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Effects of genetic variation at the *CYP2C19/CYP2C9* locus on pharmacokinetics of chlorcycloguanil in adult Gambians

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Abstract

Aims—Antimalarial biguanides are metabolized by *CYP2C19*, thus genetic variation at the *CYP2C* locus might affect pharmacokinetics and so treatment outcome for malaria.

Materials & methods—Polymorphisms in *CYP2C19* and *CYP2C9* in 43 adult Gambians treated with chlorproguanil/dapsone for uncomplicated malaria were assessed. Chlorcycloguanil pharmacokinetics were measured and associations with *CYP2C19* and *CYP2C9* alleles and *CYP2C19* metabolizer groups investigated.

Results—All *CYP2C19/CYP2C9* alleles obeyed Hardy–Weinberg equilibrium. There were 15 *CYP2C19/2C9* haplotypes with a common haplotype frequency of 0.23. Participants with the *CYP2C19*17* allele had higher chlorcycloguanil area under the concentration versus time curve at 24 h (AUC_{0-24}) than those without (geometric means: 317 vs 216 ng.h/ml; ratio of geometric means: 1.46; 95% CI: 1.03 to 2.09; $p = 0.0363$) and higher C_{max} (geometric mean ratio: 1.52; 95% CI: 1.13 to 2.05; $p = 0.0071$).

Conclusion—*CYP2C19*17* determines antimalarial biguanide metabolic profile at the *CYP2C19/CYP2C9* locus.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Keywords

chlorcycloguanil; CYP2C19; CYP2C9; drug metabolism; pharmacokinetics; polymorphisms

The polymorphic enzymes CYP2C9 and CYP2C19 are part of the cytochrome P450 (CYP) superfamily of enzymes and metabolize approximately a third of all clinically important drugs [1,2]. For CYP2C19, individuals have been grouped phenotypically according to metabolism of *S*-mephenytoin into poor and extensive metabolizers. Poor metabolizers represent 0.02–0.05 of Europeans and 0.13–0.23 of Asians. The *CYP2C19*2* and *CYP2C19*3* alleles contribute to the poor metabolizer phenotype with *CYP2C19*2* being found in Europeans and Asians and *CYP2C19*3* common only in Asians. *CYP2C19*17* is a recently described gain-of-function polymorphism leading to increased transcription and has a frequency of 0.18 in Swedes and Ethiopians and 0.04 in Chinese [3]. Whilst there are substantial published genotypic data from Europeans and Asians, there is limited allelic information currently available for Africans, particularly in sub-Saharan Africa.

The antimalarial biguanide proguanil is commonly used for malaria prophylaxis in combination with chloroquine in North Africa, Middle East and Central Asia and in variable risk areas of South Asia, Southeast Asia, Central and South America and the Caribbean. Proguanil is also used for prophylaxis in combination with atovaquone in sub-Saharan Africa, Oceania and high-risk areas of South and Southeast Asia, Central and South America, and the Caribbean [101]. The WHO recommends the chloroquine/proguanil combination for treatment of uncomplicated *Plasmodium falciparum* and *vivax* malaria in Southeast Asia and for prevention of malaria during pregnancy in Togo, South Africa, Swaziland and Botswana [4].

Antimalarial biguanides are converted to their active metabolite by cytochrome P450 enzymes and these metabolites inhibit the malaria parasite's dihydrofolate reductase enzyme. Other metabolites such as 4-chlorophenylbiguanide and dichlorophenylbiguanide are also produced; however, they are not thought to have antimalarial activity [5-7]. In the atovaquone–proguanil combination, synergism arises from enhancement of the membrane collapsing activity of atovaquone by proguanil, however, in other combinations and when used alone the effect on folate metabolism is thought to be most important [8].

The biguanides have the advantage of relatively short duration of action and thus may be less likely to select resistant parasites than other antimalarial compounds [9]. Biguanide combinations that have been evaluated for malaria therapy include chlorproguanil–dapson (Lapdap™) and chlorproguanil–dapson–artesunate (CDA, Dacart™), which have completed Phase III studies. However, due to concerns about increased risk of anemia, particularly in patients who are glucose-6-phosphate dehydrogenase deficient, the development of CDA has been terminated and Lapdap withdrawn from the market [102]. Lapdap side effects were thought to be related to dapsone metabolites.

Chlorproguanil is converted to its active metabolite chlorcycloguanil by CYP2C19 [10], which is subsequently renally excreted [11]. Thus mutations affecting the function of CYP2C19 might affect treatment outcome by influencing pharmacokinetic parameters of the drug *in vivo*. Dapsone is converted to its toxic hydroxylamine metabolite by the cytochrome P450 CYP2C9, which is structurally similar to CYP2C19 [12], but the role of CYP2C9 in chlorproguanil metabolism is not known.

We determined allele frequencies for *CYP2C19* and *CYP2C9* and investigated the effects of fast and slow metabolism alleles on chlorcycloguanil pharmacokinetics for the first time in

West Africa. Our aim was to determine the magnitude of inherited differences in drug activation and hence whether these genetic effects might influence treatment response.

Materials & methods

Study subjects

A total of 43 adult residents of Farafenni and surrounding villages (The Gambia) (18 female), median age 27 years (range 17–60 years), with uncomplicated malaria who were treated with chlorproguanil/dapsone at a target dose 2–2.5 mg/kg body weight and gave informed consent to donate a blood sample for genetic analysis.

Another set of 85 DNA samples from participants in a previous investigation [13] who had given broad consent for genetic studies formed the sample set from Sukuta in the western region of The Gambia, which is approximately 122 km from Farafenni in the north. The two study populations are ethnically similar with the majority coming from the Mandinka, Wolof and Fula tribes. This second set was used for PCR optimization and to determine allele frequencies and linkage disequilibrium (LD) patterns. The Medical Research Council (MRC) Scientific Coordinating Committee and the MRC/Gambia Government Joint Ethics Committee gave ethical approval of the studies (L2004.80, SCC981 29th November 2004).

Drug & metabolite assays

Following protein precipitation plasma concentrations of chlorproguanil and chlorcycloguanil were determined using HPLC tandem mass spectroscopy (HPLC/MS/MS) on an API3000 or API5000 (Sciex, Concord, ON, Canada). The method was validated based on a percentage bias of less than $\pm 15\%$ and a within- and between-assay precision of 15%. The maximum percentage bias observed was 7.5% for chlorproguanil and 12.1% for chlorcycloguanil. The maximum within- and between-assay precision observed were 10.1 and 2.7% for chlorproguanil and 10.9 and 9.4% for chlorcycloguanil. The stability of chlorproguanil and chlorcycloguanil was validated in human plasma stored at room temperature for at least 20 h and at -80°C for at least 10 months. The lower limit of quantitation was 2 ng/ml for chlorproguanil, 4 ng/ml for chlorcycloguanil using a 50 μl aliquot of blood anticoagulated with lithium heparin.

Full pharmacokinetic sampling was performed after the first of three daily doses. Plasma concentrations were used to determine pharmacokinetic parameters for chlorcycloguanil including area under the concentration versus time curve at 24 h (AUC_{0-24}) and at the end of sampling (AUC_{0-t}), the maximum plasma concentration (C_{max}) and the time to achieve this (t_{max}) by means of non-compartmental methods using WinNonlin Professional Ed 4.1 (Pharsight Corp., CA, USA) at GlaxoSmithKline, UK. The higher limit of quantitation was 400 ng/ml for chlorproguanil and chlorcycloguanil.

Genotyping

DNA was extracted and typed for common SNPs using the Taqman[®] Drug Metabolizing Genotyping system where available [103] or allele specific PCR using primers detailed in Supplementary Table 1. The SNPs in the exons of *CYP2C19* and *CYP2C9* were chosen because they are known to affect enzyme structure and/or function, we also added SNPs in the 5' region of *CYP2C19*, which might affect promoter function. The haplotypes present across the two genes were determined using PHASE 2.1 [14] and effects on drug metabolite pharmacokinetics were analyzed.

Classification of metabolizer groups

A simple genetic model based on *CYP2C19* genotype status was used to classify participants *a priori* into fast, intermediate and slow metabolizer groups. Fast metabolizers were defined as having one or more *CYP2C19**17 alleles, intermediate had no fast or slow metabolizing alleles and slow metabolizers had no *CYP2C19**17 alleles and one or more *CYP2C19**2/*13 alleles. A similar grouping has been used in previous studies [3,15,16]. *CYP2C19**1 cannot be determined with certainty due to the continuous refinement of genetic techniques. The recently identified *17 allele was very common; the majority of Gambians in our study are fast metabolizers (Table 1).

Statistical analysis

Pharmacokinetic parameters of chlorcycloguanil: AUC_{0-24} , AUC_{0-t} , C_{max} and t_{max} were log-transformed and compared between genotypic groups defined by individual haplotype or allele using analysis of variance or t-test in SPSS 11.5 (SPSS Inc., IL, USA). Differences were expressed in terms of the ratio of geometric means, p-values were not adjusted for multiple testing.

Results

Allele & haplotype frequencies for *CYP2C19* & *CYP2C9* in Gambians

Allele frequencies are broadly similar to those established in West-African Yoruba from the HapMap project, which often offers the only published source of allele frequency data in West Africans [104]. Allele frequencies in African-Americans were included from the ABI database [105] and were similar except for rs7067866 where the variant allele had a somewhat higher frequency in Gambians (Figure 1). Whilst these databases provide useful information on the diversity of alleles present in people of African descent participants were not selected randomly and thus caution should be taken when using this as reference data for frequencies of alleles.

Haplotype analysis generated 15 haplotypes, Hap2 and Hap10 had frequencies of 0.19 and 0.01, respectively and carried the gain-of-function *CYP2C19**17 variant allele and the promoter variant allele rs7067866, which were always found together. The loss-of-function *CYP2C19**2 allele was found on Hap 4 (0.11) and Hap15 (0.01), whilst Hap13 (0.01) and Hap14 (0.01) carried *CYP2C19**13. The decreased function *CYP2C9* alleles *2, *9 and *11A and the increased function *CYP2C9**8 allele were carried on haplotypes with low frequencies less than 0.05. A stratified analysis by geographical site showed similar allele frequencies in Sukuta and Faraffeni (Figure 1).

There was a high degree of LD ($D' = 1$) between *CYP2C19* and *CYP2C9* as shown in the haplotype block diagram (Figure 2) generated from Haploview 4.1 [106]. Interestingly pharmacokinetic values for *CYP2C9**11 followed those of *CYP2C19**2 although the two loci are not in LD (Figure 2).

Association of *CYP2C19* & *CYP2C9* genetic markers with chlorcycloguanil pharmacokinetic parameters

The *CYP2C19**17 gain-of-function allele had the greatest effect on chlorcycloguanil pharmacokinetic parameters increasing both AUC and C_{max} . There was a trend for shorter time to reach peak metabolite concentrations in those with the fast metabolizing *CYP2C19**17 allele compared with those without. There were trends in the opposite direction for the *CYP2C19**2 loss-of-function allele with reduced AUC and C_{max} and longer t_{max} ; however, these trends did not reach statistical significance. Pharmacokinetic parameters for *CYP2C9**8 tended to follow those for *CYP2C19**2.

The metabolite ratio (AUC chlorcycloguanil/AUC chlorproguanil), which is a phenotypic measure of CYP2C19 activity was analyzed. AUC_{0-t} and C_{max} metabolite ratios were higher in *CYP2C19*17* carriers compared with noncarriers although these trends did not reach statistical significance (ratio AUC_{0-t}: 0.31 vs 0.20; $p = 0.090$; C_{max}: 0.23 vs 0.19; $p = 0.069$). There were also nonsignificant trends in the opposite direction for the *CYP2C19*2* loss-of-function allele with reduced AUC and C_{max} and longer t_{max} (Table 2).

Chlorcycloguanil AUC₀₋₂₄ was significantly higher in fast when compared with intermediate and slow metabolizer groups, as was AUC_{0-t} and C_{max} (Table 1). Fast metabolizers had a higher AUC_{0-t} (geometric mean: 335 ng.h/ml) than intermediate (geometric mean ratio: 1.62; 95% CI: 1.12–2.35; $p = 0.0133$), and slow metabolizers (1.58; 95% CI: 0.97–2.60; $p = 0.0672$). One participant with both *CYP2C19*2* and *CYP2C19*17* had increased AUC and C_{max} values indicating that the effect of *CYP2C19*17* overcomes that of *CYP2C19*2* resulting in rapid metabolism (Table 1).

Discussion

This study identifies *CYP2C19*17* as the major genetic determinant for activation of antimalarial biguanides at the *CYP2C19/CYP2C9* locus. People with the *CYP2C19*17* allele have increased AUC and C_{max} reflecting increased exposure to the active antimalarial metabolite. Fast metabolizers achieve approximately double the maximum plasma concentration of active drug when compared with slow metabolizers.

We found the frequency of the *CYP2C19*17* fast metabolizing allele to be similar to that reported in Europeans, which is substantially higher than found in Asians [3]. The frequency is similar to that quoted in the Hapmap project for Yoruba in Ibadan, Nigeria [104]. The *CYP2C19*2* slow metabolizing allele frequency in Gambians was also similar to that in Europeans and African-Americans [17]. *CYP2C19*3* and *CYP2C19*9* variants have not been previously found in people of African descent and this is in accordance with our findings in Gambians. The *CYP2C9*11* minor allele occurred with a frequency of 0.03 similar to its frequency of 0.027 in a Beninese population and whilst one study found *CYP2C9*2* to be absent in Beninese, we found a frequency of 0.01 in Gambians [18].

The difference between chlorcycloguanil pharmacokinetics for *CYP2C19*2* was surprisingly small compared with the effects of *CYP2C19*17*. Although there was a trend for lower AUC with the slow metabolizing allele, as would be expected, this was not statistically significant in our study. This may be due to the contribution of other P450 isoforms such as CYP3A4 in the biotransformation of antimalarial biguanides [19], which might mask small differences between *CYP2C19*2* subgroups. In addition, the sampling time used was relatively short given the average half-life of the metabolite, which is approximately 15 h. Extended sampling, which could have been undertaken at the end of treatment, would have given a more complete picture and may have identified smaller differences between metabolizer groups. However in the context of clinical treatment, the fact that we observed effects on AUC within a normal dosage regimen adds weight to the argument that these genetic variants could be important from a clinical translational perspective.

A study in the malaria endemic island of Vanuatu showed that *CYP2C19*2* poor metabolizers treated with proguanil experienced similar antimalarial activity when compared with extensive metabolizers suggesting that biguanides may have some intrinsic activity that does not depend on biotransformation by CYP2C19 although in this study the effects of *CYP2C19*17* which we find to be the major determinant of chlorcycloguanil levels in Africans, were not evaluated [20]. Nevertheless there remains a possibility that there may be

intrinsic activity of the parent biguanide or other active metabolites of chlorproguanil. The discussion here largely assumes acceptance that chlorcycloguanil is the active agent, which seems to reflect current scientific opinion.

A limitation of our study was the relatively small number of participants, which generated insufficient data to estimate effects on homozygotes. A similar study in a different cohort involving a larger population with pharmacokinetic profile of chlorcycloguanil would be useful to validate our results and explore the effects in genetic subgroups.

An important question is whether the differences that we observed between genotype groups will translate into differences in the effectiveness of clinical treatment. CYP2C19 forms an active antiestrogenic metabolite from tamoxifen, an agent used to treat breast cancer. Clinical treatment outcome in women taking tamoxifen for breast cancer was measured by recurrences of breast cancer, relapse-free periods and event-free survival rates; all indices were better in women with *CYP2C19*17* than those without the *CYP2C19*17* variant [16]. Several studies have shown that the *CYP2C19* genotype affects clinical outcome of omeprazole treatment for peptic ulcers [21,22]. In one study the omeprazole AUC was estimated to be approximately 40% lower in subjects heterozygous or homozygous for *CYP2C19*17* when compared with subjects homozygous for the wild-type [3]. This is similar to the AUC effect size that we observed for *CYP2C19*17* on chlorcycloguanil and adds weight to the suggestion that genetic variations might govern to some extent the clinical response to treatment with antimalarial biguanides.

We have shown in adults for the first time the importance of the *CYP2C19*17* allele on metabolism of antimalarial biguanides, however, in practice children bear the greatest burden from malaria. Whilst *CYP2C19* allele frequencies will be similar in children and adults, the effects on pharmacokinetic parameters may be different. Thus it is important to assess the effects on clinical outcome in this group. Work is now in progress to examine the effect of *CYP2C19* genotype on clinical outcome in a randomized controlled trial of chlorproguanil/dapsone in children with uncomplicated malaria.

In each population where a biguanide is used in combination with another antimalarial there will be a subset of people, defined by *CYP2C19* genotype, who have potentially subtherapeutic biguanide levels thus encouraging development of resistance by the malaria parasite. The size of this underdosed group will depend on *CYP2C19* allele frequency in the population. Thus one would expect the effect to be particularly evident in Asia where the frequency of *CYP2C19*17* is low and poor metabolizing alleles (*CYP2C19*2* and *CYP2C19*3*) are common. It is interesting to note in this context that resistance to antimalarials is a particular problem in the Asian subcontinent.

Conclusion

This study sheds important light on metabolism of antimalarial biguanides, which remain a key part of WHO guidelines on malaria. New biguanide combinations may be designed in future for routine treatment and malaria prophylaxis, although none are currently known to be under development. We have identified an important potential determinant of clinical efficacy and a possible mediator of parasite resistance. Clinical trials of any biguanide combinations, particularly in geographical areas where slow metabolism is common, should evaluate *CYP2C19*17* as a potential modifier of pharmacokinetics and treatment outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Executive summary

- Up to 500 million cases of malaria occur each year worldwide and over one million people die – mostly young children in sub-Saharan Africa.
- Malaria causes 10% of all deaths in developing countries.
- Antimalarial biguanides (chlorproguanil/proguanil) are cheap and effective antimalarial drugs that are widely used for prophylaxis and in some circumstances for malaria treatment.
- The antimalarial biguanides are transformed to an active triazine metabolite by CYP2C19, thus polymorphisms in *CYP2C19* might affect pharmacokinetics and treatment outcome.

Materials & methods

- *CYP2C19* and *CYP2C9* polymorphisms were typed in 43 adult subjects with uncomplicated malaria treated with chlorproguanil/dapsone.
- Genetic variations were then correlated with pharmacokinetic parameters for chlorcycloguanil.

Results

- There were high levels of linkage disequilibrium between the *CYP2C19* and *CYP2C9* genes.
- Allele frequencies from two different sites in The Gambia for the common functional variants (*CYP2C19*2* and *CYP2C19*17*) were similar to those in West African Yoruba from the HapMap database and in Europeans.
- Chlorcycloguanil AUC_{0-24h}, AUC_{0-t} and C_{max} were significantly higher in fast compared with intermediate and slow metabolizers.
- *CYP2C19*17* was the main single determinant of chlorcycloguanil levels at the *CYP2C19/C9* genetic locus.

Discussion

- The size of the effect of *CYP2C19*17* on chlorcycloguanil pharmacokinetics is similar to that seen with other substrates where genetic variation is linked to clinical outcome (omeprazole and tamoxifen).
- Studies are in progress to investigate the effects of *CYP2C19* genetic variants on outcome of treatment for malaria in children.
- *CYP2C19* allele frequencies vary considerably by geographic location, therefore, this information might contribute to public health policy decisions on malaria treatment in different countries.
- In populations such as those in the Asian subcontinent where fast metabolizers are infrequent there will be a subset of people with potentially subtherapeutic biguanide levels, which might contribute to development of resistance in the malaria parasite.

Figure 1. Allele frequencies and haplotypes in 43 Gambian adults following genotyping of polymorphic *CYP2C19* and *CYP2C9* alleles by PCR-sequence specific primers and Taqman[®] real-time PCR

CYP2C19 minor allele frequency is compared with allele frequencies of West-African Yorubas from the HapMap project. Haplotypes were determined by PHASE version 2.1. The SNPs are arranged according to contig positions as given by the NCBI reference assembly. The positions of the SNPs in promoter, intron and exons of the genes are indicated.

*Minor allele frequencies for African-Americans from Applied Biosystems (CA, USA) [105] were used where allele frequencies for HapMap Yoruba were not available.

Figure 2. Haplotype block diagram of *CYP2C19* and *CYP2C9*

The diagram was generated using the Haploview program [106]. The NCBI rs ID numbers of the polymorphisms in *CYP2C19* and *CYP2C9* that were investigated are indicated. Three main haplotype blocks were identified with strong LD generally between the two genes (D' values > 0.9). Block 1 contained the *CYP2C19**17 marker, which was in strong LD with the nearby promoter SNP rs7067866. Block 2 contained the *CYP2C19**2 allele. Block 3 comprised two SNPs in *CYP2C9*. The diagram also shows each haplotype in a block with its population frequency and connections from one block to the next. In the crossing areas, a value of multiallelic D' is indicated, which represents the level of recombination between the two blocks. Interestingly the decreased function *CYP2C9**8 allele is in complete LD with both the gain-of-function *CYP2C19**17 allele ($D' = 1$) and the reduced function *CYP2C19**2 allele ($D' = 1$).

LD: Linkage disequilibrium; LOD: Log of the likelihood odds ratio.

Table 1
Pharmacokinetic parameters of chlorcydoguanil in fast, intermediate and slow metabolizer groups.

Type of metabolizer	Fast (n = 18)	Intermediate (n = 13)	Slow (n = 10)	p-value from one-way analysis of variance
AUC ₀₋₂₄ (geometric mean, range) ng.h/ml	334.8 (127.4–734.4)	206.8 (101.6–444.7)	211.3 (93.9–460.7)	0.032
AUCW _{0-t} (geometric mean, range) ng.h/ml	341 (28.3–734.6)	207.9 (101.7–445.6)	192.8 (78.9–468.1)	0.009
C _{max} (geometric mean, range) ng/ml	22.5 (9.3–40.5)	14 (7.0–26.5)	14.2 (6.4–30.1)	0.009
t _{max} (geometric mean, range) h	10.3 (5.0–18.4)	10.8 (3.3–24.3)	13 (8.4–18.6)	0.404

Geometric means of pharmacokinetic parameters of chlorcycloguanil in fast (*CYP2C19*17*), intermediate (neither *CYP2C19*17* nor **2*, **13*) and poor (*CYP2C19*2*, **13*) metabolizers. There was a significant decreasing trend of chlorcycloguanil AUC and C_{max} from fast to slow metabolizers. Chlorcycloguanil geometric mean for t_{max} was the same (10 h) in fast and intermediate metabolizers and did not differ significantly in slow metabolizers (13 h). AUC: Area under the curve; C_{max}: Maximum plasma concentration; t_{max}: Time to achieve maximum plasma concentration.

Table 2
CYP2C genetic markers and their association with geometric means (ranges) of pharmacokinetic parameters of chlorcycloguanil.

	AUC ₀₋₂₄ ng.h/ml (geometric mean [range])			p-value *	AUC ₀₋₄ ng.h/ml (geometric mean [range])			p-value *	C _{max} ng/ml (geometric mean [range])			p-value *	t _{max} h (geometric mean [range])			p-value *
	WW ‡	WM	MM		WW	WM	MM		WW	WM	MM		WW	WM	MM	
CYP2C19																
rs7067866 WW 14 WM 25 MM 2	325 (239-443)	257 (94-734)	232 (97-632)	0.877	326 (239-326)	263 (94-735)	229 (79-635)	0.854	20.6 (16-26)	18 (7-40)	16 (6-40)	0.971	14 (10-18)	11 (5-24)	10 (3-24)	0.770
rs12248560 (*17) WW 23 WM+MM 18	217 (94-461)	317 (127-734)		0.036	209 (79-468)	326 (128-735)		0.011	14 (6-30)	22 (9-40)		0.007	12 (3-24)	10(5-18)		0.353
rs11568729 WW 33 WM+MM 5	257 (94-734)	228(156-280)		0.678	257 (79-735)	229(157-281)		0.692	18 (6-40)	16(13-20)		0.612	11 (3-24)	13 (8-24)		0.347
rs4986894 WW 31 WM+MM 9	264 (102-734)	214(94-551)		0.358	274 (102-735)	193(79-552)		0.107	18 (7-41)	15(6-38)		0.278	10 (3-24)	13(8-19)		0.146
rs4244285 (*2) WW 31 WM+MM 10	266 (102-734)	216(94-551)		0.325	276 (102-735)	196 (79-552)		0.099	18 (7-40)	15(6-38)		0.210	10 (3-24)	13(8-19)		0.143
rs4417205 WW 29 WM 10 MM 2	276 (127-734)	202 (94-551)	182	0.294	279 (128-735)	185 (79-552)	305 (182-511)	0.124	18 (9-40)	14 (6-38)	22 (12-40)	0.384	11 (3-24)	12 (8-19)	13 (10-18)	0.705
rs7879685 (*13) WW 40 WM+MM1	249 (94-734)	461		0.274	250 (79-735)	468		0.280	17 (6-40)	29		0.299	11 (3-24)	10		0.837
CYP2C9																
rs799853 (*2) WW 40 WM 1	254 (94-734)	201		0.678	255 (79-735)	203		0.693	17 (6-40)	12		0.472	11 (3-24)	8		0.529
rs7900194 (*8) WW 39 WM 2	253 (94-734)	245 (135-445)		0.936	254 (79-735)	246(136-446)		0.940	40 (6-40)	16(10-27)		0.886	11 (5-24)	6(3-10)		0.031
rs2256871 (*9) WW 37 WM 4	255 (94-734)	232(102-580)		0.748	256 (79-735)	234(102-585)		0.764	18 (6-40)	15 (7-40)		0.477	11 (3-24)	10 (5-24)		0.618
rs28371685 (*11) WW 38 WM 3	263 (94-734)	160(102-227)		0.136	263 (79-735)	161 (102-228)		0.153	18 (6-40)	11 (7-20)		0.101	11 (3-24)	12(8-24)		0.850

* Statistical analyses were performed on log-transformed variables by analysis of variance (where there were sufficient numbers in MM group) and t-test (where numbers in MM were insufficient and therefore merged with WM to give WM+MM).

[‡]The three genotypic groups are: homozygous for the common allele (WW); heterozygotes (WM); homozygous variant (MM). AUC: Area under the curve; C_{max}: Maximum plasma concentration; t_{max}: Time to achieve maximum plasma concentration.