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# Differential Genotype Dependent Inhibition of CYP2C9 in Humans

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**ABSTRACT:**

**The effects of genetic polymorphisms in drug-metabolizing enzymes (e.g., *CYP2C9*3*) on drug clearance have been well characterized but much less is known about whether these polymorphisms alter susceptibility to drug-drug interactions. Previous in vitro work has demonstrated that genotype-dependent inhibition of CYP2C9 mediated flurbiprofen metabolism, suggesting the possibility of genotype-dependent inhibition interactions in vivo. In the current study, flurbiprofen was used as a probe substrate and fluconazole as a prototypical inhibitor to investigate whether genotype-dependent inhibition of CYP2C9 occurs in vivo. From 189 healthy volunteers who were genotyped for CYP2C9 polymorphisms, 11 control subjects (*CYP2C9*1/*1*), 9 heterozygous and 2 homozygous for the *CYP2C9*3* allele participated in the pharmacokinetic drug interaction study. Subjects received a single 50-mg oral dose of flurbiprofen alone or after administration of either 200 or 400 mg of fluconazole for 7 days using an open, randomized, crossover design. Flurbiprofen and fluconazole plasma concentrations along with flurbiprofen and 4′-hydroxyflurbiprofen urinary excretion were monitored. Flurbiprofen apparent oral clearance differed significantly among the three genotype groups ($p < 0.05$) at baseline but not after pretreatment with 400 mg of fluconazole for 7 days. Changes in flurbiprofen apparent oral clearance after fluconazole coadministration were gene dose-dependent, with virtually no change occurring in **3/*3* subjects. Analysis of fractional clearances suggested that the fraction metabolized by CYP2C9, as influenced by genotype, determined the degree of drug interaction observed. In summary, the presence of *CYP2C9*3* alleles (either one or two alleles) can alter the degree of drug interaction observed upon coadministration of inhibitors.**

The cytochrome P450 (P450) superfamily of enzymes plays an important role in the oxidation of numerous xenobiotics, with CYP2C9 accounting for 10 to 20% of the P450 protein content in human liver. CYP2C9 has been reported to catalyze approximately 20% of P450-mediated drug oxidation reactions (Shimada et al., 1994; Rendic and Dicarlo, 1997; Miners and Birkett, 1998), including agents such as nonsteroidal anti-inflammatory drugs, tolbutamide, losartan, and the narrow therapeutic index drugs warfarin and phenytoin (Rettie et al., 1992; Stearns et al., 1995; Tracy et al., 1995; Bajpai et al., 1996; Miners and Birkett, 1996; Hamman et al., 1997; Niemi et al., 2002; Yan et al., 2005). To date, 30 CYP2C9 allelic variants located within the coding region have been reported (http://www.imm.ki.SE/CYPalleles). In particular, the **3* allele ($Ile^{359}Leu$), which is expressed at an allele frequency of 15% (Lee et al., 2002), results in significantly reduced oral clearance for several CYP2C9 substrates (Aithal et al., 1999; Lee et al., 2003b; Vianna-Jorge et al., 2004; Guo et al., 2005; Perini et al., 2005) and has been associated with an increased frequency of adverse events after warfarin or phenytoin administration (Kidd et al., 2001; Higashi et al., 2002).

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Certain diseases or the presence of comorbid conditions may necessitate coadministration of multiple medications, increasing the chances of drug-drug interactions. However, genetic polymorphisms in drug-metabolizing enzymes are not routinely evaluated for their impact on drug interactions during clinical studies. Genotype-dependent inhibition has been demonstrated with CYP2D6 and CYP2C19 genetic polymorphisms (Hamelin et al., 2000; Lessard et al., 2001; LLerena et al., 2001; Lindh et al., 2003; Yasui-Furukori et al., 2004a,b; Uno et al., 2006), but these polymorphisms resulted in expression of inactive proteins that should not be subject to inhibition. No clinical studies have explored the effect of single nucleotide polymorphisms (SNPs) resulting in reduced activity enzymes (e.g., *CYP2C9*3*) and their effect on the degree of inhibition interactions observed. Recently, in vitro studies from our laboratory using five commonly used probe substrates and 28 inhibitors demonstrated genotype- as well as substrate-dependent inhibition of CYP2C9 (Kumar et al., 2006b). To evaluate the potential in vivo significance of these findings, a clinical study using an open, randomized, crossover design, was conducted with the CYP2C9 probe substrate flurbiprofen (Hutzler et al., 2001; Greenblatt et al., 2006; Zgheib et al., 2007) and the prototypical CYP2C9 inhibitor fluconazole (Venkatakrishnan et al., 2000) in individuals of the *CYP2C9*1/*1*, **1/*3*, and **3/*3* genotypes to characterize the magnitude of the drug interaction in subjects with different *CYP2C9* polymorphisms.

## Materials and Methods

**Subject Selection.** The study protocol was reviewed and approved by the Institutional Review Board, University of Minnesota, Minneapolis, MN. A

**ABBREVIATIONS:** P450, cytochrome P450; AUC, area under the curve; ANOVA, analysis of variance.

total of 189 healthy subjects were enrolled in the genotype screening phase of the study. After obtaining written informed consent, 30 ml of blood was drawn and stored at room temperature for DNA isolation. All subjects were nonsmokers and were not taking any medicine at the time of enrollment, except oral contraceptives.

**DNA Isolation.** The whole blood sample was mixed thoroughly with red blood cell lysis buffer (preheated to 37°C). The mixture was allowed to incubate in the water bath at 37°C for 15 min. The samples were then centrifuged for 15 min at 3000 rpm. The pellets were washed repeatedly with red blood cell lysis buffer until the pellet became white in color. The pellet was then dissolved in 3 ml of white blood cell lysis buffer and incubated at 37°C overnight. To each tube, 10 $\mu$l of proteinase K was added, and the mixture was vortexed and incubated for 24 h at 37°C. Ammonium acetate (1.5 ml of 7.5 M) was added to precipitate the DNA. Proteins were then removed by centrifuging at 3000 rpm for 20 min, and 10 ml of absolute ethanol was added to the supernatant. The mixture was then gently inverted to allow the DNA to precipitate. The precipitated DNA was then suspended in 3 ml of TE buffer (Sigma-Aldrich, St. Louis, MO). Quantitation of DNA was carried out by measuring the sample absorbance at 260 nm.

**Determination of CYP2C9 Genotype.** DNA samples were diluted to 20 ng/$\mu$l, and single nucleotide polymorphisms were determined by TaqMan-based allele discrimation assay kits (Applied Biosystems, Foster City, CA). The genotyping reaction was conducted in 96-well plates, and the reaction components were 1 $\mu$l of diluted DNA, 1.25 $\mu$l of primer, 12.5 $\mu$l of TaqMan universal master mix (Applied Biosystems), and water to a total volume of 25 $\mu$l. The thermocycler protocol was as follows: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 92°C for 15 s and 60°C for 1 min. Reactions were analyzed on a Prism model 7500 sequence detection system (Applied Biosystems). The SNP database reference numbers were *CYP2C9*2* = rs1799853 and *CYP2C9*3* = rs1057910.

**Drug Interaction Study Design.** From the 189 genotyped subjects, 11 *CYP2C9*1/*1*, 9 *CYP2C9*1/*3*, and 2 *CYP2C9*3/*3* subjects further consented to participate in the drug interaction study. All subjects were healthy as determined by physical examination, medical history, vital signs, and routine biochemical and urinalysis tests. Subjects were instructed to abstain from alcohol for 3 days and caffeine-containing food, chocolate, and beverages for 2 days before the pharmacokinetic study. Female subjects of child-bearing potential underwent a urine pregnancy test.

An open, randomized, crossover design with a 1 week washout was used. Subjects received either 50 mg of flurbiprofen as a tablet (Mylan Pharmaceuticals Inc., Morgantown, WV) alone or 200 or 400 mg of fluconazole as a tablet (Ivax Pharmaceuticals Inc., Miami, FL) for 7 days followed by 50 mg of flurbiprofen on the 7th day. Compliance with fluconazole administration was assessed by pill count as well as examination of subject diaries. Subjects were required to fast overnight before the study day. On the morning of each period, subjects were admitted to the Clinical Research Center and remained there for 12 h, were dismissed, and then returned the next morning for collection of the 24-h blood sample and return of the urine collection. Fluconazole was administered in the morning as scheduled, and flurbiprofen administered orally 2 h later. A light snack was allowed 2 h after flurbiprofen dosing.

**Plasma and Urine Collection.** Blood samples (7 ml) were collected into heparinized tubes at 0 min (before flurbiprofen administration) and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after the administration of flurbiprofen and immediately centrifuged for 10 min at 3200 rpm, and the resulting plasma was aliquoted and stored at −20°C. Total voided urine was collected before flurbiprofen administration and at intervals of 0 to 12 h and 12 to 24 h after flurbiprofen administration and kept refrigerated throughout the collection period. At the end of each collection interval, total urine volume was recorded and two 25-ml aliquots were stored at −20°C for later analysis.

**Analysis of Flurbiprofen Concentrations in Plasma and Flurbiprofen and 4′-Hydroxyflurbiprofen Concentrations in Urine.** Flurbiprofen and 4′-hydroxyflurbiprofen concentrations were quantified by high-performance liquid chromatographic according to methods previously developed in our laboratory (Hutzler et al., 2000). Acid hydrolysis, as previously described, was used to cleave glucuronides of flurbiprofen and 4′-hydroxyflurbiprofen, before analysis.

**Analysis of Fluconazole in Plasma.** Fluconazole plasma concentrations were quantitated by a method described previously (Cociglio et al., 1996).

**In Vitro Assessment of Genotype-Dependent Inhibition.** The ability of fluconazole to inhibit expressed, reconstituted CYP2C9.1, an equimolar mixture of CYP2C9.1/CYP2C9.3, and CYP2C9.3 was determined according to methods previously established in our laboratory (Kumar et al., 2006a). Briefly, fluconazole (0, 10, 50, and 100 $\mu$M) was incubated with three concentrations of flurbiprofen (5, 25, and 50 $\mu$M) with each of the enzyme preparations listed above and the formation of 4′-hydroxyflurbiprofen was monitored.

**Noncompartmental Pharmacokinetic Analysis.** Flurbiprofen pharmacokinetic parameters were estimated from plasma concentration-time data by standard noncompartmental methods (WinNonlin v5.2, Pharsight, Palo Alto, CA). The area under the concentration-time curve ($AUC_{0-inf}$) of flurbiprofen was calculated using the linear trapezoidal rule with extrapolation to infinity. Apparent oral clearance (CL/F) of flurbiprofen was calculated as dose/$AUC_{0-inf}$. The 4′-hydroxyflurbiprofen formation clearance ($CL_{f,m}$) was calculated using eq. 1:

$$CL_{f,m} = \frac{\text{Amt. 4'OHFexcreted}_{0-24\ hr}}{\text{flurbiprofen}AUC_{0-24\ hr}} \quad (1)$$

where Amt. 4′OHFexcreted$_{0-24\ hr}$ is the amount of 4′-hydroxyflurbiprofen metabolite (both as 4′-hydroxyflurbiprofen and its glucuronide conjugate) excreted in the urine during the 24-h collection interval.

**Statistical Analysis.** Sample size calculations were based on the study conducted by Zgheib et al. (2007) in which 78% inhibition of 4′-hydroxyflurbiprofen formation clearance was observed after pretreatment with 7 doses of 400 mg of fluconazole. To detect a 30% difference in inhibition between *CYP2C9*1/*1* and *CYP2C9*1/*3* with power of 90% and 5% chance of type I error, it was estimated that a sample size of at least $n = 8$ per group would be necessary. One-way ANOVA was applied to compare demographics such as age, body weight, height, and creatinine concentration between the three CYP2C9 genotype groups. Before statistical analysis, flurbiprofen pharmacokinetic parameters (e.g., CL/F, $AUC_{0-inf}$, and half-life) were log-transformed, and two-way repeated-measure ANOVA was then applied. Pairwise multiple comparisons were performed using the Holm-Sidak method. A Friedman repeated-measure ANOVA on ranks was performed on $CL_{f,m}$, $C_{max}$, $T_{max}$ and V/F because of non-normalized distributions. Pairwise comparisons were made using the Tukey-Kramer test. Fluconazole pharmacokinetic parameters ($AUC_{0-24}$, $C_{max}$, and half-life) across groups and treatment periods were compared using one-way analysis of variance with a $p$ value of 0.05 or less regarded as statistically significant. All statistical analyses were conducted with SigmaStat 3.1 (Systat Software, Point Richmond, CA).

**Model-Based Pharmacokinetic Analysis.** A nonlinear mixed-effects modeling approach was used for both pharmacokinetic parameter estimation and to develop a global model for the flurbiprofen data that included a pharmacokinetic submodel and a drug interaction submodel. All flurbiprofen concentrations from all drug interaction arms in subjects with all three genotypes were simultaneously modeled using NONMEM VI (ICON U.S., Ellicott City, MD) (Beal et al., 2006) implemented with Compaq Visual Fortran v6.6 and PDxPop 2.2a, using a first-order conditional estimation algorithm that allowed for an interaction between the ETA and EPSILON levels of random effects. For the pharmacokinetic submodel, a one-compartment model with first-order absorption and first-order elimination adequately described the flurbiprofen concentration-time profiles. The link between the drug interaction model and the pharmacokinetic model was adapted from the Rowland-Matin equation (Rowland and Matin, 1973). Flurbiprofen clearance was modeled as a function of the genotypic CL2C9, CLnon2C9, the fluconazole concentration ($I$), and the $K_i$ for the fluconazole-CYP2C9 interaction:

$$CL = \frac{CL2C9}{1 + \frac{I}{K_i}} + CLnon2C9 \quad (2)$$

where CL2C9 represents CYP2C9 metabolic clearance and CLnon2C9 represents non-CYP2C9 clearance. It is important to note that CL2C9 and $K_i$ can both take on different values across different genotypes.

In the population model, the typical values of the uninhibited CL2C9 for each of the three genotypes were estimated as regression parameters. The typical value of CLnon2C9 was also estimated as a regression parameter,

TABLE 1

*Prevalence of CYP2C9*2 and CYP2C9*3 alleles in the study population (individuals screened for genotype, before inclusion in the pharmacokinetic study)*

| SNPs | No. of Subjects ($n = 187$)[a] | |
|---|---|---|
| | Homozygous | Heterozygous |
| *CYP2C9*2* | 2 | 23 |
| *CYP2C9*3* | 2 | 18 |

[a] *CYP2C9*1*1* − $n = 142$.

assuming that it is not dependent upon genotype and is shared across all genotypes and inhibitor concentrations. Flurbiprofen volume of distribution, first-order absorption rate constant, and an absorption lag time were also estimated and assumed to be log normally distributed in the population. The between-subject variability for each was expressed as a coefficient of variation. Residual unexplained variability was modeled with a proportional error model and expressed as a coefficient of variation.

Parameters of the drug interaction submodel were based on previous literature (Kunze and Trager, 1996), wherein the average steady-state concentration was reported to be 60 $\mu$M after 400 mg/day fluconazole for 6 days. This value was used in the regression equation as *I* for the 400 mg of fluconazole interaction treatment arm. Assuming linear pharmacokinetics of fluconazole, the value for *I* in the 200 mg of fluconazole treatment arm was taken to be 30 $\mu$M. This same study reported the average in vivo $K_i$ for the fluconazole/CYP2C9 interaction to be 22 $\mu$M. We assumed that all of these subjects were of the predominant **1/*1* genotype. Our laboratory determined the in vitro $K_i$ values to have a **1/*3* to **1/*1* ratio of 1.55; similarly, the **3/*3* to **1/*1* in vitro ratio was 2.00 (see *Results*). If we assume that the in vitro ratio approximates the in vivo ratio, these ratios were used to estimate the value of $K_i$ in **1/*3* and **3/*3* genotypes from the literature in vivo value.

Because there were only two **3/*3* subjects, a likelihood ratio test was applied to determine whether the information from these two subjects was sufficiently strong to reject the hypothesis that CL2C9 in **3/*3* subjects was no different from the CL2C9 in **1/*3* subjects. Using the maximum likelihood objective function in NONMEM, the difference in objective function values between a full and nested model is approximately $\chi^2$ distributed with degrees of freedom equal to the difference in the number of parameters between the two competing models. A decrease in objective function value of 3.8 or greater indicates that the more complicated model is superior to the nested model ($p < 0.05$, $\chi^2$, df = 1). We also determined whether the model that independently estimated the clearances of each genotypic group in each treatment arm (nine clearance parameters) was significantly different from the model that included all of the assumptions made in the drug interaction submodel (four clearance parameters). With 5 df, a decrease in objective function value of 11.1 results in $\alpha = 0.05$.

## Results

After obtaining informed consent, 189 healthy volunteers were genotyped for CYP2C9 alleles. Two subjects were not further evaluated as one subject presented with hypertension and in the other case, phlebotomy was unsuccessful. Of these subjects, 40.2% were male, 72.3% were white, 12.7% were African-American, 8.5% were Asian, two individuals were Pacific-Islander, and three did not self-identify. The median age was 25 years (range 18–61). The allele frequencies for all individuals screened are reported in Table 1. Twenty-two subjects (11 *CYP2C9*1/*1*, 9 *CYP2C9*1/*3*, and 2 *CYP2C9*3/*3*) were then enrolled into the drug interaction study and their demographics are presented in Table 2. Before flurbiprofen administration, one *CYP2C9*1/*3* genotype subject withdrew from the study for personal reasons and, hence, was excluded from the analysis. No significant adverse events were reported by any subjects.

Mean flurbiprofen plasma concentration-time profiles before and after pretreatment with either 200 or 400 mg of fluconazole in *CYP2C9*1/*1*, *CYP2C9*1/*3*, and *CYP2C9*3/*3* genotype individuals are depicted in Fig. 1, A to C, respectively. Pharmacokinetic parameters derived from noncompartmental analysis of individual data are presented in Table 3. With respect to genotype, by comparing the control period versus 7-day pretreatment with 200 mg of fluconazole, statistically significant differences were noted in apparent oral clearance, AUC, and half-life between treatment periods in the *CYP2C9*1/*1* and *CYP2C9*1/*3* genotype groups ($p < 0.01$). However, no statistically significant differences in oral clearance or AUC were noted in the *CYP2C9*3/*3* group, comparing control versus 200 mg of fluconazole.

Flurbiprofen oral clearance, AUC, and half-life differed significantly between baseline and 400 mg of fluconazole pretreatment in subjects with either the *CYP2C9*1/*1* or *CYP2C9*1/*3* genotypes ($p < 0.01$). These differences remained, when the parameters for the 200 and 400 mg doses of fluconazole were compared. However, no statistically significant differences in the above parameters among any of the periods (baseline versus 200 mg of fluconazole versus 400 mg of fluconazole) were observed in the *CYP2C9*3/*3* genotype group. Finally, no statistically significant differences were noted in $C_{max}$, $T_{max}$ and V/F across periods in any of the three genotype groups.

Figure 2 is an interaction plot of median oral clearance (25th and 75th percentile) for each of the three genotype groups during the three periods (baseline and 200 mg or 400 mg of fluconazole). At baseline, median oral clearance in *CYP2C9*1/*1* individuals differed significantly ($p < 0.05$) from that in the *CYP2C9*1/*3* and *CYP2C9*3/*3* individuals. After 200 mg of fluconazole, individual oral clearances in all three genotype groups decreased, but the median oral clearance in *CYP2C9*1/*1* individuals was not statistically different ($p = 0.084$) from that of *CYP2C9*1/*3* individuals. However, median oral clearance in the *CYP2C9*1/*1* group was statistically different ($p = 0.015$) from that for *CYP2C9*3/*3* individuals after 200 mg of fluconazole. Finally, no differences were noted ($p > 0.05$) in flurbiprofen median oral clearance across all genotypes after 400 mg of fluconazole pretreatment. In the *CYP2C9*3/*3* individuals, no significant decrease in apparent oral clearance was observed at either of the fluconazole doses. Similar trends were seen in the $AUC_{0-inf}$, and this was reflected by a 2.4- and 3.2-fold increase in flurbiprofen $AUC_{0-inf}$ in *CYP2C9*1/*3* and *CYP2C9*3/*3* groups, but almost no change was observed in the *CYP2C9*3/*3* group following coadministration of 400 mg of fluconazole for 7 days (Fig. 3).

The 4′-hydroxyflurbiprofen formation clearances ($CL_{f,m}$) for the three periods are presented in Table 3. At both baseline and 200 mg of fluconazole coadministration, the formation clearance in individuals with either one or two **3* alleles varied significantly from that for wild-type individuals. However, after 400 mg of fluconazole coadministration, only individuals with two **3* alleles differed statistically from those carrying only the **1* allele.

Mean plasma concentration-time profiles of fluconazole after seven doses of 200 and 400 mg in the three genotype groups are shown in Fig. 4, and the pharmacokinetics parameters are summarized in Table 4. No statistically significant differences were noted in $AUC_{0-24}$, half-life, or $C_{max}$ for a given dose of fluconazole among the three genotype groups.

In vitro studies of fluconazole inhibition of flurbiprofen metabolism were conducted to provide the framework for the in vitro-in vivo correlations and model-based analysis (see below). In expressed enzyme preparations of CYP2C9.1 (simulate wild-type **1/*1* individuals), the $K_i$ value was 11 $\mu$M for fluconazole inhibition of flurbiprofen 4′-hydroxylation. In an equimolar mixture of CYP2C9.1/CYP2C9.3 (simulate heterozygote **1/*3* individuals), the fluconazole $K_i$ was 17 $\mu$M. Finally, with the CYP2C9.3 protein (simulate homozygote **3/*3*

TABLE 2

*Demographics of subjects participating in drug interaction study (n = 21)*

Data are presented as median (range), except for *CYP2C9*3/*3* genotype group in which individual data are presented. No significant differences among the CYP2C9 genotype groups, *CYP2C9*1/*1*, *CYP2C9*1/*3*, and *CYP2C9*3/*3*, were noted with respect to age [$F(2,18) = 0.95$, $P = 0.41$], body weight [$F(2,18) = 1.38$, $P = 0.28$)], height [$F(2,18) = 0.48$, $P = 0.63$], or creatinine concentrations [$F(2,18) = 1.2$, $P = 0.32$].

| Demographics | *CYP2C9*1/*1* | *CYP2C9*1/*3* | *CYP2C9*3/*3* |
|---|---|---|---|
| No. subjects | 11 | 8 | 2 |
| Age (years) | 25 (19–36) | 23 (19–28) | (25, 29) |
| Gender (male/female) | 4/7 | 3/5 | 2/0 |
| Body weight (kg) | 73.7 (51–108) | 66.9 (49–84) | (77, 85) |
| Height (cm) | 166 (154–193) | 167 (160–189) | (177, 179) |
| Creatinine concentration | 0.87 (0.7–1.27) | 0.86 (0.76–1.03) | (1, 1.1) |

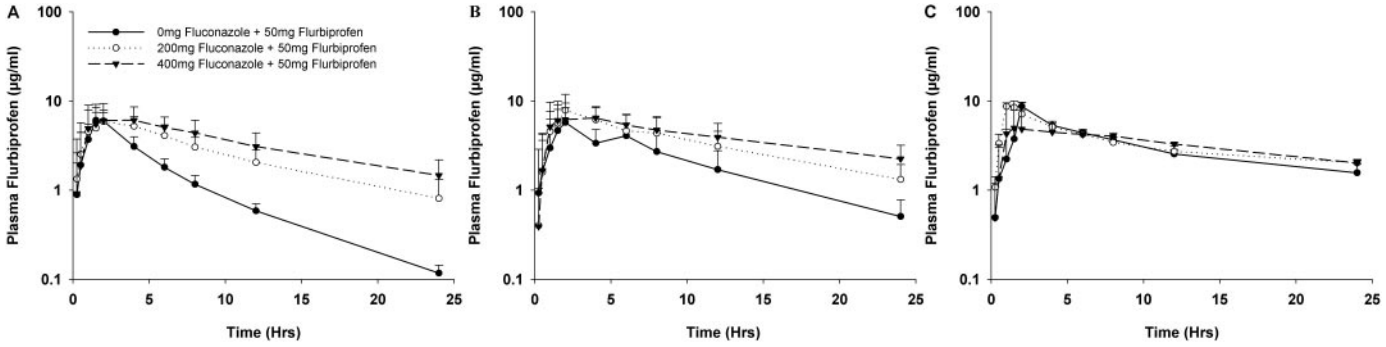


FIG. 1. Mean ± S.D. plasma flurbiprofen concentration-time profiles after oral administration of flurbiprofen tablet (50 mg) alone (●) and after seven doses of fluconazole: 200 mg (○) and 400 mg (▼) in 11 *CYP2C9*1/*1* subjects (A), 8 *CYP2C9*1/*3* subjects (B), and 2 *CYP2C9*3/*3* subjects (C).

TABLE 3

*Noncompartmental pharmacokinetic parameters of flurbiprofen before (baseline) and after pretreatment with 200 or 400 mg of fluconazole for 7 days*

Data are shown as median (range), except for *CYP2C9*3/*3* genotype group, in which individual data are presented.

| Pharmacokinetic Parameters | Units | *CYP2C9*1/*1* ($n = 11$) | *CYP2C9*1/*3* ($n = 8$) | *CYP2C9*3/*3* ($n = 2$) |
|---|---|---|---|---|
| Baseline | | | | |
| CL/F | Liters · hour$^{-1}$ | 1.6 (1.2–2.2) | 0.9 (0.5–2)* | (0.4, 0.6)*† |
| $AUC_{0-inf}$ | Micrograms · milliliter$^{-1}$ · hour | 30.8 (23.2–42.7) | 53.7 (24.6–112)* | (85.8, 119)*† |
| $T_{1/2}$ | Hours | 4.8 (4.2–6.1) | 7.2 (5.5–7.5)* | (9.7, 13.8)*† |
| $C_{max}$ | Micrograms · milliliter$^{-1}$ | 7.6 (4.1–9.5) | 8.9 (4.2–10.7) | (8, 9.4) |
| $T_{max}$ | Hours | 1.5 (0.5–2.0) | 2.0 (1.5–6.1) | (2.0, 2.0) |
| V/F | Liters | 10.8 (7.8–13.8) | 9.2 (3.9–22) | (8.2, 8.4) |
| $CL_{f,m}$ | Liters · hour$^{-1}$ | 0.67 (0.43–0.95) | 0.35 (0.15–0.86)* | (0.05, 0.05)*† |
| 200 mg of fluconazole coadministration | | | | |
| CL/F | Liters · hour$^{-1}$ | 0.8 (0.3–1.1) | 0.5 (0.3–1.1) | (0.3, 0.4)* |
| $AUC_{0-inf}$ | Micrograms · milliliter$^{-1}$ · hour | 62.3 (46.1–150) | 96.1 (44.1–197) | (136, 152)* |
| $T_{1/2}$ | Hours | 7.8 (6.1–16.3) | 9.2 (8.0–13.2) | (18.9, 22.6)*† |
| $C_{max}$ | Micrograms · milliliter$^{-1}$ | 5.9 (4–10.2) | 7.7 (5.2–15.5) | (8.6, 8.6) |
| $T_{max}$ | Hours | 2.0 (1.0–4.0) | 2.0 (1.8–4.0) | (1, 1.5) |
| V/F | Liters | 8.3 (6.1–13.7) | 7.5 (3.2–14.6) | (10, 10.8) |
| $CL_{f,m}$ | Liters · hour$^{-1}$ | 0.28 (0.13–0.37) | 0.14 (0.06–0.34)* | (0.02, 0.03)* |
| 400 mg of fluconazole coadministration | | | | |
| CL/F | Liters · hour$^{-1}$ | 0.5 (0.24–0.82) | 0.4 (0.2–0.8) | (0.4, 0.4) |
| $AUC_{0-inf}$ | Micrograms · milliliter$^{-1}$ · hour | 93.2 (60.7–206) | 133 (65.4–257) | (119, 133) |
| $T_{1/2}$ | Hours | 9.9 (7.4–12.5) | 13.7 (12.2–17.1)* | (16.1, 16.1)* |
| $C_{max}$ | Micrograms · milliliter$^{-1}$ | 7.5 (4.7–14.4) | 8.4 (3.7–12.2) | (4.3, 8.5) |
| $T_{max}$ | Hours | 2.0 (1.0–4.0) | 1.5 (1.0–4.0) | (1.5, 8) |
| V/F | Liters | 7.5 (4.2–11.0) | 7.1 (4.5–16.4) | (8.8, 9.8) |
| $CL_{f,m}$ | Liters · hour$^{-1}$ | 0.17 (0.07–0.34) | 0.08 (0.04–0.23) | (0.02, 0.02)* |

*$P < 0.05$ between *CYP2C9*1/*1* and *CYP2C9*1/*3* or *CYP2C9*3/*3*.
†$P < 0.05$ between *CYP2C9*1/*3* and *CYP2C9*3/*3*.

individuals) the fluconazole $K_i$ was 23 $\mu$M. Thus, the $K_i$ of fluconazole inhibition was altered in a gene dose-dependent fashion.

Results from the model-based analysis (eq. 2) of the drug interaction are presented in Table 5. Only the values of the uninhibited clearance, CL2C9, for each genotype were estimated. The clearances as the result of the drug interaction (i.e., the reduced clearances) were modeled in the drug interaction submodel as $1/(1 + I/K_i)$. These values of flurbiprofen clearance and the magnitude of the drug interaction are all consistent with the noncompartmental results. Diagnostic plots of weighted residuals versus time or predicted concentration were randomly distributed around zero and suggested no reason to reject the model. The plot of observed versus predicted concentration indicated a good fit of the observed data under the model. The attempt to combine CL2C9 in the **3/*3* subjects with the CL2C9 in **1/*3* subjects as a single parameter resulted in a significantly inferior model ($p < 0.05$, $\chi^2$, df = 1) as indicated by an increase in the objective

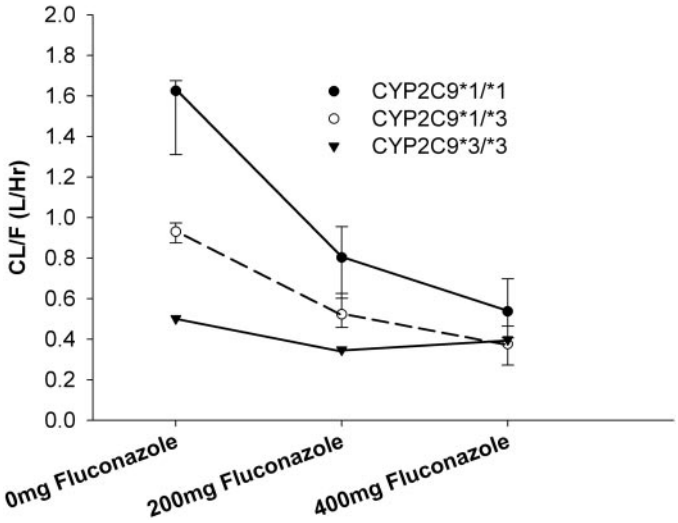


FIG. 2. Interaction plot of flurbiprofen apparent oral clearance (median ± 25th percentile) of the three genotype groups at each of the three treatment periods. Values are taken from Table 3.

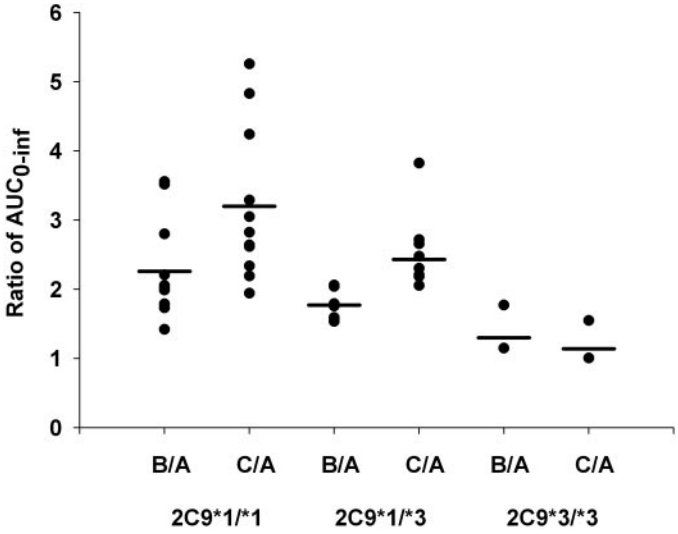


FIG. 3. Ratio of individual $AUC_{0-inf}$ for flurbiprofen with fluconazole (200 or 400 mg)/flurbiprofen alone with means (line) of the three genotype groups. A, baseline; B, 200 mg of fluconazole; C, 400 mg of fluconazole.

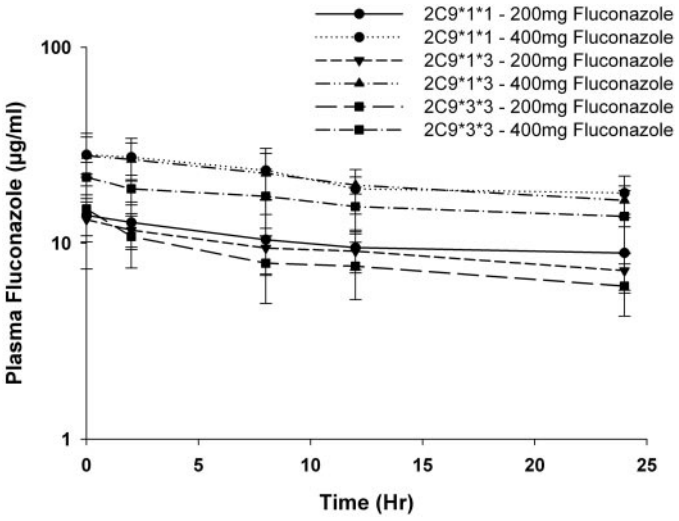


FIG. 4. Mean ± S.D. plasma fluconazole concentration versus time profiles after oral administration of seven doses of 200 or 400 mg of fluconazole tablets in 11 *CYP2C9*1/*1*, 8 *CYP2C9*1/*3*, and 2 *CYP2C9*3/*3* subjects.

function value of 5.2 units. When nine CL2C9 typical values were estimated (one for each genotype and level of drug interaction), the objective function value decreased by 4.8 units compared with the model incorporating the in vivo and in vitro drug interaction model (not statistically significant ($p > 0.05$, $\chi^2$, df = 5).

## Discussion

The effects of genetic polymorphisms on rates of drug metabolism are well known, but how these polymorphisms impact susceptibility to drug-drug interactions is less clear. In particular, whether reduced function proteins (e.g., CYP2C9.3) are inhibited to the same extent in vivo as wild-type proteins (e.g., CYP2C9.1) has not been determined. This knowledge is potentially clinically important because differential dosage adjustments may be needed in individuals with the *CYP2C9*3* genotype compared with the more prevalent wild-type-expressing individuals, when a known interacting drug is coadministered. Results of the present in vivo interaction study demonstrated that genotype-dependent inhibition of flurbiprofen oral clearance occurs when it is coadministered with fluconazole in subjects with none, one, or two *CYP2C9*3* alleles.

CYP2C9 exclusively catalyzes the oxidative metabolism of flurbiprofen to its 4′-hydroxy (primary) oxidative metabolite (Tracy et al., 1995, 1996), and this reaction has been demonstrated to be a reliable probe of CYP2C9 activity in vivo (Lee et al., 2003b; Zgheib et al., 2007). Differences in flurbiprofen pharmacokinetics have been evaluated previously in individuals genotyped for *CYP2C9*1/*2* and *CYP2C9*1/*3*, and reduced oral clearance was noted in individuals with both genotypes compared with individuals with the *CYP2C9*1/*1* genotype (Lee et al., 2003a). Fluconazole is a prototypical CYP2C9 inhibitor, with nearly complete bioavailability and less than 10% of a dose being metabolized (Shiba et al., 1990). Greenblatt et al. (2006) reported a reduction of flurbiprofen oral clearance, after two doses of 200 mg of fluconazole, to approximately 55% of the baseline value (Greenblatt et al., 2006), similar to the current results in wild-type individuals. In another study, the formation clearance of 4′-hydroxyflurbiprofen (as an indicator of CYP2C9 activity) decreased by 69 and 78% from baseline after single and seven doses of 200 mg of fluconazole, respectively (Zgheib et al., 2007), again similar to the current results. However, in neither study were the subjects' genotypes known.

In vitro studies from our laboratory (Kumar et al., 2006b) reported genotype-dependent inhibition of CYP2C9, and in the current work we report a 2-fold difference in fluconazole $K_i$ values (with flurbiprofen as a substrate) between the CYP2C9.1 and CYP2C9.3 proteins, suggesting that genotype-dependent inhibition in vivo was possible. Pretreatment with seven doses of either 200 or 400 mg of fluconazole significantly decreased flurbiprofen oral clearance in *CYP2C9*1/*1* and *CYP2C9*1/*3* individuals (Fig. 1, A and B) in a gene dose-dependent manner, compared with the control phase. In contrast, flurbiprofen plasma concentrations were unchanged among the three periods in the two individuals homozygous for *CYP2C9*3* (Fig. 1C). Although fluconazole is largely excreted unchanged, it was necessary to determine whether the CYP2C9 genotype in any way altered fluconazole clearance and, thus, accounted for the genotype-dependent inhibition. However, neither fluconazole plasma concentrations nor clearance was impacted by genotype, demonstrating that the inhibition observed was not due to altered fluconazole disposition.

Clearance values estimated from the population model-based drug

TABLE 4

*Noncompartmental pharmacokinetic parameters of fluconazole after 200 mg or 400 mg coadministration for 7 days*

Data are shown as median (range), except for *CYP2C9*3/*3* genotype group, in which individual data are presented.

| Pharmacokinetic Parameters | Units | *CYP2C9*1/*1* ($n$ = 11) | *CYP2C9*1/*3* ($n$ = 8) | *CYP2C9*3/*3* ($n$ = 2) |
|---|---|---|---|---|
| 200 mg of fluconazole | | | | |
| $AUC_{0-24}$ | Micrograms · milliliter$^{-1}$ · hour | 265 (187–395) | 248 (142–293) | 158, 262 |
| $T_{1/2}$ | Hours | 31.6 (13.3–46.4) | 27.3 (14.7–46.7) | 34.6, 51.6 |
| $C_{max}$ | Micrograms · milliliter$^{-1}$ | 14.5 (11.3–19.6) | 14.4 (9.2–16.0) | 9.6, 20.3 |
| 400 mg of fluconazole | | | | |
| $AUC_{0-24}$ | Micrograms · milliliter$^{-1}$ · hour | 543 (362–742) | 515 (474–722) | 330, 490 |
| $T_{1/2}$ | Hours | 35.4 (17.9–54.9) | 42.0 (15.5, 55.2) | (49.5, 51.1) |
| $C_{max}$ | Micrograms · milliliter$^{-1}$ | 27.8 (19.6–42.1) | 26.4 (23.3–47.0) | 18.8, 24.6 |

TABLE 5

*Model-based population pharmacokinetic parameters of flurbiprofen*

| Parameter | Estimate | 95% Confidence Interval |
|---|---|---|
| CL2C9, uninhibited (liters/h) | | |
| *CYP2C9*1/*1* | 1.38 | (1.15, 1.61) |
| *CYP2C9*1/*3* | 0.805 | (0.580, 1.03) |
| *CYP2C9*3/*3* | 0.384 | (0.215, 0.553) |
| CLnon2C9 (liters/h) | 0.198 | (0.0792, 0.317) |
| V (liters) | 8.40 | (7.34, 9.46) |
| $K_a$ (hr$^{-1}$) | 2.13 | (1.18, 3.08) |
| LAG (h) | 0.200 | (0.181, 0.219) |
| Variability (CV%) | | |
| CL | 25.6 | (5.9, 35.8) |
| V | 26.1 | (12.4, 34.6) |
| $K_a$ | 94.2 | (63.1, 117) |
| LAG | 14.0 | (1.4, 19.7) |
| RUV | 47.7 | (43.5, 51.7) |

V, flurbiprofen volume of distribution; $K_a$, flurbiprofen first-order absorption rate constant; LAG, flurbiprofen absorption lag time; RUV, residual unexplained variability; CV, coefficient of variation.

interaction analysis were consistent with those from the noncompartmental analysis. It is particularly noteworthy that the clearances of flurbiprofen at two different levels of fluconazole interaction were closely predicted from a drug interaction model. For example, in the *CYP2C9*1/*3* group, the uninhibited clearance was estimated to be 0.9 liter/h. In the model-based approach, a value of 0.81 liter/h was estimated. In the 200 mg of fluconazole drug interaction arm, the noncompartmental approach provided a clearance of 0.5 liter/h; this compares to 0.428 liter/h under the drug interaction model. Values that were comparable between the two analysis approaches were also noted at the 400 mg of fluconazole interaction level. These findings are encouraging and provide support to the notion that in vivo drug interactions may be reasonably predicted from in vitro data under an appropriate model.

With respect to 4′-hydroxyflurbiprofen formation clearances ($CL_{f,m}$), significant differences were noted at baseline (no fluconazole) among the three genotype groups (Table 4). These differences disappeared at the highest dose of fluconazole (400 mg) between the **1/*1* and **1/*3* groups, but the $CL_{f,m}$ value for the *CYP2C9*3/*3* individuals remained statistically different. Interestingly, a substantial reduction in $CL_{f,m}$ was noted with administration of either 200 or 400 mg of fluconazole, regardless of genotype, compared with the control situation (no fluconazole). For example, in *CYP2C9*1/*1* individuals, the $CL_{f,m}$ was reduced 75% at the 400 mg of fluconazole dose, and $CL_{f,m}$ was reduced 77 and 60% in the *CYP2C9*1/*3* and *CYP2C9*3/*3* groups, respectively. Given that fluconazole at this dose inhibits several P450s (Venkatakrishnan et al., 2000), the possibility that this reduction in formation clearance may be due to inhibition of residual flurbiprofen hydroxylase activity carried out by other P450s, such as CYP3A4 or CYP2C19, cannot be discounted. Thus, fluconazole does inhibit the CYP2C9.3 protein by a similar percentage as with the CYP2C9.1 protein, yet no change in oral clearance of flurbiprofen is noted in *CYP2C9*3/*3* individuals. This apparent paradox can be explained by the relatively low percentage contribution of this $CL_{f,m}$ of 4′-hydroxyflurbiprofen to the total clearance in *CYP2C9*3/*3* individuals. For example, formation of 4′-hydroxyflurbiprofen accounted for 42% of total clearance in the absence of fluconazole and 34% of total clearance with 400 mg of fluconazole in **1/*1* subjects. In contrast, in *CYP2C9*3/*3* individuals, although the $CL_{f,m}$ was reduced by 60% after coadministration of 400 mg of fluconazole, this pathway of clearance was only 10% of total clearance in the control period and was reduced to 5% with 400 mg of fluconazole. Because this pathway represented such a small proportion of the total clearance, the effect of CYP2C9 inhibition was minimal in these homozygotic variant individuals. This finding has important implications for prediction of drug-drug interactions because one must consider both genotype and fraction metabolized by a given pathway when predicting these interactions. Further studies are needed to test this hypothesis with drugs that have greater and lesser fractions metabolized by a given pathway in individuals with variant genotypes.

Some studies have reported the impact of genetic polymorphisms in CYP2C19 and CYP2D6 on drug-drug interactions in humans. Yasui-Furukori et al. (2004a,b) studied the degree of inhibition of omeprazole and lansoprazole metabolism after coadministration of the CYP2C19 inhibitor fluvoxamine, in subjects genotyped for CYP2C19 polymorphisms. This study, in concurrence with similar studies by others (Uno et al., 2006), concluded that extensive metabolizers experience a greater extent of CYP2C19 inhibition upon coadministration of inhibitors compared with CYP2C19 poor metabolizers. Similarly, studies have been conducted in extensive versus poor metabolizers of CYP2D6 and the extent of drug-drug interactions was compared. These studies have consistently reported a greater degree of inhibition of wild-type CYP2D6 enzyme compared with variant forms of CYP2D6 (Hamelin et al., 2000; Lessard et al., 2001; LLerena et al., 2001; Lindh et al., 2003). The variant alleles of CYP2C19, as well as those of CYP2D6, in the above studies cause either a splicing defect or a frame shift resulting in either premature termination of translation or a truncated protein. Thus, these genotype-dependent inhibition results for CYP2C19 and CYP2D6 polymorphisms identified in these studies are not surprising, given that the polymorphisms result in the expression of inactive proteins, such that no residual activity is present to be inhibited. The present findings suggest that despite CYP2C9.3 protein maintaining ~20 to 30% residual activity compared with the CYP2C9.1 enzyme, with respect to fluconazole inhibition, the CYP2C9.3 enzyme in vivo behaves as if it were inactive in that the contribution to overall clearance of the drug is so minor that inhibition has little effect on overall clearance.

These findings of genotype-dependent inhibition in an enzyme with residual activity have potentially important clinical implications. If

one were to reduce the dose of a target drug based on interaction potential of the inhibitor without considering genotype, the net result could be underdosing of the patient and potentially suboptimal therapy. Thus, if these results are more generally applicable, one may also need to consider both genotype status and fraction metabolized for drugs metabolized by polymorphic enzymes in situations in which combinations of drugs that are known to interact must be used. Additional studies are underway to determine whether this phenomenon in *CYP2C9*3* subjects occurs with other substrates or is limited to the studied combination of flurbiprofen and fluconazole.

## References


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

Bajpai M, Roskos LK, Shen DD, and Levy RH (1996) Roles of cytochrome P4502C9 and cytochrome P4502C19 in the stereoselective metabolism of phenytoin to its major metabolite. *Drug Metab Dispos* **24:**1401–1403.

Beal SL, Sheiner LB, and Boeckmann AJ (2006) *NONMEM Users Guides (1989–2006)*. Ellicott City, MD, Icon Development Solutions.

Cociglio M, Brandissou S, Alric R, and Bressolle F (1996) High-performance liquid chromatographic determination of fluconazole in plasma. *J Chromatogr B Biomed Appl* **686:**11–17.

Greenblatt DJ, von Moltke LL, Perloff ES, Luo Y, Harmatz JS, and Zinny MA (2006) Interaction of flurbiprofen with cranberry juice, grape juice, tea, and fluconazole: in vitro and clinical studies. *Clin Pharmacol Ther* **79:**125–133.

Guo Y, Zhang Y, Wang Y, Chen X, Si D, Zhong D, Fawcett JP, and Zhou H (2005) Role of CYP2C9 and its variants (CYP2C9*3 and CYP2C9*13) in the metabolism of lornoxicam in humans. *Drug Metab Dispos* **33:**749–753.

Hamelin BA, Bouayad A, Methot J, Jobin J, Desgagnes P, Poirier P, Allaire J, Dumesnil J, and Turgeon J (2000) Significant interaction between the nonprescription antihistamine diphenhydramine and the CYP2D6 substrate metoprolol in healthy men with high or low CYP2D6 activity. *Clin Pharmacol Ther* **67:**466–477.

Hamman MA, Thompson GA, and Hall SD (1997) Regioselective and stereoselective metabolism of ibuprofen by human cytochrome P450 2C. *Biochem Pharmacol* **54:**33–41.

Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, and Rettie AE (2002) Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* **287:**1690–1698.

Hutzler JM, Frye RF, Korzekwa KR, Branch RA, Huang S, and Tracy TS (2001) Minimal in vivo activation of CYP2C9-mediated flurbiprofen metabolism by dapsone. *Eur J Pharm Sci* **14:**47–52.

Hutzler JM, Frye RF, and Tracy TS (2000) Sensitive and specific high-performance liquid chromatographic assay for 4′-hydroxyflurbiprofen and flurbiprofen in human urine and plasma. *J Chromatogr B Biomed Sci Appl* **749:**119–125.

Kidd RS, Curry TB, Gallagher S, Edeki T, Blaisdell J, and Goldstein JA (2001) Identification of a null allele of CYP2C9 in an African-American exhibiting toxicity to phenytoin. *Pharmacogenetics* **11:**803–808.

Kumar V, Locuson CW, Sham YY, and Tracy TS (2006a) Amiodarone analog-dependent effects on CYP2C9-mediated metabolism and kinetic profiles. *Drug Metab Dispos* **34:**1688–1696.

Kumar V, Wahlstrom JL, Rock DA, Warren CJ, Gorman LA, and Tracy TS (2006b) CYP2C9 inhibition: impact of probe selection and pharmacogenetics on in vitro inhibition profiles. *Drug Metab Dispos* **34:**1966–1975.

Kunze KL and Trager WF (1996) Warfarin-fluconazole. III. A rational approach to management of a metabolically based drug interaction. *Drug Metab Dispos* **24:**429–435.

Lee CR, Goldstein JA, and Pieper JA (2002) Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics* **12:**251–263.

Lee CR, Pieper JA, Frye RF, Hinderliter AL, Blaisdell JA, and Goldstein JA (2003a) Differences in flurbiprofen pharmacokinetics between CYP2C9*1/*1, *1/*2, and *1/*3 genotypes. *Eur J Clin Pharmacol* **58:**791–794.

Lee CR, Pieper JA, Frye RF, Hinderliter AL, Blaisdell JA, and Goldstein JA (2003b) Tolbutamide, flurbiprofen, and losartan as probes of CYP2C9 activity in humans. *J Clin Pharmacol* **43:**84–91.

Lessard E, Yessine MA, Hamelin BA, Gauvin C, Labbe L, O'Hara G, LeBlanc J, and Turgeon J (2001) Diphenhydramine alters the disposition of venlafaxine through inhibition of CYP2D6 activity in humans. *J Clin Psychopharmacol* **21:**175–184.

Lindh JD, Annas A, Meurling L, Dahl ML, and AL-Shurbaji A (2003) Effect of ketoconazole on venlafaxine plasma concentrations in extensive and poor metabolisers of debrisoquine. *Eur J Clin Pharmacol* **59:**401–406.

LLerena A, Berecz R, de la RA, Fernandez-Salguero P, and Dorado P (2001) Effect of thioridazine dosage on the debrisoquine hydroxylation phenotype in psychiatric patients with different CYP2D6 genotypes. *Ther Drug Monit* **23:**616–620.

Miners JO and Birkett DJ (1996) Use of tolbutamide as a substrate probe for human hepatic cytochrome P450 2C9. *Methods Enzymol* **272:**139–145.

Miners JO and Birkett DJ (1998) Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol* **45:**525–538.

Niemi M, Cascorbi I, Timm R, Kroemer HK, Neuvonen PJ, and Kivisto KT (2002) Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. *Clin Pharmacol Ther* **72:**326–332.

Perini JA, Vianna-Jorge R, Brogliato AR, and Suarez-Kurtz G (2005) Influence of CYP2C9 genotypes on the pharmacokinetics and pharmacodynamics of piroxicam. *Clin Pharmacol Ther* **78:**362–369.

Rendic S and Dicarlo FJ (1997) Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. *Drug Metab Rev* **29:**413–580.

Rettie AE, Korzekwa KR, Kunze KL, Lawrence RF, Eddy AC, Aoyama T, Gelboin HV, Gonzalez FJ, and Trager WF (1992) Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: a role for P-4502C9 in the etiology of (*S*)-warfarin-drug interactions. *Chem Res Toxicol* **5:**54–59.

Rowland M and Matin SB (1973) Kinetics of drug-drug interactions. *J Pharmacokinet Biopharm* **1:**553–567.

Shiba K, Saito A, and Miyahara T (1990) Safety and pharmacokinetics of single oral and intravenous doses of fluconazole in healthy subjects. *Clin Ther* **12:**206–215.

Shimada T, Yamazaki H, Mimura M, Inui Y, and Guengerich FP (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270:**414–423.

Stearns RA, Chakravarty PK, Chen R, and Chiu SH (1995) Biotransformation of losartan to its active carboxylic acid metabolite in human liver microsomes: role of cytochrome P4502C and 3A subfamily members. *Drug Metab Dispos* **23:**207–215.

Tracy TS, Marra C, Wrighton SA, Gonzalez FJ, and Korzekwa KR (1996) Studies of flurbiprofen 4′-hydroxylation—additional evidence suggesting the sole involvement of cytochrome P450 2C9. *Biochem Pharmacol* **52:**1305–1309.

Tracy TS, Rosenbluth BW, Wrighton SA, Gonzalez FJ, and Korzekwa KR (1995) Role of cytochrome P450 2C9 and an allelic variant in the 4′-hydroxylation of (*R*)- and (*S*)-flurbiprofen. *Biochem Pharmacol* **49:**1269–1275.

Uno T, Shimizu M, Yasui-Furukori N, Sugawara K, and Tateishi T (2006) Different effects of fluvoxamine on rabeprazole pharmacokinetics in relation to CYP2C19 genotype status. *Br J Clin Pharmacol* **61:**309–314.

Venkatakrishnan K, von Moltke LL, and Greenblatt DJ (2000) Effects of the antifungal agents on oxidative drug metabolism: clinical relevance. *Clin Pharmacokinet* **38:**111–180.

Vianna-Jorge R, Perini JA, Rondinelli E, and Suarez-Kurtz G (2004) CYP2C9 genotypes and the pharmacokinetics of tenoxicam in Brazilians. *Clin Pharmacol Ther* **76:**18–26.

Yan Z, Li J, Huebert N, Caldwell GW, Du Y, and Zhong H (2005) Detection of a novel reactive metabolite of diclofenac: evidence for CYP2C9-mediated bioactivation via arene oxides. *Drug Metab Dispos* **33:**706–713.

Yasui-Furukori N, Saito M, Uno T, Takahata T, Sugawara K, and Tateishi T (2004a) Effects of fluvoxamine on lansoprazole pharmacokinetics in relation to CYP2C19 genotypes. *J Clin Pharmacol* **44:**1223–1229.

Yasui-Furukori N, Takahata T, Nakagami T, Yoshiya G, Inoue Y, Kaneko S, and Tateishi T (2004b) Different inhibitory effect of fluvoxamine on omeprazole metabolism between CYP2C19 genotypes. *Br J Clin Pharmacol* **57:**487–494.

Zgheib NK, Frye RF, Tracy TS, Romkes M, and Branch RA (2007) Evaluation of flurbiprofen urinary ratios as in vivo indices for CYP2C9 activity. *Br J Clin Pharmacol* **63:**477–487.


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