*Address correspondence to this author at the Pharmacogenomics Laboratory, Department of Pharmacology, JIPMER, Pondicherry – 605006, India; E-mail: adithan@dataone.in

Table 1. Enzyme Activity of Allelic Variants of CYP2C9 and CYP2C19

Gene	Enzyme Activity			
	Increased	Normal	Decreased	Absent
<i>CYP2C9</i>	*7	*1	*2, *3, *5, *11, *12, *14, *16, *18	*15
<i>CYP2C19</i>	*17	*1A, *1B, *1C,	-	*2B, *3A, *3B, *4, *5A, *5B, *6, *7, *8

*CYP2C9*4* was identified during the genetic analysis of 32 Japanese epileptic patients [22]. Twenty-one of them were selected based on the ratio of the concentration of 5-(para-hydroxyphenyl)-phenylhydantoin(5-HPPH) to phenytoin, serum concentration to daily dose, and insufficient response at usual dosage regimens. They characterized an allelic variant 1076T>C on exon 7, which led to an amino acid substitution from isoleucine to threonine at residue 359. Since the substrate recognition site SRS5 of CYP2C9 spans residues 359 – 369 [23], this mutation may cause a change in substrate binding.

*CYP2C9*5* was evidenced from a study in which 140 European American and 120 African American genomic samples were subjected to single-strand conformational polymorphism (SSCP) analysis, and later, grown in *Trichoplusia ni* cells and expressed in a baculovirus system [24]. The variant, due to a C1080G transversion on exon 7 leads to an Asp360Glu substitution in the encoded protein which increased the Km value by 12-fold for (S)-warfarin, 5-fold for diclofenac and 3-fold for lauric acid, when compared to *CYP2C9*1*. A population of 328 Japanese individuals was genotyped for this variant and the frequency of distribution was found to be 0.005.

*CYP2C9*6* was identified in a 64-year African-American female who was placed on oral phenytoin therapy 100mg t.i.d. after hospitalization for status epilepticus [25]. Thirteen days after discharge, she presented with slurred speech, mental confusion, memory loss and inability to stand. Phenytoin levels were measured by fluorescence polarization and the plasma concentration-time profile analyzed. Its half-life was 310 h. (12.9 days), and the AUC was 5.8-fold higher than in an expected CYP2C9 EM. Genotyping revealed that the individual did not carry any of the known variants for *CYP2C9* (*2 or *3), or *CYP2C19* (*2, *3, *4, *5, *6, *7 and *8). Direct sequencing of the exons and intron-exon junctions revealed that the individual was homozygous for 818delA on exon 5. The frequency of *CYP2C9*6* in African-Americans from mid-Tennessee was 0.6%.

*CYP2C9*7, *8, *9, *10, *11 and *12*

The upstream region, coding regions and intron-exon junctions of *CYP2C9* were sequenced using lymphoblastoid cell lines of 92 subjects from the three major racial groups [26]. Of the 23 SNPs identified in the exons and exon-intron junctions and 15 SNPs in the promoter region, 6 novel alleles produced coding changes: 55C>A coding for L19I (*CYP2C19*7*), 449G>A coding for R150H (*8), 752A>G leading to H251R (*9), 815A>G resulting in E272G (*10), 1003C>T coding for R335W (*11) and 1465C>T coding for

P489S (*12). The new alleles were expressed in a bacterial cDNA expression system and their catalytic activity was assessed *in vitro* using tolbutamide as a substrate. Neither *CYP2C9*7* nor *10 exhibited altered activity, while *9 showed a slight decrease in activity. *CYP2C9*11* formed a defective protein with 60-80% catalytic activity, and Km was increased three-fold when compared to the wild-type. *CYP2C9*12* produced a 40% decrease in catalytic activity.

1137T>C

*CYP2C19*21*→*CYP2C9*21* a recent study reported a variant allele 1137T>C on exon 7, that led to an erroneous genotyping of a Japanese individual on warfarin therapy as *CYP2C9*3/*3* [27]. His genotyping results erroneously showed that he was *CYP2C9*3/*3*, which was surprising because his INR was normalized from 3.21 to 1.52 with a maintenance dose of warfarin (1.5 mg/day), without any bleeding complications. Usually, a *CYP2C9*3/*3* would be associated with a lesser dose, higher INR and bleeding complications. The individual was later found to be a compound heterozygote for the *3 and the new variant 1137T>C.

*CYP2C9*13*

A Chinese individual whose apparent genotype *CYP2C9*1/*3* did not agree with his PM status for both tolbutamide and lornoxicam was found to be a carrier of a novel T269C transversion allele on exon 2 causing amino acid change Leu90Pro in the encoded protein [28]. He was a heterozygote for *CYP2C9*3* and had a novel mutation, which was further designated as *CYP2C9*13*. Genotyping of 147 Chinese subjects showed that the frequency of this allele is 1.02% in the Chinese population.

*CYP2C9*14, *15, *16, *17, *18, & *19*

A prospective study of Asian patients on stable warfarin therapy identified novel mutations in the promoter, exonic, intronic and 3' untranslated regions of *CYP2C9* [29]. Recently, new variants of *CYP2C9* were reported from Asian subjects in Singapore [30]. Following sequencing, clones of the variant alleles were expressed in a bacterial cDNA expression system. Using tolbutamide as a probe, the catalytic activity of *CYP2C9*14* and *16 was found to be 80-90% lesser, and that of *CYP2C9*17* and *19 was 30-40% lesser when compared to the wild-type allele. *CYP2C9*15* and *18 were null alleles.

*CYP2C9*21, *22, *23*

The alleles *CYP2C9*21* (89C>T → Pro³⁰Leu), *22 (121A>G → Asn⁴¹Asp) and *23 (226G>A → Val⁷⁶Met)

were identified during a haplotype analysis of *CYP2C9*→*CYP2C9* in a European American population [31]. The variants were present in this population at a frequency of 0.5%, 0.3% and 0.5% respectively.

CYP2C9 – INTER-ETHNIC DISTRIBUTION

The marked inter-ethnic difference in the distribution of *CYP2C9* alleles and genotypes makes it an important component for the study of inter-individual differences in drug metabolism. Many global populations have been genotyped for the *CYP2C9* polymorphism, the results of which are compared in this section.

Caucasian populations including Turkish [10], Russian [32], Croatian [33,34], Spanish [35], French [36] and Belgian [37] showed a similar trend in the distribution of *CYP2C9**2 and *3 alleles, which was higher than in East Asian populations (Table 2).

The Spanish population showed a frequency of 16.2% for *3, the highest reported among Caucasian populations [35,38,39]. The PM genotype was present in 10% of individuals.

Bolivians (South American population) had a lower frequency of *CYP2C9**2 when compared to other Caucasians (Europeans and North Americans), but was higher than East Asians [40]. The frequencies were similar to Canadian Native Indian [41], African-American [20] and Ethiopian [42] populations. The PM phenotype in the Bolivian population was 0.4%.

In the Brazilian population, the frequency of *CYP2C9* alleles was similar to other Caucasians. However, they differed among the colored groups, being 2.5 to 3 times lower in Black Brazilians [43]. Mexican-Americans, comprising Hispanic individuals, exhibited a prevalence of *CYP2C9**2 and *3 which was similar to Caucasians, slightly higher than Africans, but lower than East Asians [38].

Arab populations such as the Faroese [44] and Egyptian populations [45] also had similar allelic frequency compared to Caucasians.

The prevalence of the *CYP2C9* polymorphism in South Indians [46] is distinct from both Caucasians and Chinese. While the frequency of *2 is higher than in Chinese and lower than in Caucasians, *3 is present in a frequency comparable to that in Caucasians.

Among the East Asians, *CYP2C9**2 is extremely rare, being reported only in 0.1% of Chinese [36,47,48]. The allele was absent in Japanese [49], Vietnamese Kinh [50], and Korean [51] populations. The frequency of *3 by meta-analysis was calculated to be 3.3% for Chinese and 4.5% for Japanese [12].

No variant alleles have been detected in the Inuit population of Canada. In addition to the common alleles, rare mutants such as *CYP2C9**1B was observed in Russian subjects from the region of Voronezh at a frequency of 24.8% [32]. *CYP2C9**5 is present in 0.8% Tanzanians [52] and 1.8% Beninese individuals [37]. An Afro-American individual who presented with phenytoin toxicity was found to possess the *CYP2C9**6 allele [25]. The prevalence of *6 in African-

Americans is 0.6%. *CYP2C9**11 was identified in 2.7% Beninese subjects [37]. Two percent of Chinese individuals are carriers of the *CYP2C9**13 allele [53].

CYP2C9 SUBSTRATES

Phenytoin

Of the known substrates of *CYP2C9*, phenytoin is the most important, owing to its zero-order (concentration-dependent) pharmacokinetics, which exhibits wide inter-individual variation (Table 3). This variation has, to some extent, been attributed to genetic factors such as the *CYP2C9* polymorphism. Phenytoin is metabolized almost extensively by the *CYP2C9* enzyme and to a minor extent by *CYP2C19* to its major metabolite 5-(para-hydroxyphenyl)-phenylhydantoin (5-HPPH). *In vitro* studies have suggested a significant reduction in *CYP2C9* activity with the *3 allele, but only a slight reduction with *2 [5].

In a group of 101 Turkish volunteers administered 300 mg phenytoin each for the phenotyping of *CYP2C9*, a significant difference in phenytoin trough levels as well as metabolic ratio was observed 12 hrs. after the test dose, when the *CYP2C9**1/*1 individuals were compared to those with *CYP2C9**1/*2, *CYP2C9**2/*2 and *CYP2C9**1/*3 [10].

The relative contribution of *CYP2C9* and *CYP2C19* on phenytoin metabolism *in vitro* was studied using sulfaphenazole and omeprazole as selective index substrate inhibitors of *CYP2C9* and *CYP2C19* respectively [58]. Phenytoin clearance was impaired by sulfaphenazole whereas omeprazole produced minimal inhibition of phenytoin, suggesting that *CYP2C19* has a minor role in the 4-hydroxylation of phenytoin while *CYP2C9* has the major role.

A further study revealed that the number of mutant alleles of *CYP2C9* is a major determinant, the *MDR1**T alleles contributed to the prediction of phenytoin plasma levels, but *CYP2C19* did not influence inter-individual variability in phenytoin metabolism [59]. An investigation of 35 patients from the Therapeutic Drug Monitoring program of the University of Vienna, Austria to evaluate the use of *CYP2C9* and *MDR1* genotyping in routine practice also showed that the dose-corrected plasma levels of phenytoin increased with number of mutant alleles for *CYP2C9* and *MDR1**T in the clinical setting.

The effect of the *CYP2C9* genotype on phenytoin dose requirement in relation to serum concentration was investigated in a group of 36 mentally retarded Dutch patients with epilepsy, who were receiving long-term phenytoin therapy [55]. Patients carrying at least one mutant allele required a 37% lower maintenance dose of phenytoin for a steady state serum concentration of 10-20 mg/L, when compared to those who were homozygous for the wild-type. A low maintenance dose of <200 mg/day sufficed to reach an effective serum concentration of phenytoin in carriers of mutant alleles, whereas patients who were *CYP2C9**1/*1 required >300 mg/day to attain therapeutic levels.

The optimal dose for phenytoin was deduced based on the *CYP2C9* and *CYP2C19* genetic polymorphisms [56]. Subjects included 169 patients who were receiving pheny-

Table 2. Inter-Ethnic Frequencies of CYP2C9*2 and *3

Population	n	*1	*2	*3	References
Turkish	499	79.4	10.6	10	[10]
Russian	290	72.6	10.5	6.7	[32]
Croatian	200	74	16.5	9.5	[33]
Croatian	177	83.9	12.4	3.7	[34]
Spanish	102	74	16	10	[35]
Spaniards	152	74	16	10	[38]
Spanish	157	69.5	14.3	16.2	[39]
French	151	77	15	8	[36]
Belgian	121	82.2	10	7.4	[37]
Bolivian	778	92.2	4.8	3.0	[40]
Brazilian	331	84.9	8.6	6.5	[43]
Beninese	111	95.5	0	0	[37]
Mexican-American	169	86	8	6	[38]
Faroese	312	85.9	8.8	5.3	[44]
Egyptian	247	82	12	6	[45]
Canadian Native Indian_(100%)	114	91	3	6	[41]
Inuit	151	100	0	0	[41]
South Indian	346	88	4	8	[46]
Chinese	235	96.4	0	3.6	[48]
Chinese	265	95.1	0	4.9	[47]
Chinese	394	96.3	0.1	3.6	[36]
Japanese	140	98.9	0	1.1	[49]
Korean	574	98.9	0	1.1	[51]
Vietnamese Kinh	157	97.8	0	2.2	[50]

toin for at least one month prior to the study. They were divided into 5 groups based on their genotype. The V_{max} was lower and K_m higher in the poor metabolizers than in the extensive metabolizers. Based on these values, the authors recommended dosage ranges for the various patient groups.

Poor metabolizers required only 2-3mg/kg/d, compared to 5-7 mg/kg/d for EMs.

However, in a study to assess the usefulness of prior genotyping in 20 routinely treated Japanese patients, V_{max} and K_m showed wide inter-individual variability, and no sig-

Table 3. Influence of CYP2C9 on Phenytoin Metabolism and Dose

S.No.	Finding	Reference
1.	*3 causes more reduction in activity, compared to *2	[54]
2.	Trough levels of phenytoin in plasma depends on CYP2C9 genotype	[10]
3.	Carriers of one mutant allele require 37% lower dose	[55]
4.	Dose: 2-3 mg/kg/d for PMs, 5-7 mg/kg/d for EMs	[56]
5.	Genotyping of CYP2C9 not very useful in determining dosage	[57]

nificant correlation was observed between unbound drug and metabolic activity [57]. These findings precluded the usefulness of genotyping in predicting the clinical outcome of phenytoin therapy.

Warfarin

Warfarin is an oral anticoagulant administered as a racemic mixture. The S-form is three times more potent than the R-form and is metabolized by CYP2C9. The dose requirement of warfarin shows wide inter-individual variation, higher plasma concentrations leading to excessive bleeding complications [60]. The odds ratio for the patient group requiring a low dose with at least one variant allele is found to be 6.21, and risk of bleeding complications is 3.7, when compared to controls.

In African-American individuals, the presence of CYP2C9*2 and *3 has been associated with a significant reduction in warfarin dose in 29% of the patients [61]. The authors claim that "the use of genotypic information to prescribe more accurate doses of warfarin may increase the safety and efficacy of this medication".

In European American individuals, the CYP2C9*2 and *3 variants combined were associated with reduced maintenance doses, longer time for stabilization of dosing and increased risk of bleeding [31].

In 201 Caucasian patients on stable warfarin therapy for at least 2 months, the CYP2C9 genotype, age, weight, concurrent medication and indication for treatment explained 29% of the variability in warfarin doses [62]. However, the CYP2C9 genotype could not be identified as a risk factor for warfarin bleeding.

Subjects with the CYP2C9*1/*11 genotype require a 33% reduction in warfarin dose, when compared to controls with the CYP2C9*1/*1 genotype. *In vitro* expression studies in insect cells reveal increased thermal lability of CYP2C9*11, which leads to reduced enzyme activity *in vivo* [63].

Attempts to tailor warfarin dose include a retrospectively developed algorithm which was tried in 48 orthopedic patients [64]. Although individuals with variant alleles achieved rapid stable therapeutic dose, they were still at a 3.6-fold increased risk for adverse events. The authors conclude that a prospective pharmacogenetics-based dosing is feasible, but would require further evaluation.

A recent study attempted to quantify the influence of CYP2C9 genotype, demographic factors and concomitant drug treatment on warfarin metabolism and maintenance dose [65]. The mean dose in carriers of zero, one and two mutant alleles was 4.88, 2.71 and 1.64 mg/day respectively. This data can be utilized in the initiation phase of genotype-based warfarin therapy.

Acenocoumarol

Mean oral plasma clearance of S-acenocoumarol is 45% lower and half-life is twice as long in the CYP2C9*1/*3 genotype group. The activity of the *3 variant is also decreased to 85% *in vitro*, for the hydroxylation of S-acenocoumarol [66].

In two cases of over-anticoagulation after 3 to 4 doses of acenocoumarol therapy 4mg/day, without bleeding symptoms, [67] the INR was more than 9, and the drug was stopped to bring it to the normal range of 2-3. Upon DNA sequencing, both patients were found to be homozygous carriers of CYP2C9*3. Thus, the influence of the CYP2C9 genetic polymorphism on acenocoumarol is clinically relevant during the initiation of therapy.

Oral Hypoglycemic Agents

Although no significant influence of CYP2C9*2 was found on the pharmacokinetic parameters of glyburide and glimepiride in Finnish volunteers, carriers of CYP2C9*3 showed a significant increase in median AUC_(0-a), C_{max} and t_{1/2} of glyburide as well as in the AUC_(0-a) and t_{1/2} of glimepiride when compared to wild-type genotype [21]. In carriers of the CYP2C9*3/*3 genotype, oral clearance of glyburide was significantly lower and insulin concentrations significantly higher than in CYP2C9*1/*1.

The functional significance of the CYP2C9 genetic polymorphism was also studied using the widely-used oral hypoglycemic agent tolbutamide [68]. The increase in AUC_(0-a) and decrease in oral clearance of tolbutamide was greater with the *1/*3 genotype than with *1/*2, when compared to wild-type, indicating that the *3 allele is severely detrimental to CYP2C9 enzyme activity.

Following the study on the functional significance of the CYP2C9 polymorphism with a 500 mg dose of tolbutamide, the possibility of low-dose tolbutamide for phenotyping of CYP2C9 was investigated, to circumvent the possibility of hypoglycemia due to higher blood levels of the drug in healthy subjects [69]. The results revealed that low-dose tolbutamide followed by a 24 hr. sample can be used as a probe for the phenotyping of CYP2C9.

Fluvastatin

Fluvastatin is a 3-hydroxy-3-methylglutaryl-coenzyme A inhibitor used in therapy of hypercholesterolemia. Kircheiner *et al.* studied the enantio-specific impact of the *2 and *3 genotypes on the pharmacokinetics and pharmacodynamics of fluvastatin in healthy volunteers [70]. Although the pharmacokinetics of both enantiomers showed statistically significant differences according to the number of *3 alleles, no effect of *2 was observed. Although the total serum cholesterol and low-density lipoprotein concentrations decreased during the 14-day period, there was no correlation with the CYP2C9 genotype.

Non Steroidal Anti-Inflammatory Drugs

Ibuprofen, one of the most widely used anti-inflammatory drugs, is available as a racemic mixture of R(-) and S(+) ibuprofen. Although no significant differences on the pharmacokinetics have been observed based on the *2 allele, the population mean clearance of CYP2C9*3/*3 individuals was 50% lower and the elimination half-life significantly higher than CYP2C9*1/*1. The AUC values were twice as high in carriers of CYP2C9*3/*3 genotype compared with carriers of the *1/*1 genotype [71].

The CYP2C9 genotype accounted for 59% of the variability in AUC_{0-a} and 52% in the variability in oral clearance,

formation clearance to the hydroxy-metabolite, and 0-24h metabolic ratio of flurbiprofen. *CYP2C9**2 was not found to have a significant impact on flurbiprofen pharmacokinetics, whereas the *CYP2C9**3 allele produced a 1.7-fold greater $AUC_{(0-\infty)}$, a significantly lower oral clearance (44%) and formation clearance (39%), compared to the wild-type [72].

Celecoxib is a selective COX-2 inhibitor, which is widely prescribed for its anti-inflammatory and analgesic properties in the treatment of rheumatoid arthritis. The primary metabolic pathway involves methyl-hydroxylation, catalyzed by *CYP2C9*. In view of the fact that the *CYP2C9* gene is polymorphically expressed, this is one of the first drugs for which caution was advised by the manufacturer, Pfizer, when prescribed to poor metabolizers of this enzyme [73]. However, neither a definition of poor metabolizers, nor the optimal dose in such a case was indicated.

A significant effect of the *3 allele has been detected with a three-fold reduced oral clearance of celecoxib and a 1.5-fold prolonged elimination half-life when comparing those with *CYP2C9**3/*3 with *CYP2C9**1/*1 [74].

The pharmacogenetic basis of the metabolism of **diclofenac** has produced variable results *in vitro* and *in vivo*. Diclofenac, a phenyl acetic acid derivative, is used to treat rheumatoid arthritis and other fever or pain-related conditions. In an attempt to study the effect of *CYP2C9* on the *in vivo* pharmacokinetics of diclofenac, a 50 mg oral dose was administered to 12 healthy subjects, and pharmacokinetic parameters were estimated from plasma and urinary samples over a period of 12 hours [75]. No significant differences were found in mean diclofenac and 4-hydroxydiclofenac concentrations between the wild-type and *3 genotype groups at any sampling time points. There were also no differences in any of the kinetic parameters for disposition or clearance of either parent drug or metabolite between the genotype groups. The authors attributed their finding to the possible substrate specificity of the *CYP2C9* polymorphism on drug metabolism.

The *in vivo* effect of *CYP2C9**2 and *3 were studied on diclofenac pharmacokinetics after a 50 mg oral dose, along with *ex vivo* measurement of prostaglandin E_2 and thromboxane A_2 levels as surrogate markers for pharmacodynamic properties of the drug, in relation to COX-2 and COX-1 activity [76]. In this study also, neither variant allele showed an influence on the pharmacokinetic or pharmacodynamic parameters of diclofenac.

However, on account of the good clinical tolerance of diclofenac along with the fact that no ideal probe was available for phenotyping *CYP2C9*, the use of a single 50mg dose of enteric-coated diclofenac was investigated as an *in vivo* probe for *CYP2C9* [77]. Although the clearance of diclofenac in heterozygous subjects was lower than in the wild-type, this did not reach statistical proportions, possibly because of the low sample size in the study.

Antihypertensive Agents

Losartan is a highly selective angiotensin-II receptor antagonist which is metabolized to the active carboxylic acid metabolite E-3174, and is widely used as an anti-

hypertensive agent. The *in vivo* impact of the *CYP2C9* genotype on losartan oxidation revealed that the plasma $AUC_{\text{losartan}}/AUC_{\text{E-3174}}$ was 2-3-fold higher in carriers of the *3 allele, and 30 fold higher in the single *3 homozygous subject. The urinary ratio of losartan/E-3174 followed the same trend, being 40-fold higher in the *3/*3 individual. Carriers of *CYP2C9**2 *per se* did not show a significant change in pharmacokinetic parameters, unless they were compound heterozygotes for *2 and *3 alleles [78].

In the Japanese population also, after administration of a single 25 mg dose of losartan, the plasma AUC ratio, MR_{4h} , MR_{6h} and urinary MR_{4-8h} in the *CYP2C9**1/*3 group were significantly lower than in *1/*1. The *3 variant was also found to reduce the hypotensive effect of losartan [79].

An 89 yr old man developed dizziness 2 days after **candesartan** was added to his treatment regimen for severe hypertension and chronic heart failure [80]. The symptoms alleviated after candesartan was withdrawn. The genotype of the patient was identified as *CYP2C9**1/*3. An estimation of his pharmacokinetic parameters after administration of 4mg candesartan cilexetil showed a 2.5 fold increase in $AUC_{(0-\infty)}$ and mean resident time, with a 48.3% reduction in oral clearance, compared to previously described patient data. This study further reiterated that the *CYP2C9**1/*3 genotype led to decreased clearance and increased plasma concentration of substrates, in this case candesartan, and therefore enhanced its hypotensive effect.

The association between the *CYP2C9**3 variant and **irbesartan** metabolism was studied in Chinese subjects [48]. After administration of 150 mg irbesartan for 26 days, *CYP2C9**3 carriers were found to have higher plasma concentrations of the drug, suggesting a significant influence of this allele on the metabolism of irbesartan.

CLINICAL RELEVANCE OF THE *CYP2C9* POLYMORPHISM

Adverse Drug Reactions & Drug Toxicity

The genetic polymorphism of *CYP2C9* has been reported to be associated with adverse reactions to its drug substrates. Some of the clinically important reports are shown in Table 4.

Association with Disease States

The *CYP2C9* polymorphism has been shown to influence the predisposition to various diseases such as depression, myocardial infarction, hypertension and certain cancers (Table 5).

CYP2C19 - Discovery

The *CYP2C9* polymorphism has been shown to influence the predisposition to various diseases such as depression, myocardial infarction, hypertension and certain cancers (Table 5). The discovery of the genetic polymorphism of the enzyme S-mephenytoin hydroxylase was made in 1979 at Vanderbilt University, during a study on the stereo-selective metabolism of (S)-mephenytoin. One subject experienced such extreme sedation after 5 days at a dose of 300 mg/day that he withdrew from the study. His urinary metabolic profile showed a deficiency in (S)-mephenytoin-4'-hydroxylase.

Table 4. Toxicological Relevance of the CYP2C9 Polymorphism

Adverse effect	n	Conclusion	Reference
Hypoglycemia after sulphonylureas	20 diabetic patients with hypoglycaemia	More common in subjects with *2/*2 or *3/*3	[81]
Phenytoin-induced cutaneous ADRs	10 patients, 39 exposed controls, 169 healthy controls	*3 more common in patients, so maybe predisposing factor	[82]
Phenytoin induced gingival hyperplasia	28 patients, 56 controls	*3 associated with increased concentration/dose ratio, no direct correlation	[83]
NSAID induced over-anticoagulation	cohort of 973 patients	Risk 2.98 times higher with *2, 10.8 times higher with *3.	[84]
NSAID induced gastrointestinal bleeding	94 with bleeding, 124 with no bleeding after NSAID use	Odds ratio was 2.5 for heterozygotes, & 3.7 for homozygous carriers	[85]

Kupfer *et al.* in 1984 showed that the deficiency in (S)-mephenytoin hydroxylase is a monogenic trait. Studies in 1993 by Wrighton *et al.* revealed that (S)-mephenytoin hydroxylase is the cytochrome P450 2C19 (*CYP2C19*) [92]. Goldstein and co-workers cloned individual CYP2C enzymes in 1994 and tested activity towards (S)-mephenytoin *in vitro* [93].

CYP2C19 Alleles

Of the 21 variant alleles of *CYP2C19* that have been described to date, the two most common variants are *CYP2C19*2* and *3, both of which result in the total absence of enzyme activity [94].

CYP2C19*2

*CYP2C19*2*, the most common variant allele of *CYP2C19*, is the result of a single base pair 681G>A mutation on exon 5, leading to an aberrant splice site [95]. This change alters the reading frame of mRNA from amino acid 215, and produces a stop codon 20 bp downstream, leading to a truncated protein. This variant accounted for the poor metabolizer phenotype of 7 out of 10 Caucasian and 10 out of 17 Japanese individuals.

CYP2C19*3

Following the characterization of *CYP2C19*2*, another mutation was identified in the seven Japanese individuals

who were poor metabolizers of mephenytoin, but were not homozygous for *CYP2C19*2* [96]. This novel mutation was *CYP2C19*3*, consisting of a 636G>A on exon 4, leading to amino acid change Ile³⁵⁹Leu. The *CYP2C19*3* explained the poor metabolizer phenotype of the remaining 7 Japanese subjects. This allele was however, not identified in Caucasian individuals.

CYP2C19*4

After the discovery that *CYP2C19*2* and *3 account for >99% of Oriental poor metabolizers but only ~87% of Caucasian PMs, the search was on to identify additional *CYP2C19* defective alleles in Caucasians. An American subject, whose PM status could not be explained by *CYP2C19*2* or *3, based on previous studies, was found to be heterozygous for the *CYP2C19*4* allele [97]. *CYP2C19*4* is characterized by an A>G mutation at the first base of exon 1. This alters the initiation codon from ATG to GTG, and therefore no protein is coded for. Genotyping of 173 European Caucasian individuals revealed only one additional *CYP2C19*4* allele. The frequency of this allele was therefore, 0.6% in Caucasians.

CYP2C19*5

The frequencies of *CYP2C19*2* and *3 were compared in the Chinese Han (n = 101) and Bai populations (n = 102) [98]. Phenotyping was done after administering 100mg racemic mephenytoin. One Bai phenotypic PM outlier ap-

Table 5. CYP2C9 and Association with Disease

Disease	n	Conclusion	Reference
Lung cancer	329 patients and 700 controls	No significant association	[86]
Major depressive disorder	70 patients, 138 controls	Relative risk 3.14 in carriers of *3	[87]
Acute myocardial infarction	1702 patients, 1503 controls	Females with *2 or *3 have an odds ratio of 1.3, 1.1 in males	[88]
Hypertension	239 patients, 265 controls	*3 has secondary protective effect in females	[47]
Hepatocellular carcinoma	50 HCV-infected livers, 6 normal livers	Level of CYP2C9 lower in tumor, may be used as marker	[89]
Colorectal cancer	377 cases, 326 controls	No association	[90]
Colorectal cancer	273 cases, 453 controls	*1/*2 confers decreased risk in men, and increased risk in women	[91]

peared to be of the EM genotype. On sequencing the entire *CYP2C19* gene, the subject was found to be heterozygous for a new mutation 1297C>T on exon 9, which leads to amino acid substitution Arg⁴³³Trp in the heme-binding region.

CYP2C19*6, *2B

Genomic DNA of a Swiss PM outlier whose phenotype could not be explained by the common *CYP2C19* alleles [95,96], was amplified across all exons of *CYP2C19* [97]. Sequence analysis showed that the subject was heterozygous for *CYP2C19*2* and two new mutations in exon 2 and exon 3. The mutation in exon 3 was 395G>A, leading to substitution Arg¹³²Gln, and was designated *CYP2C19*6*. *CYP2C19*2B* is due to 276G>C on exon 2, causing amino acid change Glu⁹²Asp. On the basis of a family study, and further using a 172 French European Caucasian control population, *CYP2C19*2B* was found to cosegregate with *CYP2C19*2*, and accounted for 15% of the *2 alleles in this population. No additional *CYP2C19*6* was detected, the frequency being 0.9%. Site directed mutagenesis of this novel allele followed by expression in a bacterial system revealed negligible catalytic activity.

CYP2C19*7, *8

Genomic DNA was sequenced from a Danish PM outlier and a French PM outlier, whose genotypes were discordant with the PM phenotype for mephenytoin metabolism, across all exons [99]. The Danish individual was found to be heterozygous for a novel 2T>A in the conserved GT splice junction donor site, and later designated as *CYP2C19*7*. *CYP2C19*8* was discovered in a French subject who had enrolled in a previous lung cancer case control study [100], whose genotype did not agree with phenotype. Sequencing of the exons and intron-exon junctions revealed heterozygosity for *CYP2C19*8*, consisting of a T358C in exon 3 that leads to Trp¹²⁰Arg, and a seven-fold lower activity for the substrate tolbutamide *in vitro*.

CYP2C19*9, *10, *11, *12, *13, *14, *15

Blaisdell *et al.* prepared lymphoblastoid cell lines from 92 humans of the three major racial groups belonging to different ethnic ancestries [101]. They included 24 Caucasians, 24 Asians, 24 Blacks and 20 of unknown racial heritage. Each exon, exon-intron junction and -1.46 kb of the upstream region was sequenced, followed by cloning and expression in a bacterial expression system. A total of 26 SNPs were detected in the exons, introns and 3'-noncoding regions of *CYP2C19* and 13 in the promoter region. *CYP2C19*9* (Arg¹⁴⁴His) produced a modest (23%) reduction in V_{max} and a 16% decrease in K_m of the enzyme. *CYP2C19*10* (Pro²²⁷Leu) increased the K_m for mephenytoin 2.5-fold and decreased the V_{max} by five-fold. *CYP2C19*11* is characterized by a Arg¹⁵⁰His change. *CYP2C19*12* (Stop⁴⁹¹Cys) destroys the stop codon, producing a protein with additional amino acids *in vitro*, which is unstable. The functional consequences of this SNP is not clear. *CYP2C19*13* results from a Arg⁴¹⁰Cys amino acid substitution. *CYP2C19*14* (Leu¹⁷Pro) and *CYP2C19*15* (Ile¹⁹Leu) could be functionally characterized. Based on the functional assays, the authors suggest that *CYP2C19*10* in the African-American population may represent a new defective allele producing

dramatic differences in the metabolism of *CYP2C19* substrates.

CYP2C19*16

All the exons and introns of *CYP2C19* were sequenced in a Japanese subject who was a poor metabolizer of mephobarbital [102]. They identified a novel mutation 1324C>T which led to amino acid change Arg⁴⁴²Cys. Since this SNP is in exon 9 close to the heme-binding site, it is proposed to result in decreased capacity of substrates for *CYP2C19*.

CYP2C19*17

Recently, a novel allele *CYP2C19*17* was identified in 18% of Swedes and Ethiopians, and 4% of Chinese individuals. This allele is characterized by -806C>T and -3402C>T in the 5'-regulatory region of *CYP2C19*. Carriers of this variant were found to have a significantly lower AUC of omeprazole, suggesting that this would give rise to an ultra-rapid metabolizer phenotype for *CYP2C19*.

Inter-ethnic variation

Numerous studies that have been done to establish the frequencies of poor metabolizers in various populations have proved the ethnic specificity of the *CYP2C19* genetic polymorphism (Table 6).

Among the *CYP2C19* alleles, *2 is the most common, ranging from 9-26% in Caucasians [10,13,32,33,37,42,103] and 20-35% in Indians and East Asians. *CYP2C19*3* is relatively rare in Caucasians, Indians and Africans (0-2%). It however occurs in 2-10% of East Asian individuals.

South American individuals from Bolivia had lower *2 and *3 allele frequencies when compared to other Caucasian populations [40].

The occurrence of *CYP2C19*2* was 15% in Jewish-Israeli [106] and Saudi Arabian [105] populations. This is significantly different from the Faroese [44], in which only 2.6% are carriers of *2. The prevalence of mutant alleles in these Arab populations is similar to Caucasians and lower than Indians and East Asians. Among 4 different Israeli ethnic groups, *CYP2C19*3* was identified only in one Bedouin subject, who was heterozygous for the allele [117]. A total of 4 individuals (2.5%) had the predicted PM genotype.

Canadian native Indians have a frequency distribution of 19.1% for *CYP2C19*2*, while *CYP2C19*3* was absent [107]. This frequency is significantly different from both Caucasian and Asian populations.

The highest prevalence of the *CYP2C19*2* and *3 variant alleles has been reported from the island population of Vanuatu [118]. In a study conducted to assess the effect of *CYP2C19* genotypes on the disposition of proguanil in 100 malaria patients, the frequencies of *CYP2C19*2* and *3 were found to be 57% and 25% respectively. Sixty eight percent of the individuals had the poor metabolizer genotype. Genotyping of 5538 individuals from 24 populations on 16 different islands of Vanuatu revealed the average PM genotype frequency to be 61%, higher than in any other population.

The South Indian population has a relatively high prevalence of *2 alleles (35%) [46]. The PM genotype frequency

Table 6. Inter-Ethnic Distribution of *CYP2C19* Alleles

Population	n	<i>CYP2C19</i> *2	<i>CYP2C19</i> *3	Reference
Turkish	404	12	0.4	[10]
Russian	290	11.4	0.3	[32]
Belgian	121	9.1	0	[37]
Dutch	765	13.3	0.2	[103]
Croatian	200	15.0	0	[33]
Italian	360	11.1	0	[104]
Bolivian	778	7.8	0.1	[40]
Saudi Arabians	97	15	0	[105]
Faroese	312	2.9	0	[44]
Jewish Israeli	140	15	1	[106]
Canadian Indians	159	19.1	0	[107]
Vanuatu	100	57	25	[15]
Vanuatu	5538	63.3	14.4	[15]
South Indian	341	35	1	[46,108]
North Indian	121	30	0	[109]
Beninese	111	13.0	0	[37]
African	922	17.3	0.4	[110]
Tanzanian	251	17.9	0.6	[111]
African	216	10	0	[112]
Ethiopian	114	14	2	[113]
African American	108	25	0	[105]
Chinese Han	101	36.6	7.5	[98]
Chinese Bai	202	25.7	5.5	[98]
Chinese Dai	193	30.3	3.4	[114]
Chinese Taiwanese	118	32	5.5	[105]
Japanese	53	23	10.4	[105]
Japanese	140	35	11	[49]
Filipino	52	39	8	[105]
Korean	103	21	12	[115]
North-Eastern Thai	107	27	2	[116]

was 12.6%. In North Indians, *2 was present at a frequency of 30%, while *3 was absent [109]. This means that the *2 frequency was similar to that in Orientals, but this population also resembled Caucasians in the total absence of the *3 allele.

A meta-analysis of *CYP2C19* distribution in Africans and black Americans showed frequencies of *CYP2C19**1, *2 and *3 were 82.3%, 17.3% and 0.4% respectively [110]. The frequency of PM genotypes in Blacks was 3.7%. Individuals

of African descent thus have a low prevalence of *CYP2C19* variant alleles.

The incidence of *2 in African Americans was 25%, which was higher than that reported in Africans or Caucasians [105]. It was similar to Caucasians in that no *3 allele was detected. The authors attribute the variation in allele frequencies between Africans and African-Americans of different geographical locations to be due to the Caucasian admixture in the gene pool of these regions.

Among the major Chinese ethnic groups, the Han population had a slightly higher prevalence of *CYP2C19* mutations than Bai and Dai [98,114]. The prevalence of the PM genotype was 14% in Chinese, 16% in Koreans [115] and 11% for Japanese. Frequencies of *2 ranged from 23 - 35% in Japanese [105], whereas it was 39% in Filipinos. The North-Eastern Thai population has a lower frequency of *3 when compared to other East Asian populations [116].

Substrates

Omeprazole

Omeprazole, a proton pump inhibitor is extensively hydroxylated by *CYP2C19* to its primary metabolite 5-hydroxy-omeprazole and to a lesser extent by *CYP3A4* to omeprazole sulphone. It is used as a probe for establishing the genotype-phenotype relationship for *CYP2C19* (Table 7) [122].

Based on an antimode of 14.4 for HI of omeprazole, 14% of South Indian individuals were classified as phenotypic PMs. This frequency was similar to that found in North Indians and Orientals, but higher than in Caucasians and Africans [119].

*CYP2C19**2 could explain only 43% of poor metabolizers in the North Indian population, suggesting the presence of additional defective alleles. The *in vitro* association was investigated between the *CYP2C19* genotype and omeprazole hydroxylation in the North Indian population [123]. The study was done from microsomes obtained from 15 liver samples, and the individuals were also genotyped for *CYP2C19**2 and *3. North Indian PMs demonstrated only 11% activity of omeprazole hydroxylase compared to EMs. Those who were heterozygous showed an intermediate activity of omeprazole hydroxylase, which was 52% of that in EMs. This pattern of intermediate activity however has not been observed *in vivo*. A concordance was thus observed between the *CYP2C19* genotype and *in vitro* activity of omeprazole hydroxylase.

To determine whether the effect of omeprazole depends on the *CYP2C19* genotype, sixteen healthy Japanese individuals of known *CYP2C19* genotype were administered a single oral dose of 20 mg omeprazole or placebo [124]. The mean 24-hr intragastric pH values differed significantly among the 3 genotype groups after omeprazole administration. A significant correlation was observed between the

CYP2C19 genotype and omeprazole AUC as well as the AUC of omeprazole and intragastric pH.

In a study on 108 patients, the *H. pylori* eradication rate for extensive metabolizers was 41% and 83% with dual and triple drug regimens (containing omeprazole), while all poor metabolizers had a 100% eradication rate. In subjects who received a quadruple regimen (which did not include omeprazole), the eradication rate was similar for extensive and poor metabolizers. Therefore, a dual therapy regimen would suffice for total eradication of *H. pylori* infection in poor metabolizers of *CYP2C19* [11].

In an attempt to predict the optimal dose of omeprazole based on the *CYP2C19* genotype, seven healthy Japanese subjects were administered a single oral dose of 20, 40 and 80 mg with an interval of at least one week wash-out period [125]. Levels of omeprazole and its metabolites were estimated and the AUC calculated. The AUC of 20 mg omeprazole in poor metabolizers was similar to the AUC in extensive metabolizers after a dose of 80 mg.

Lansoprazole

Lansoprazole is a proton pump inhibitor that is used in the treatment of peptic ulcer and gastro-esophageal reflux disease. It is extensively metabolized in the liver to 5-hydroxy lansoprazole and lansoprazole sulphone. Kim *et al.* evaluated the enantio-specific disposition of lansoprazole in 12 Korean subjects [126]. Each subject received a 30 mg enteric-coated formulation of lansoprazole, and blood samples were drawn over a period of 24 hrs. The protein binding of the enantiomers was determined and pharmacokinetic parameters were estimated. The effect of the *CYP2C19* genetic polymorphism was less significant than on other proton pump inhibitors such as omeprazole and pantoprazole.

Another study investigated the pharmacokinetics of lansoprazole and 5-hydroxy lansoprazole among different *CYP2C19* genotype groups in healthy Japanese subjects [127]. After administering 60 mg of lansoprazole, blood was drawn over a period of 24 hrs. From the pharmacokinetic analysis of lansoprazole and its metabolites, the C_{max} differed among the three groups for the (S) enantiomer. The AUC of the (S) enantiomer was 13 fold higher than the (R) enantiomer, suggesting a stereo-specific disposition of the drug by *CYP2C19*.

Table 7. Phenotyping Studies Using Omeprazole as Probe

Population	n	Antimode	PM (%)	Reference
Korean	103	6.95	12.6	[115]
North-Eastern Thai	107	7	6.5	[116]
South Indian	300	14.4	14	[119]
North Indians	100	50	11	[120]
Bantu Tanzanians	207	7	14.5	[111]
Malaysian	142	6	14.1	[121]

Proguanil

The metabolism of proguanil was studied in relation to S-mephenytoin 4'-hydroxylation in an Indonesian population [128]. After administering a single oral dose of 200 mg proguanil hydrochloride to 24 healthy volunteers, venous blood samples were collected over a period of 72 hrs. and pharmacokinetic parameters of plasma proguanil, cycloguanil and 4-chlorophenylbiguanide were assessed. The bioactivation of proguanil to cycloguanil was found to cosegregate with the genetically determined 4'-hydroxylation of S-mephenytoin, thereby suggesting that it was metabolized by CYP2C19.

Similar results were found in a study on proguanil metabolism done in Tanzanians [129]. Each of the 216 subjects took 100 mg of racemic mephenytoin and urine was collected for 8 hrs. Then, 1 week later, they were given 200 mg proguanil, and urine was collected for 8 hrs. The mephenytoin S/R ratio statistically correlated with the proguanil/cycloguanil and proguanil/4-chlorophenylbiguanide ratio, again indicating the role of CYP2C19 in proguanil bioactivation to cycloguanil.

The oxidation of proguanil was studied in 89 unrelated healthy Turkish volunteers, to investigate the pattern of oxidation and to ascertain whether the metabolism cosegregates with that of S-mephenytoin [130]. A 0-8 hr urine sample was used for the analysis, after administration of 200 mg proguanil orally. At least two weeks later, the same subjects were given 100 mg mephenytoin. Based on antimode value of 15, the frequency of PMs was found to 5.6%. The PMs of proguanil were also identified as PMs of mephenytoin, and there was correlation between the oxidation of proguanil and the hydroxylation of mephenytoin.

The impact of the CYP2C19 genotype was studied on proguanil oxidation in the Caucasian population, in which all volunteers were administered 100 mg of proguanil and urine was collected over 8 hrs [131]. A gene dose effect for proguanil oxidation was observed, confirming the role of CYP2C19 in the *in vivo* formation of cycloguanil.

The genetic polymorphism of CYP2C19 was studied in the South Pacific Polynesian population using proguanil as a probe [132]. Subjects included 59 unrelated healthy individuals, at least 75 - 100% Polynesian in origin. They were given 200 mg proguanil and urine was collected over 8 hrs. Using an antimode of 10 as for the Caucasian population, the PM phenotype frequency was 13.6%.

In order to establish the impact of CYP2C19 genotype and phenotype on proguanil metabolism, 25 Bantu Tanzanians were divided into three groups based on their CYP2C19 genotype, administered 200 mg proguanil and plasma samples drawn over 72 hrs, and urine collected over 9 hrs. for the pharmacokinetic study [133]. Individuals who were of the mt/mt genotype were found to have lower metabolic capacity than individuals who were wt/wt or wt/mt.

Proguanil was used as a probe for CYP2C19 to establish the prevalence of PMs in a Nigerian population [134]. The study was conducted in 126 unrelated volunteers of Nigerian origin. Urine voided during 8 hrs. was collected after intake

of 200 mg proguanil. Using an antimode value of 10, the PM frequency in this population was found to be 4.8%.

Carisoprodol

Carisoprodol is a skeletal muscle relaxant, which is N-dealkylated by CYP2C19 to meprobamate [135]. The association between the CYP2C19 genotype and carisoprodol: meprobamate ratio was studied in a 'real life' setting. Two polarized groups of subjects with apparent high and low metabolic ratios, based on a cut-off point of 1.0, were selected from a total sample of 358 cases of carisoprodol-drugged drivers. DNA samples from 94 anonymous healthy subjects formed the control group. The frequency of CYP2C19*2 was significantly higher in the high-ratio group, due mainly to the number of heterozygous individuals. The authors suggest that these heterozygous individuals should be considered intermediate metabolizers of the drug. The metabolism of carisoprodol seems to follow a gene-dosage effect, reflecting the number of active CYP2C19 alleles.

Clobazam

Clobazam is an anti-epileptic agent which shows wide inter-individual variability in metabolism. In a preliminary study on the possible involvement of CYP2C19 in clobazam metabolism, epileptic patients on stable clobazam therapy were included for reference values of the drug and its metabolite, and for CYP2C19 genotyping [136]. In the predicted CYP2C19 PM patients, the metabolic ratio was 10-27 fold higher than control epileptic patients. This data indicated that patients with one or two CYP2C19*2 alleles may be at a higher risk of adverse effects with clobazam therapy.

A gene-dose effect was observed for clobazam metabolism based on the CYP2C19 genotype. The N-desmethylclobazam/clobazam dose ratio in patients with 2 mutant alleles was 6-fold higher than in wild-type patients.

Clobazam metabolism was characterized *in vitro* by using cDNA expressed P450 and P450 specific inhibitors. N-desmethylclobazam was 4'-hydroxylated to 4'-hydroxydesmethylclobazam by CYP2C19 [137]. Further, 22 Japanese epileptic patients on clobazam were found to have a significantly higher N-desmethylclobazam/clobazam metabolic ratio, if they had mutant allele CYP2C19*2, compared to the wild-type.

Antidepressants

It is well established that the CYP2D6 polymorphism has an impact on most drugs affecting the CNS, such as anxiolytics, antidepressants and anti-psychotics. The relationship between the CYP2D6, CYP2C19 and CYP2C9 genotypes and steady-state plasma concentration of antidepressants was determined in 136 Caucasian depressed inpatients in a clinical setting [138]. Two PMs of CYP2C19 showed dose-corrected plasma concentrations that were more than 2-fold that of the drug-specific median, but on the whole, CYP2C19 played only a minor role in the metabolism of antidepressants, when compared to CYP2D6.

The effect of the CYP2C19 genotypes on the concomitant administration of fluvoxamine with alprazolam was studied in 23 Japanese patients [139]. While patients with no or one mutant allele showed more than 100% increase in alprazo-

lam levels after administration of fluvoxamine, those with 2 mutant alleles showed not more than a 50% increase. There was a wide variation in the drug interactions, which were related to the *CYP2C19* genotypes.

Clomipramine

To evaluate the impact of *CYP2C19* on clomipramine, 51 Japanese patients were administered 250 mg/day clomipramine orally for 2 weeks to attain steady-state concentrations [140]. Subjects who were homozygous for *CYP2C19**2 had 75% higher concentrations of the drug, thus suggesting a role of *CYP2C19* in predicting those with a risk of high plasma concentrations with clomipramine.

Citalopram

Citalopram is an SSRI used in the treatment of depression. The pharmacokinetics of the drug and its metabolites in relation to the *CYP2C19* genotype were estimated in a population of 19 healthy Swedish volunteers [141]. The subjects were administered 10 mg citalopram twice daily for 7 days. On the 7th day, blood and urine samples were collected over a period of 12 hours. The AUC of citalopram was found to be significantly greater in poor metabolizers of mephenytoin, indicating a role of *CYP2C19* in the metabolism of this drug.

Venlafaxine

To assess the impact of *CYP2C19* genotypes on the pharmacokinetics of the antidepressant venlafaxine, 28 unrelated healthy Japanese males were administered the drug, and plasma concentrations of the drug and its metabolite O-desmethylvenlafaxine measured [142]. *CYP2C19* was found to be one of the genetic determinants of venlafaxine metabolism.

Amitriptyline

The concordance between *CYP2C19* genotypes and amitriptyline metabolic ratios was assessed in a series of 202 postmortem toxicology cases [143]. A positive correlation was found between the proportion of demethylated metabolites and number of functional copies of *CYP2C19*. The presence of concordance suggests the feasibility of postmortem pharmacogenetic analysis.

Cyclophosphamide

Cyclophosphamide, a prodrug used as an anticancer agent, is metabolized to its active metabolite 4-hydroxy cyclophosphamide. In an *in vitro* study to evaluate the contribution of *CYP2C19* on the activation of cyclophosphamide, microsomes of 32 human livers were incubated with cyclophosphamide and the kinetic parameters measured [144].

They were then incubated with R-omeprazole, and quantification of the compound done by HPLC. A statistically significant correlation was found between the 4-hydroxylation of cyclophosphamide and 5'-hydroxylation of omeprazole, indicating that *CYP2C19* plays a role in the metabolism of this drug.

Bortezomib

A reversible inhibitor of the 20S proteasome, bortezomib is suggested for use in cancer chemotherapy. The relative contribution of various CYP450s to its metabolism was studied *in vitro* using human liver microsomes [145]. *CYP2C19* was found to play a major role in the metabolism of this drug.

Thalidomide

The possibility of *CYP2C19* playing a role in the therapeutic response to thalidomide in prostate cancer was investigated in a case-control study [146]. In the case-control study, patients who were *CYP2C19**2/*2 failed to show a decline in prostate-specific antigen. The results of this study were inconclusive and warrant further confirmation.

Oral Contraceptives

The effect of oral contraceptives on *CYP2C19* activity was assessed using probe drugs mephenytoin and omeprazole [147]. The study population included Swedish healthy volunteers who had already been phenotyped for *CYP2C19* using the probe drugs in earlier studies, and were now genotyped for *CYP2C19**2. The mephenytoin S/R ratio was 2.5 fold and the omeprazole metabolic ratio 2 fold higher in women who took oral contraceptives than in those who did not. Oral contraceptives were thus found to inhibit *CYP2C19* activity, both in carriers of the *CYP2C19**1 and *2 allele.

The use of oral contraceptives causes a 60% larger AUC_(0-∞) of carisoprodol in both extensive and intermediate metabolizers and longer t_{1/2} in extensive metabolizers. There was, however, no influence of oral contraceptives on the degree of adverse effects with carisoprodol.

Clinical Implications

The *CYP2C19* genetic polymorphism has been known to increase the predisposition to adverse reactions with certain drug substrates (Table 8).

Association with Disease States

The relationship between the *CYP2C19* genotype and phenotype was investigated in 16 patients with advanced stages of cancer of the lung, colon, breast, stomach, pancreas, or esophagus [155]. Based on the normit plot of the

Table 8. Clinical Implication of the *CYP2C19* Polymorphism

Adverse Drug Reaction (ADR)	Conclusion	Reference
Side-effects to clobazam	Carrier of *2, predisposed to ADR with clobazam	[148]
Cardiotoxicity to terolidine	*2 higher in patient group	[149]
Adverse drug reactions to cyclophosphamide	*2 carriers have a lower predisposition to ovarian failure	[150]

Table 9. CYP2C19 and Association with Disease

Disease	n	Conclusion	Reference
Colorectal cancer	490 patients, 593 controls	*2 plays a protective role against colorectal cancer	[151]
Hepatocellular carcinoma following HCV infection	38 patients with cirrhosis	PM phenotype susceptible to hepatocellular carcinoma	[152]
Systemic lupus erythematosus	70 patients, 161 controls	No association	[153]
Hepatocellular carcinoma	84 Japanese	No association	[154]

log hydroxylation index of omeprazole, and an antimode value of 1.0, four patients had a PM phenotype, in spite of an EM genotype. Moreover, the plot had shifted to the right, on being compared to the reference population, indicating a decreased metabolic capacity of CYP2C19 substrates in patients with cancer. This may influence the efficacy and toxicity of chemotherapeutic agents (Table 9).

CONCLUSION

The fact that CYP2C9 and CYP2C19 together metabolize only ~18% of currently used drugs (in contrast to CYP3A4 which metabolizes ~50%) by no means decreases their importance in pharmacogenetic studies. This is so particularly due to the nature of drugs they metabolize, and the clinical implications that follow. An increasing number of case reports describing adverse events to these agents owing to a PM genotype emphasize the benefits of prior genotyping. In the rapidly emerging era of 'personalized medicine', drug companies are already including pharmacogenetic data in clinical trials [156], and DNA testing services provide tests for the physician which allows him to provide 'tailored' drug therapy, as opposed to the conventional 'trial and error' prescribing. Tests which are already available include those for CYP450s 2C9, 2C19, 2D6 and 1A2, for vascular disorders, the treatment of depression, or pain management [157,158]. These can be employed taking into account the prevalence of polymorphisms in the particular population. For instance, CYP2C9 variants are more common in Caucasians, whereas the CYP2C19*2 polymorphism is of importance to Chinese and Indians. The science of Pharmacogenetics has a lot left to be discovered, towards individualized drug therapy.

REFERENCES

- Raucy JL, Mueller L, Duan K, Allen SW, Strom S, Lasker JM. Expression and induction of CYP2C P450 enzymes in primary cultures of human hepatocytes. *J Pharmacol Exp Ther* 2002; 302(2): 475-82.
- Nelson DR, Koymans L, Kamataki T, *et al.* P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 1996; 6(1): 1-42.
- van der WJ, Steijns LS. Cytochrome P450 enzyme system: genetic polymorphisms and impact on clinical pharmacology. *Ann Clin Biochem* 1999; 36 (Pt 6): 722-9.
- Wolf CR, Smith G. *Pharmacogenetics*. *Br Med Bull* 1999; 55: 366-86.
- Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics* 2002; 12(3): 251-63.
- Daly AK. Pharmacogenetics of the major polymorphic metabolizing enzymes. *Fundam Clin Pharmacol* 2003; 17(1): 27-41.
- Goldstein JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *Br J Clin Pharmacol* 2001; 52(4): 349-55.
- Homepage of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (CYP2C19) 2006 [cited 2006]; Available from: URL: <http://www.imm.ki.se/cypalleles/cyp2c19.htm>
- Homepage of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (CYP2C9) 2006 [cited]; Available from: URL: <http://www.cypalleles.ki.se/cyp2c9.htm>
- Aynacioglu AS, Brockmoller J, Bauer S, *et al.* Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. *Br J Clin Pharmacol* 1999; 48(3): 409-15.
- Tanigawara Y, Aoyama N, Kita T, *et al.* CYP2C19 genotype-related efficacy of omeprazole for the treatment of infection caused by *Helicobacter pylori*. *Clin Pharmacol Ther* 1999; 66(5): 528-34.
- Xie HG, Prasad HC, Kim RB, Stein CM. CYP2C9 allelic variants: ethnic distribution and functional significance. *Adv Drug Deliv Rev* 2002 18; 54(10): 1257-70.
- Xie HG, Stein CM, Kim RB, Wilkinson GR, Flockhart DA, Wood AJ. Allelic, genotypic and phenotypic distributions of S-mephenytoin 4'-hydroxylase (CYP2C19) in healthy Caucasian populations of European descent throughout the world. *Pharmacogenetics* 1999; 9(5): 539-49.
- Xie HG. Genetic variations of S-mephenytoin 4'-hydroxylase (CYP2C19) in the Chinese population. *Life Sci* 2000; 66(14): L175-L181.
- Kaneko A, Lum JK, Yaviong L, *et al.* High and variable frequencies of CYP2C19 mutations: medical consequences of poor drug metabolism in Vanuatu and other Pacific islands. *Pharmacogenetics* 1999; 9(5): 581-90.
- Transon C, Lecoer S, Leemann T, Beaune P, Dayer P. Interindividual variability in catalytic activity and immunoreactivity of three major human liver cytochrome P450 isozymes. *Eur J Clin Pharmacol* 1996; 51(1): 79-85.
- National Centre for Biotechnology Information 2006 January 17 [cited 2006 Feb 5]; Available from: URL: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene&cmd=retrieve&dopt=default&list_uids=1559
- Solus JF, Arietta BJ, Harris JR, *et al.* Genetic variation in eleven phase I drug metabolism genes in an ethnically diverse population. *Pharmacogenomics* 2004; 5(7): 895-931.
- Crespi CL, Miller VP. The R144C change in the CYP2C9*2 allele alters interaction of the cytochrome P450 with NADPH: cytochrome P450 oxidoreductase. *Pharmacogenetics* 1997; 7(3): 203-10.
- Sullivan-Klose TH, Ghanayem BI, Bell DA, *et al.* The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics* 1996; 6(4): 341-9.
- Niemi M, Cascorbi I, Timm R, Kroemer HK, Neuvonen PJ, Kivisto KT. Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. *Clin Pharmacol Ther* 2002; 72(3): 326-32.
- Imai J, Ieiri I, Mamiya K, *et al.* Polymorphism of the cytochrome P450 (CYP) 2C9 gene in Japanese epileptic patients: genetic analysis of the CYP2C9 locus. *Pharmacogenetics* 2000; 10(1): 85-9.
- Bhasker CR, Miners JO, Coulter S, Birkett DJ. Allelic and functional variability of cytochrome P4502C9. *Pharmacogenetics* 1997; 7(1): 51-8.
- Dickmann LJ, Rettie AE, Kneller MB, *et al.* Identification and functional characterization of a new CYP2C9 variant (CYP2C9*5) expressed among African Americans. *Mol Pharmacol* 2001; 60(2): 382-7.

- [25] Kidd RS, Curry TB, Gallagher S, Edeki T, Blaisdell J, Goldstein JA. Identification of a null allele of CYP2C9 in an African-American exhibiting toxicity to phenytoin. *Pharmacogenetics* 2001; 11(9): 803-8.
- [26] Blaisdell J, Jorge-Nebert LF, Coulter S, *et al.* Discovery of new potentially defective alleles of human CYP2C9. *Pharmacogenetics* 2004; 14(8): 527-37.
- [27] Okuda R, Izumoto H, Nishiki M, *et al.* A novel CYP2C9 variant that caused erroneous genotyping in a patient on warfarin therapy. *Pharmacogenetics* 2004; 14(10): 707-9.
- [28] Si D, Guo Y, Zhang Y, Yang L, Zhou H, Zhong D. Identification of a novel variant CYP2C9 allele in Chinese. *Pharmacogenetics* 2004; 14(7): 465-9.
- [29] Zhao F, Loke C, Rankin SC, *et al.* Novel CYP2C9 genetic variants in Asian subjects and their influence on maintenance warfarin dose. *Clin Pharmacol Ther* 2004; 76(3): 210-9.
- [30] Delozier TC, Lee SC, Coulter SJ, Goh BC, Goldstein JA. Functional characterization of novel allelic variants of CYP2C9 recently discovered in Southeast Asians. *J Pharmacol Exp Ther* 2005; 11.
- [31] Veenstra DL, Blough DK, Higashi MK, *et al.* CYP2C9 haplotype structure in European American warfarin patients and association with clinical outcomes. *Clin Pharmacol Ther* 2005; 77(5): 353-64.
- [32] Gaikovitch EA, Cascorbi I, Mrozikiewicz PM, *et al.* Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. *Eur J Clin Pharmacol* 2003; 59(4): 303-12.
- [33] Bozina N, Granic P, Lalic Z, Tramisak I, Lovric M, Stavljenic-Rukavina A. Genetic polymorphisms of cytochromes P450: CYP2C9, CYP2C19, and CYP2D6 in Croatian population. *Croat Med J* 2003; 44(4): 425-8.
- [34] Topic E, Stefanovic M, Samardzija M. Association between the CYP2C9 polymorphism and the drug metabolism phenotype. *Clin Chem Lab Med* 2004; 42(1): 72-8.
- [35] Dorado P, Berecz R, Norberto MJ, Yasar U, Dahl ML, Llerena A. CYP2C9 genotypes and diclofenac metabolism in Spanish healthy volunteers. *Eur J Clin Pharmacol* 2003; 59(3): 221-5.
- [36] Yang JQ, Morin S, Verstuyft C, *et al.* Frequency of cytochrome P450 2C9 allelic variants in the Chinese and French populations. *Fundam Clin Pharmacol* 2003; 17(3): 373-6.
- [37] Allabi AC, Gala JL, Desager JP, Heusterspreute M, Horsmans Y. Genetic polymorphisms of CYP2C9 and CYP2C19 in the Beninese and Belgian populations. *Br J Clin Pharmacol* 2003; 56(6): 653-7.
- [38] Llerena A, Dorado P, O'Kirwan F, Jepson R, Licinio J, Wong ML. Lower frequency of CYP2C9*2 in Mexican-Americans compared to Spaniards. *Pharmacogenomics J* 2004; 4(6): 403-6.
- [39] Garcia-Martin E, Martinez C, Ladero JM, Gamito FJ, Agundez JA. High frequency of mutations related to impaired CYP2C9 metabolism in a Caucasian population. *Eur J Clin Pharmacol* 2001; 57(1): 47-9.
- [40] Bravo-Villalta HV, Yamamoto K, Nakamura K, Baya A, Okada Y, Horiuchi R. Genetic polymorphism of CYP2C9 and CYP2C19 in a Bolivian population: an investigative and comparative study. *Eur J Clin Pharmacol* 2005; 61(3): 179-84.
- [41] Gaedigk A, Casley WL, Tyndale RF, Sellers EM, Jurima-Romet M, Leeder JS. Cytochrome P4502C9 (CYP2C9) allele frequencies in Canadian Native Indian and Inuit populations. *Can J Physiol Pharmacol* 2001; 79(10): 841-7.
- [42] Scordo MG, Aklillu E, Yasar U, Dahl ML, Spina E, Ingelman-Sundberg M. Genetic polymorphism of cytochrome P450 2C9 in a Caucasian and a black African population. *Br J Clin Pharmacol* 2001; 52(4): 447-50.
- [43] Vianna-Jorge R, Perini JA, Rondinelli E, Suarez-Kurtz G. CYP2C9 genotypes and the pharmacokinetics of tenoxicam in Brazilians. *Clin Pharmacol Ther* 2004; 76(1): 18-26.
- [44] Halling J, Petersen MS, Damkier P, *et al.* Polymorphism of CYP2D6, CYP2C19, CYP2C9 and CYP2C8 in the Faroese population. *Eur J Clin Pharmacol* 2005; 16.
- [45] Hamdy SI, Hiratsuka M, Narahara K, *et al.* Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. *Br J Clin Pharmacol* 2002; 53(6): 596-603.
- [46] Jose R, Chandrasekaran A, Sam SS, *et al.* CYP2C9 and CYP2C19 genetic polymorphisms: frequencies in the south Indian population. *Fundam Clin Pharmacol* 2005; 19(1): 101-5.
- [47] Yu BN, Luo CH, Wang D, *et al.* CYP2C9 allele variants in Chinese hypertension patients and healthy controls. *Clin Chim Acta* 2004; 348(1-2): 57-61.
- [48] Hong X, Zhang S, Mao G, *et al.* CYP2C9*3 allelic variant is associated with metabolism of irbesartan in Chinese population. *Eur J Clin Pharmacol* 2005; 11.
- [49] Kimura M, Ieiri I, Mamiya K, Urae A, Higuchi S. Genetic polymorphism of cytochrome P450s, CYP2C19, and CYP2C9 in a Japanese population. *Ther Drug Monit* 1998; 20(3): 243-7.
- [50] Lee SS, Kim KM, Thi-Le H, Yea SS, Cha IJ, Shin JG. Genetic polymorphism of CYP2C9 in a Vietnamese Kinh population. *Ther Drug Monit* 2005; 27(2): 208-10.
- [51] Yoon YR, Shon JH, Kim MK, *et al.* Frequency of cytochrome P450 2C9 mutant alleles in a Korean population. *Br J Clin Pharmacol* 2001; 51(3): 277-80.
- [52] Yasar U, Aklillu E, Canaparo R, *et al.* Analysis of CYP2C9*5 in Caucasian, Oriental and black-African populations. *Eur J Clin Pharmacol* 2002; 58(8): 555-8.
- [53] Guo Y, Zhang Y, Wang Y, *et al.* Role of CYP2C9 and its variants (CYP2C9*3 and CYP2C9*13) in the metabolism of lornoxicam in humans. *Drug Metab Dispos* 2005; 33(6): 749-53.
- [54] Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics* 2002; 12(3): 251-63.
- [55] van der WJ, Steijns LS, van Weelden MJ, de HK. The effect of genetic polymorphism of cytochrome P450 CYP2C9 on phenytoin dose requirement. *Pharmacogenetics* 2001; 11(4): 287-91.
- [56] Hung CC, Lin CJ, Chen CC, Chang CJ, Liou HH. Dosage recommendation of phenytoin for patients with epilepsy with different CYP2C9/CYP2C19 polymorphisms. *Ther Drug Monit* 2004; 26(5): 534-40.
- [57] Taguchi M, Hongou K, Yagi S, *et al.* Evaluation of phenytoin dosage regimens based on genotyping of CYP2C subfamily in routinely treated Japanese patients. *Drug Metab Pharmacokinet* 2005; 20(2): 107-12.
- [58] Giancarlo GM, Venkatakrishnan K, Granda BW, von Moltke LL, Greenblatt DJ. Relative contributions of CYP2C9 and 2C19 to phenytoin 4-hydroxylation *in vitro*: inhibition by sulfaphenazole, omeprazole, and ticlopidine. *Eur J Clin Pharmacol* 2001; 57(1): 31-6.
- [59] Kerb R, Aynacioglu AS, Brockmoller J, *et al.* The predictive value of MDR1, CYP2C9, and CYP2C19 polymorphisms for phenytoin plasma levels. *Pharmacogenomics J* 2001; 1(3): 204-10.
- [60] Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 1999; 27; 353(9154): 717-9.
- [61] Freeman BD, Zehnbauser BA, McGrath S, Borecki I, Buchman TG. Cytochrome P450 polymorphisms are associated with reduced warfarin dose. *Surgery* 2000 Aug; 128(2): 281-5.
- [62] Wadelius M, Sorlin K, Wallerman O, *et al.* Warfarin sensitivity related to CYP2C9, CYP3A5, ABCB1 (MDR1) and other factors. *Pharmacogenomics J* 2004; 4(1): 40-8.
- [63] Tai G, Farin F, Rieder MJ, *et al.* In-vitro and in-vivo effects of the CYP2C9*11 polymorphism on warfarin metabolism and dose. *Pharmacogenet Genomics* 2005; 15(7): 475-81.
- [64] Voora D, Eby C, Linder MW, *et al.* Prospective dosing of warfarin based on cytochrome P-450 2C9 genotype. *Thromb Haemost* 2005; 93(4): 700-5.
- [65] Herman D, Locatelli I, Grabnar I, *et al.* Influence of CYP2C9 polymorphisms, demographic factors and concomitant drug therapy on warfarin metabolism and maintenance dose. *Pharmacogenomics J* 2005; 5(3): 193-202.
- [66] Thijssen HH, Ritzen B. Acenocoumarol pharmacokinetics in relation to cytochrome P450 2C9 genotype. *Clin Pharmacol Ther* 2003; 74(1): 61-8.
- [67] Verstuyft C, Morin S, Robert A, Loriot MA, Beaune P, Jaillon P, *et al.* Early acenocoumarol overanticoagulation among cytochrome P450 2C9 poor metabolizers. *Pharmacogenetics* 2001; 11(8): 735-7.
- [68] Lee CR, Pieper JA, Hinderliter AL, Blaisdell JA, Goldstein JA. Evaluation of cytochrome P4502C9 metabolic activity with tolbutamide in CYP2C91 heterozygotes. *Clin Pharmacol Ther* 2002; 72(5): 562-71.

- [69] Jetter A, Kinzig-Schippers M, Skott A, *et al.* Cytochrome P450 2C9 phenotyping using low-dose tolbutamide. *Eur J Clin Pharmacol* 2004; 60(3): 165-71.
- [70] Kirchheiner J, Kudlicz D, Meisel C, *et al.* Influence of CYP2C9 polymorphisms on the pharmacokinetics and cholesterol-lowering activity of (-)-3S,5R-fluvastatin and (+)-3R,5S-fluvastatin in healthy volunteers. *Clin Pharmacol Ther* 2003; 74(2): 186-94.
- [71] Kirchheiner J, Meineke I, Freytag G, Meisel C, Roots I, Brockmoller J. Enantiospecific effects of cytochrome P450 2C9 amino acid variants on ibuprofen pharmacokinetics and on the inhibition of cyclooxygenases 1 and 2. *Clin Pharmacol Ther* 2002; 72(1): 62-75.
- [72] Lee CR, Pieper JA, Frye RF, Hinderliter AL, Blaisdell JA, Goldstein JA. Differences in flurbiprofen pharmacokinetics between CYP2C9*1/*1, *1/*2, and *1/*3 genotypes. *Eur J Clin Pharmacol* 2003; 58(12): 791-4.
- [73] Product monograph: CELEBREX Celecoxib capsules, 100 mg and 200 mg 2005 [cited 2006]; Available from: URL: www.pfizer.ca/our%20products/prescription%20pharmaceuticals/default.asp?s=1&id=7&doc=enmonograph
- [74] Kirchheiner J, Stormer E, Meisel C, Steinbach N, Roots I, Brockmoller J. Influence of CYP2C9 genetic polymorphisms on pharmacokinetics of celecoxib and its metabolites. *Pharmacogenetics* 2003; 13(8): 473-80.
- [75] Shimamoto J, Ieiri I, Urae A, *et al.* Lack of differences in diclofenac (a substrate for CYP2C9) pharmacokinetics in healthy volunteers with respect to the single CYP2C9*3 allele. *Eur J Clin Pharmacol* 2000; 56(1): 65-8.
- [76] Kirchheiner J, Meineke I, Steinbach N, Meisel C, Roots I, Brockmoller J. Pharmacokinetics of diclofenac and inhibition of cyclooxygenases 1 and 2: no relationship to the CYP2C9 genetic polymorphism in humans. *Br J Clin Pharmacol* 2003; 55(1): 51-61.
- [77] Morin S, Llorca MA, Poirier JM, *et al.* Is diclofenac a valuable CYP2C9 probe in humans? *Eur J Clin Pharmacol* 2001; 56(11): 793-7.
- [78] Yasar U, Tybring G, Hidestrand M, *et al.* Role of CYP2C9 polymorphism in losartan oxidation. *Drug Metab Dispos* 2001; 29(7): 1051-6.
- [79] Sekino K, Kubota T, Okada Y, *et al.* Effect of the single CYP2C9*3 allele on pharmacokinetics and pharmacodynamics of losartan in healthy Japanese subjects. *Eur J Clin Pharmacol* 2003; 59(8-9): 589-92.
- [80] Uchida S, Watanabe H, Nishio S, *et al.* Altered pharmacokinetics and excessive hypotensive effect of candesartan in a patient with the CYP2C9/3 genotype. *Clin Pharmacol Ther* 2003; 74(5): 505-8.
- [81] Holstein A, Plaschke A, Ptak M, *et al.* Association between CYP2C9 slow metabolizer genotypes and severe hypoglycaemia on medication with sulphonylurea hypoglycaemic agents. *Br J Clin Pharmacol* 2005; 60(1): 103-6.
- [82] Lee AY, Kim MJ, Chey WY, Choi J, Kim BG. Genetic polymorphism of cytochrome P450 2C9 in diphenylhydantoin-induced cutaneous adverse drug reactions. *Eur J Clin Pharmacol* 2004; 60(3): 155-9.
- [83] Soga Y, Nishimura F, Ohtsuka Y, *et al.* CYP2C9 polymorphisms, phenytoin metabolism and gingival overgrowth in epileptic subjects. *Life Sci* 2004; 74(7): 827-34.
- [84] Office of the Registrar General, India 2005 December 1 [cited 2006]; Available from: URL: <http://www.censusindia.net/>
- [85] Martinez C, Blanco G, Ladero JM, *et al.* Genetic predisposition to acute gastrointestinal bleeding after NSAIDs use. *Br J Pharmacol* 2004; 141(2): 205-8.
- [86] London SJ, Daly AK, Leathart JB, Navidi WC, Idle JR. Lung cancer risk in relation to the CYP2C9*1/CYP2C9*2 genetic polymorphism among African-Americans and Caucasians in Los Angeles County, California. *Pharmacogenetics* 1996; 6(6): 527-33.
- [87] Llerena A, Berez R, Dorado P, Gonzalez AP, Penas-Lledo EM, de la RA. CYP2C9 gene and susceptibility to major depressive disorder. *Pharmacogenomics J* 2003; 3(5): 300-2.
- [88] Yasar U, Bennet AM, Eliasson E, *et al.* Allelic variants of cytochromes P450 2C modify the risk for acute myocardial infarction. *Pharmacogenetics* 2003; 13(12): 715-20.
- [89] Tsunedomi R, Iizuka N, Hamamoto Y, *et al.* Patterns of expression of cytochrome P450 genes in progression of hepatitis C virus-associated hepatocellular carcinoma. *Int J Oncol* 2005; 27(3): 661-7.
- [90] Landi S, Gemignani F, Moreno V, *et al.* A comprehensive analysis of phase I and phase II metabolism gene polymorphisms and risk of colorectal cancer. *Pharmacogenet Genomics* 2005; 15(8): 535-46.
- [91] Tranah GJ, Chan AT, Giovannucci E, Ma J, Fuchs C, Hunter DJ. Epoxide hydrolase and CYP2C9 polymorphisms, cigarette smoking, and risk of colorectal carcinoma in the Nurses' Health Study and the Physicians' Health Study. *Mol Carcinog* 2005; 44(1): 21-30.
- [92] Wrighton SA, Stevens JC, Becker GW, VandenBranden M. Isolation and characterization of human liver cytochrome P450 2C19: correlation between 2C19 and S-mephenytoin 4'-hydroxylation. *Arch Biochem Biophys* 1993; 306(1): 240-5.
- [93] Goldstein JA, Faletto MB, Romkes-Sparks M, *et al.* Evidence that CYP2C19 is the major (S)-mephenytoin 4'-hydroxylase in humans. *Biochemistry* 1994; 33(7): 1743-52.
- [94] Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet* 2002; 41(12): 913-58.
- [95] De Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA. The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J Biol Chem* 1994; 269(22): 15419-22.
- [96] De Morais SM, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K, Goldstein JA. Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese. *Mol Pharmacol* 1994; 46(4): 594-8.
- [97] Ferguson RJ, De Morais SM, Benhamou S, *et al.* A new genetic defect in human CYP2C19: mutation of the initiation codon is responsible for poor metabolism of S-mephenytoin. *J Pharmacol Exp Ther* 1998; 284(1): 356-61.
- [98] Xiao ZS, Goldstein JA, Xie HG, *et al.* Differences in the incidence of the CYP2C19 polymorphism affecting the S-mephenytoin phenotype in Chinese Han and Bai populations and identification of a new rare CYP2C19 mutant allele. *J Pharmacol Exp Ther* 1997; 281(1): 604-9.
- [99] Ibeanu GC, Blaisdell J, Ferguson RJ, *et al.* A novel transversion in the intron 5 donor splice junction of CYP2C19 and a sequence polymorphism in exon 3 contribute to the poor metabolizer phenotype for the anticonvulsant drug S-mephenytoin. *J Pharmacol Exp Ther* 1999; 290(2): 635-40.
- [100] Benhamou S, Bouchardy C, Dayer P. Lung cancer risk in relation to mephenytoin hydroxylation activity. *Pharmacogenetics* 1997; 7(2): 157-9.
- [101] Blaisdell J, Mohrenweiser H, Jackson J, *et al.* Identification and functional characterization of new potentially defective alleles of human CYP2C19. *Pharmacogenetics* 2002; 12(9): 703-11.
- [102] Morita J, Kobayashi K, Wanibuchi A, *et al.* A novel single nucleotide polymorphism (SNP) of the CYP2C19 gene in a Japanese subject with lowered capacity of mephenytoin 4'-hydroxylation. *Drug Metab Pharmacokinet* 2004; 19(3): 236-8.
- [103] Tamminga WJ, Wemer J, Oosterhuis B, de Zeeuw RA, de Leij LF, Jonkman JH. The prevalence of CYP2D6 and CYP2C19 genotypes in a population of healthy Dutch volunteers. *Eur J Clin Pharmacol* 2001; 57(10): 717-22.
- [104] Scordo MG, Caputi AP, D'Arrigo C, Fava G, Spina E. Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population. *Pharmacol Res* 2004; 50(2): 195-200.
- [105] Goldstein JA, Ishizaki T, Chiba K, *et al.* Frequencies of the defective CYP2C19 alleles responsible for the mephenytoin poor metabolizer phenotype in various Oriental, Caucasian, Saudi Arabian and American black populations. *Pharmacogenetics* 1997; 7(1): 59-64.
- [106] Svirid S, Shpizen S, Leitersdorf E, Levy M, Caraco Y. Phenotypic-genotypic analysis of CYP2C19 in the Jewish Israeli population. *Clin Pharmacol Ther* 1999; 65(3): 275-82.
- [107] Nowak MP, Sellers EM, Tyndale RF. Canadian Native Indians exhibit unique CYP2A6 and CYP2C19 mutant allele frequencies. *Clin Pharmacol Ther* 1998; 64(4): 378-83.
- [108] Adithan C, Gerard N, Vasu S, Rosemary J, Shashindran CH, Krishnamoorthy R. Allele and genotype frequency of CYP2C19 in a Tamilian population. *Br J Clin Pharmacol* 2003; 56(3): 331-3.
- [109] Lamba JK, Dhiman RK, Kohli KK. Genetic polymorphism of the hepatic cytochrome P450 2C19 in north Indian subjects. *Clin Pharmacol Ther* 1998; 63(4): 422-7.

- [110] Xie HG, Kim RB, Stein CM, Wilkinson GR, Wood AJ. Genetic polymorphism of (S)-mephenytoin 4'-hydroxylation in populations of African descent. *Br J Clin Pharmacol* 1999; 48(3): 402-8.
- [111] Herrlin K, Massele AY, Jande M, *et al.* Bantu Tanzanians have a decreased capacity to metabolize omeprazole and mephenytoin in relation to their CYP2C19 genotype. *Clin Pharmacol Ther* 1998; 64(4): 391-401.
- [112] Bathum L, Skjelbo E, Mutabingwa TK, Madsen H, Horder M, Brosen K. Phenotypes and genotypes for CYP2D6 and CYP2C19 in a black Tanzanian population. *Br J Clin Pharmacol* 1999; 48(3): 395-401.
- [113] Persson I, Aklillu E, Rodrigues F, Bertilsson L, Ingelman-Sundberg M. S-mephenytoin hydroxylation phenotype and CYP2C19 genotype among Ethiopians. *Pharmacogenetics* 1996; 6(6): 521-6.
- [114] He N, Yan FX, Huang SL, *et al.* CYP2C19 genotype and S-mephenytoin 4'-hydroxylation phenotype in a Chinese Dai population. *Eur J Clin Pharmacol* 2002; 58(1): 15-8.
- [115] Roh HK, Dahl ML, Tybring G, Yamada H, Cha YN, Bertilsson L. CYP2C19 genotype and phenotype determined by omeprazole in a Korean population. *Pharmacogenetics* 1996; 6(6): 547-51.
- [116] Tassaneeyakul W, Tawalee A, Tassaneeyakul W, *et al.* Analysis of the CYP2C19 polymorphism in a North-eastern Thai population. *Pharmacogenetics* 2002; 12(3): 221-5.
- [117] Luo HR, Aloumanis V, Lin KM, Gurwitz D, Wan YJ. Polymorphisms of CYP2C19 and CYP2D6 in Israeli ethnic groups. *Am J Pharmacogenomics* 2004; 4(6): 395-401.
- [118] Kaneko A, Bergqvist Y, Taleo G, Kobayakawa T, Ishizaki T, Bjorkman A. Proguanil disposition and toxicity in malaria patients from Vanuatu with high frequencies of CYP2C19 mutations. *Pharmacogenetics* 1999; 9(3): 317-26.
- [119] Rosemary J, Adithan C, Padmaja N, Shashindran CH, Gerard N, Krishnamoorthy R. The effect of the CYP2C19 genotype on the hydroxylation index of omeprazole in South Indians. *Eur J Clin Pharmacol* 2005; 21.
- [120] Lamba JK, Dhiman RK, Kohli KK. Genetic polymorphism of the hepatic cytochrome P450 2C19 in north Indian subjects. *Clin Pharmacol Ther* 1998; 63(4): 422-7.
- [121] Pang YS, Wong LP, Lee TC, Mustafa AM, Mohamed Z, Lang CC. Genetic polymorphism of cytochrome P450 2C19 in healthy Malaysian subjects. *Br J Clin Pharmacol* 2004; 58(3): 332-5.
- [122] Balian JD, Sukhova N, Harris JW, *et al.* The hydroxylation of omeprazole correlates with S-mephenytoin metabolism: a population study. *Clin Pharmacol Ther* 1995; 57(6): 662-9.
- [123] Lamba JK, Dhiman RK, Singh R, Kohli KK. Correlation between omeprazole hydroxylase and CYP2C19 genotype in North Indians. *Eur J Clin Pharmacol* 2001; 57(9): 649-52.
- [124] Furuta T, Ohashi K, Kosuge K, *et al.* CYP2C19 genotype status and effect of omeprazole on intragastric pH in humans. *Clin Pharmacol Ther* 1999; 65(5): 552-61.
- [125] Kita T, Sakaeda T, Aoyama N, *et al.* Optimal dose of omeprazole for CYP2C19 extensive metabolizers in anti-Helicobacter pylori therapy: pharmacokinetic considerations. *Biol Pharm Bull* 2002; 25(7): 923-7.
- [126] Kim KA, Shon JH, Park JY, *et al.* Enantioselective disposition of lansoprazole in extensive and poor metabolizers of CYP2C19. *Clin Pharmacol Ther* 2002; 72(1): 90-9.
- [127] Miura M, Tada H, Yasui-Furukori N, *et al.* Pharmacokinetic differences between the enantiomers of lansoprazole and its metabolite, 5-hydroxylansoprazole, in relation to CYP2C19 genotypes. *Eur J Clin Pharmacol* 2004; 60(9): 623-8.
- [128] Setiabudy R, Kusaka M, Chiba K, Darmansjah I, Ishizaki T. Metabolic disposition of proguanil in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation recruited from an Indonesian population. *Br J Clin Pharmacol* 1995; 39: 297-303.
- [129] Skjelbo E, Mutabingwa TK, Bygbjerg I, Nielsen KK, Gram LF, Broosen K. Chloroguanide metabolism in relation to the efficacy in malaria prophylaxis and the S-mephenytoin oxidation in Tanzanians. *Clin Pharmacol Ther* 1996; 59(3): 304-11.
- [130] Basci NE, Bozkurt A, Kortunay S, Isimer A, Sayal A, Kayaalp SO. Proguanil metabolism in relation to S-mephenytoin oxidation in a Turkish population. *Br J Clin Pharmacol* 1996; 42(6): 771-3.
- [131] Hoskins JM, Shenfield GM, Gross AS. Relationship between proguanil metabolic ratio and CYP2C19 genotype in a Caucasian population. *Br J Clin Pharmacol* 1998; 46(5): 499-504.
- [132] Wanwimolruk S, Bhawan S, Coville PF, Chalcraft SC. Genetic polymorphism of debrisoquine (CYP2D6) and proguanil (CYP2C19) in South Pacific Polynesian populations. *Eur J Clin Pharmacol* 1998; 54(5): 431-5.
- [133] Herrlin K, Massele AY, Rimoy G, *et al.* Slow chloroguanide metabolism in Tanzanians compared with white subjects and Asian subjects confirms a decreased CYP2C19 activity in relation to genotype. *Clin Pharmacol Ther* 2000; 68(2): 189-98.
- [134] Bolaji OO, Sadare IO, Babalola CP, Ogunbona FA. Polymorphic oxidative metabolism of proguanil in a Nigerian population. *Eur J Clin Pharmacol* 2002; 58(8): 543-5.
- [135] Bramness JG, Skurtveit S, Fauske L, *et al.* Association between blood carisoprodol: meprobamate concentration ratios and CYP2C19 genotype in carisoprodol-drugged drivers: decreased metabolic capacity in heterozygous CYP2C19*1/CYP2C19*2 subjects? *Pharmacogenetics* 2003; 13(7): 383-8.
- [136] Contin M, Sangiorgi S, Riva R, Parmeggiani A, Albani F, Baruzzi A. Evidence of polymorphic CYP2C19 involvement in the human metabolism of N-desmethylclobazam. *Ther Drug Monit* 2002; 24(6): 737-41.
- [137] Giraud C, Tran A, Rey E, Vincent J, Treluyer JM, Pons G. *In vitro* characterization of clobazam metabolism by recombinant cytochrome P450 enzymes: importance of CYP2C19. *Drug Metab Dispos* 2004; 32(11): 1279-86.
- [138] Grammader K, Verwohlt PL, Rietschel M, *et al.* Impact of polymorphisms of cytochrome-P450 isoenzymes 2C9, 2C19 and 2D6 on plasma concentrations and clinical effects of antidepressants in a naturalistic clinical setting. *Eur J Clin Pharmacol* 2004; 60(5): 329-36.
- [139] Suzuki Y, Shioiri T, Muratake T, *et al.* Effects of concomitant fluvoxamine on the metabolism of alprazolam in Japanese psychiatric patients: interaction with CYP2C19 mutated alleles. *Eur J Clin Pharmacol* 2003; 58(12): 829-33.
- [140] Yokono A, Morita S, Someya T, Hirokane G, Okawa M, Shimoda K. The effect of CYP2C19 and CYP2D6 genotypes on the metabolism of clomipramine in Japanese psychiatric patients. *J Clin Psychopharmacol* 2001; 21(6): 549-55.
- [141] Herrlin K, Yasui-Furukori N, Tybring G, Widen J, Gustafsson LL, Bertilsson L. Metabolism of citalopram enantiomers in CYP2C19/CYP2D6 phenotyped panels of healthy Swedes. *Br J Clin Pharmacol* 2003; 56(4): 415-21.
- [142] Fukuda T, Nishida Y, Zhou Q, Yamamoto I, Kondo S, Azuma J. The impact of the CYP2D6 and CYP2C19 genotypes on venlafaxine pharmacokinetics in a Japanese population. *Eur J Clin Pharmacol* 2000; 56(2): 175-80.
- [143] Koski A, Sistonen J, Ojanpera I, Gergov M, Vuori E, Sajantila A. CYP2D6 and CYP2C19 genotypes and amitriptyline metabolite ratios in a series of medicolegal autopsies. *Forensic Sci Int* 2005; 14.
- [144] Griskevicius L, Yasar U, Sandberg M, *et al.* Bioactivation of cyclophosphamide: the role of polymorphic CYP2C enzymes. *Eur J Clin Pharmacol* 2003; 59(2): 103-9.
- [145] Uttamsingh V, Lu C, Miwa GT, Gan LS. Relative contributions of the five major human cytochromes P450, 1A2, 2C9, 2C19, 2D6 and 3A4 to the hepatic metabolism of the proteasome inhibitor bortezomib. *Drug Metab Dispos* 2005; 15.
- [146] Ando Y, Fuse E, Figg WD. Thalidomide metabolism by the CYP2C subfamily. *Clin Cancer Res* 2002; 8(6): 1964-73.
- [147] Laine K, Tybring G, Bertilsson L. No sex-related differences but significant inhibition by oral contraceptives of CYP2C19 activity as measured by the probe drugs mephenytoin and omeprazole in healthy Swedish white subjects. *Clin Pharmacol Ther* 2000; 68(2): 151-9.
- [148] Parmeggiani A, Posar A, Sangiorgi S, Giovanardi-Rossi P. Unusual side-effects due to clobazam: a case report with genetic study of CYP2C19. *Brain Dev* 2004; 26(1): 63-6.
- [149] Ford GA, Wood SM, Daly AK. CYP2D6 and CYP2C19 genotypes of patients with terodiline cardiotoxicity identified through the yellow card system. *Br J Clin Pharmacol* 2000; 50(1): 77-80.
- [150] Takada K, Arefayene M, Desta Z, *et al.* Cytochrome P450 pharmacogenetics as a predictor of toxicity and clinical response to pulse cyclophosphamide in lupus nephritis. *Arthritis Rheum* 2004; 50(7): 2202-10.
- [151] Sachse C, Smith G, Wilkie MJ, *et al.* A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. *Carcinogenesis* 2002; 23(11): 1839-49.

- [152] Chau TK, Marakami S, Kawai B, Nasu K, Kubota T, Ohnishi A. Genotype analysis of the CYP2C19 gene in HCV-seropositive patients with cirrhosis and hepatocellular carcinoma. *Life Sci* 2000; 67(14): 1719-24.
- [153] Kortunay S, Bozkurt A, Bathum L, *et al.* CYP2C19 genotype does not represent a genetic predisposition in idiopathic systemic lupus erythematosus. *Ann Rheum Dis* 1999; 58(3): 182-5.
- [154] Mochizuki J, Murakami S, Sanjo A, Takagi I, Akizuki S, Ohnishi A. Genetic polymorphisms of cytochrome P450 in patients with hepatitis C virus-associated hepatocellular carcinoma. *J Gastroenterol Hepatol* 2005; 20(8): 1191-7.
- [155] Williams ML, Bhargava P, Cherrouk I, Marshall JL, Flockhart DA, Wainer IW. A discordance of the cytochrome P450 2C19 genotype and phenotype in patients with advanced cancer. *Br J Clin Pharmacol* 2000; 49(5): 485-8.
- [156] Clinical Trials Gov: A service of the U S National Institutes of Health 2006 [cited 2006]; Available from: URL: <http://www.clinicaltrials.gov/search/term=pharmacogenetics>
- [157] Roche Diagnostics 2006 [cited 2006]; Available from: URL: http://www.roche-diagnostics.com/products_services/amplichip_cyp450.html
- [158] Genelex Corporation Drug Reaction Testing 2006 [cited 2006]; Available from: URL: <http://www.healthanddna.com/drugreactiontest.html>

Received: 03 April, 2006

Revised: 05 July, 2006

Accepted: 31 July, 2006