The Impact of *CYP2C9*11* Allelic Variant on the Pharmacokinetics of Phenytoin and (*S*)-Warfarin

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Cytochrome P450 2C9 (CYP2C9) is responsible for the oxidative metabolism of about 15% of commonly used drugs, some of which are characterized by a narrow therapeutic window. *CYP2C9* is highly polymorphic, and over 60 alleles have been described. *CYP2C9*2* and *CYP2C9*3* are the most common polymorphisms among White patients and both are associated with decreased activity. The evidence concerning the functional importance of less frequent variant alleles is scarce. The objective of the current study was to characterize the *in vivo* activity of CYP2C9 among carriers of *CYP2C9*11*, one of the "African" alleles and the fourth most common *CYP2C9* variant allele among White patients by using two prototype substrates, phenytoin and (*S*)-warfarin. Single 300-mg phenytoin and 20-mg warfarin doses were given to 150 healthy Ethiopian Jewish participants who were nonsmokers, at least one week apart. (*S*)-warfarin oral clearance and phenytoin metabolic ratio (PMR) derived from the ratio of 5-(4-hydroxyphenyl)-5-phenylhydantoin in 24-hour urine collection to plasma phenytoin 12 hours (PMR 24/12) or 24 hours (PMR 24/24) post dosing, were used as markers of CYP2C9 activity. PMR 24/12 and PMR 24/24 were reduced by 50% and 62.2%, respectively, among carriers of *CYP2C9*1/*11* (n = 13) as compared with carriers of *CYP2C9*1/*1* (n = 127) (false discovery rate (FDR) q < 0.001). The respective decrease in (*S*)-warfarin oral clearance was 52.6% (FDR q < 0.001). In conclusion, the enzyme encoded by *CYP2C9*11* is characterized by a more than 50% decrease in the enzymatic activity, resembling the extent of decrease associated with *CYP2C9*3* ("no-function allele"). Among patients of African ancestry, *CYP2C9*11* genetic analysis should be considered prior to prescribing of narrow therapeutic window drugs such as phenytoin, warfarin, nonsteroidal anti-inflammatory drugs, or siponimod.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Most of the evidence relating to *CYP2C9* genetic variants and altered pharmacokinetics of cytochrome P450 2C9 (CYP2C9) substrates is focused on *CYP2C9*2* and *CYP2C9*3*, the most common variant alleles among White patients. The functional importance of less frequent alleles, such as *CYP2C9*11*, which is more common among African populations, is not fully defined.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The study evaluated the magnitude of decrease in CYP2C9 activity among carriers of *CYP2C9*11* by using two prototype substrates of CYP2C9, phenytoin and (*S*)-warfarin.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Phenytoin metabolism as evaluated by phenytoin metabolic ratio and the oral clearance of (*S*)-warfarin were reduced by more than 50% among carriers of *CYP2C9*1/*11* as compared with carriers of *CYP2C9*1/*1* genotypes. These findings imply that *CYP2C9*11* is associated with significant reduction in CYP2C9 activity resembling the decrease associated with the *CYP2C9*3* allele.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Prior to initiation of therapy with CYP2C9 substrates characterized by a narrow therapeutic window, genetic analysis of *CYP2C9*11* should be considered, especially among African populations.

Cytochrome P450 2C9 (CYP2C9) is the most common isoform of the CYP2C subfamily accounting for about 20% of cytochrome P450 content in the liver. It is involved in the oxidative metabolism of ~15% of commonly used drugs, some of which are characterized by a narrow therapeutic window such as warfarin, phenytoin, and glimepiride.[1,2] *CYP2C9* is a highly polymorphic

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pharmacogene, and currently over 60 variant alleles have been described, many of which encode for enzyme with decreased catalytic activity.[3] Carriage of mutated alleles may presumably lead to elevated plasma concentration and enhanced frequency of adverse effect. Prominent examples are hemorrhage due to warfarin and neurotoxicity secondary to treatment with phenytoin.[4–7]

The frequency of *CYP2C9* variant alleles varies greatly between populations. Among White patients, *CYP2C9*2* and *CYP2C9*3* are the most prevalent variant alleles with allele frequency of 12.4% and 7.3%, respectively.[2,8] Most of the evidence relating to *CYP2C9* genetic variants and altered response to CYP2C9 substrates is focused on these relatively common alleles. The evidence concerning the functional importance of less frequent variant alleles is scarce and commonly based on *in vitro* data, anecdotal case reports, or altered pharmacokinetics of CYP2C9 substrate in a limited number of participants.

Together with *CYP2C9*5, CYP2C9*6*, and *CYP2C9*8, CYP2C9*11* is one of the "African" *CYP2C9* allelic variants which are commonly found among populations of African ancestry.[8,9] Based on the 1000 Genomes Project, the estimated average frequency of *CYP2C9*11* among Africans is 2.4%, ranging from <1% and up to 5.1% among Nigerians.[3] Although rarely found in White patients, it is the fourth most common allelic variants among White patients.[8] There is general agreement in the literature that *CYP2C9*11* encodes for enzyme with impaired catalytic activity. This is predominantly based on more than 10 *in vitro* studies demonstrating decreased intrinsic clearance ranging between 26% and 90% as compared with *CYP2C9*1/*1*.[10–19] Inconsistent findings were obtained in two studies that evaluated *in vivo* pharmacokinetic parameters of two different CYP2C9 substrates among carriers of *CYP2C9*1/*1* genotype as compared with a limited number of participants carrying the *CYP2C9*11* allele. In one study, the ratio of losartan to its CYP2C9-mediated metabolite (i.e., E3174) in 8-hour urine collection was not significantly different between carriers of the *CYP2C9*1/*1* genotype and three carriers of the *CYP2C9*1/*11* genotype.[20] In yet another study, phenytoin metabolic ratio (PMR) (the ratio of S-5-(4-hydroxyphenyl)-5-phenylhydantoin in 8-hour urine collection to mid-interval plasma phenytoin concentration) was significantly decreased among carriers of *CYP2C9*1/*11* ($n = 3$) or *CYP2C9*9/*11* ($n = 2$) as compared with *CYP2C9*1/*1* genotypes.[21] One additional approach to define the functional importance of rare *CYP2C9* genetic variants is to use a warfarin dose requirement as a surrogate marker of CYP2C9 activity. Four such studies have demonstrated up to a 40% decrease in warfarin dose requirement among carriers of *CYP2C9*11* as compared with *CYP2C9*1/*1* genotypes,[10,22–24] but in four additional studies no significant difference was found.[25–28] Due to the rarity of *CYP2C9*11* allele, the number of patients included in each of these studies was extremely small, ranging from one and up to five patients. Finally, a meta-analysis of seven studies among Black African participants revealed a modest 12% decrease in warfarin dose requirement among 35 patients carrying the *CYP2C9*1/*11* genotype as compared with 1474 patients carrying the *CYP2C9*1/*1* genotype.[29]

One approach that may be used to fill in the knowledge gap concerning the activity of rare alleles is to evaluate the pharmacokinetics of CYP2C9 substrates in a specific population with expected higher frequency of the rare allele. Thus, the purpose of the current study was to characterize the activity of *CYP2C9*11* by studying the pharmacokinetics of two model CYP2C9 substrates, phenytoin and warfarin, in a cohort consisting of Ethiopian Jewish participants.

METHODS

Study population

The study was conducted in the Clinical Pharmacology Unit which is affiliated with the Division of Medicine at the Hadassah-Hebrew University Medical Center. The current study is part of a large research project aimed at investigating genetic determinants of warfarin metabolism (NCT00162474). One section of this research project was designed to compare CYP2C9 *in vivo* activity among Ethiopian and non-Ethiopian Jewish participants residing in Israel.[30] Briefly, 150 Ethiopian Jewish participants were enrolled into the study. In order to be considered "Ethiopian", both the parents and the grandparents had to be born in Ethiopia prior to immigration to Israel. All participants were nonsmokers, in the age range of 18–50 years and in good health based on medical interview and complete physical examination. The presence of any chronic disease and the regular consumption of drugs including oral contraceptives and alcohol were considered to be exclusion criteria. The study protocol was approved by the Institutional Review Board of the Hadassah University Hospital, and all participants signed a consent form before enrollment.

Evaluation of CYP2C9 activity *in vivo*

The activity of CYP2C9 was evaluated in two successive stages at least 1 week apart using two model CYP2C9 substrates, phenytoin and warfarin. In the first stage, following an 8-hour fast, participants were administered at ~8:00 p.m. a single dose of 300-mg phenytoin (three capsules of Epanutin, Pfizer Ltd, 100 mg each) together with 250 mL of water. Fasting was continued for four additional hours post phenytoin intake. Immediately prior to phenytoin intake the participants were requested to empty their bladder and to start urine collection for the next 24 hours at two equal intervals, 12 hours each: 0–12 hours and 12–24 hours. The volume of each urine collection was measured and 20 mL aliquots were immediately stored at −20°C for the future analysis of 5-(4-hydroxyphenyl)-5-phenylhydantoin (p-HPPH). Two blood samples, 5 mL each were drawn 12 and 24 hours following phenytoin dosing. Plasma was separated and stored at −20°C for the measurement of plasma phenytoin concentration.

At least a week later and following an overnight fast, the participants received a single dose of 20 mg warfarin along with 200 mL of water. Fasting was continued for four additional hours post warfarin administration. Periodic blood samples were obtained through indwelling intravenous catheter immediately before and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14, 24, and through separate venipunctures 30, 36, 48, 54, 60, 72, 96, and 120 hours post warfarin administration. Plasma was immediately separated and kept frozen at −20°C until analysis. Additional plasma samples were obtained for the evaluation of international normalized ratio (INR) just prior to and at 12, 24, 36, 48, 60, 72, 96, and 120 hours post warfarin intake.

Analysis of p-HPPH in urine and plasma phenytoin

The concentration of plasma phenytoin and urinary p-HPPH were measured by two separate high-performance liquid chromatography (HPLC) methods developed in our laboratory.[30] It should be noted that only the production of (S)-p-HPPH is mediated by the activity of CYP2C9 whereas CYP2C19 mediates the production of (R)-p-HPPH.

However, as previously shown by our group, the formation clearance of (R)-p-HPPH is 30-fold lower as compared with the formation clearance of (S)-p-HPPH and the urinary excretion of (R)-p-HPPH accounts for less than 5% of total urine p-HPPH.[31] Thus, the *in vivo* activity of CYP2C9 can be reliably derived from the phenytoin metabolic ratio (PMR), defined as the molar ratio of urinary content of (total) p-HPPH excreted over 24 hours to mid-interval phenytoin plasma concentration (i.e., PMR 24/12) or to phenytoin plasma concentration 24 hours after dosing (i.e., PMR 24/24), normalized to the duration of urine collection, as published previously.[31]

Analysis of plasma (S)-warfarin and (R)-warfarin

The analysis of (*S*)-warfarin and (*R*)-warfarin enantiomers was performed using an HPLC method as described previously with some modifications.[32] The system consisted of e2695 Separation Module (Waters, Milford, MA) equipped with 2489 UV/visible detector (Waters). Separation was achieved using CHIRAL ART Cellulose-SC, Classical Analytical HPLC Column (4.6 mm Inner Diameter), S-5 µm, 250 × 4.6 mm (YMC America, Devens, MA). The limit of detection for both enantiomers was 30 ng/mL and the intraday and interday coefficient of variation was 1.7% and 3.4% for warfarin enantiomers concentration, respectively.

Genetic analysis

Genomic DNA was extracted from peripheral leukocytes using a traditional phenol-chloroform extraction procedure. Identification of *CYP2C9*2*, *CYP2C9*3*, *CYP2C9*5*, *CYP2C9*6*, *CYP2C9*8*, and *CYP2C9*11* was performed through three separate direct sequencing procedures (BGI group, Shenzhen, Guangdong, China) spanning exons 3 (*CYP2C9*2* & *CYP2C9**8), 5 (*CYP2C9*6*), and 7 (*CYP2C9*3*, *CYP2C9*5*, & *CYP2C9*11*). Participants not carrying any of the tested *CYP2C9* variant alleles were defined as carriers of the wild-type *CYP2C9*1/*1* genotype. To exclude the presence of rare allelic variants, the entire coding region of *CYP2C9* with the corresponding intronic boundaries was sequenced in carriers of the *CYP2C9*11* allele. The identification of VKORC1 haplotypes was based on the analysis of *rs9923231* (−1639G>A) using the high-resolution melting technique as published previously.[33]

Data analysis

Out of the 150 participants we identified five carriers of *CYP2C9*1/*2* and two carriers of *CYP2C9*1/*3* genotypes which were excluded from analysis. The remaining cohort consisted of 127 carriers of *CYP2C9*1/*1*, 13 carriers of *CYP2C9*1/*11*, and 3 carriers of *CYP2C9*2/*11* genotypes. No additional *CYP2C9* coding region genetic polymorphisms were identified in carriers of the *CYP2C9*11* variant allele. Phenytoin metabolic ratio was derived from the molar ratio of urinary excretion of p-HPPH over 24 hours to plasma phenytoin concentration 12 hours (i.e., PMR 24/12) or 24 hours (i.e., PMR 24/24) post dosing normalized to the duration of urine collection. The pharmacokinetics of (*R*)-warfarin and (*S*)-warfarin were evaluated by a noncompartmental method using Phenix WinNonlin software (Certara, Princeton, NJ). Elimination half-life was derived from the terminal portion of the plasma concentration-time curve. The area under the plasma concentration-time curve from time zero until 120 hours post dosing ($AUC_{0\to120}$) was calculated using the linear/log trapezoidal method and extrapolated to infinity ($AUC_{0\to\infty}$) by adding the ratio of last measured concentration (C_{120}) to elimination rate constant (K_{elim}). Oral clearance of warfarin enantiomers was derived from the ratio of warfarin dose to the respective $AUC_{0\to\infty}$ assuming complete bioavailability. Pharmacodynamic measurements were assessed by calculating the area under the INR-time curve until 120 hours ($INR_{0\to120}$). Demographic details are presented separately for each of the *CYP2C9* genotypes included in this study (**Table S1**). Comparison of pharmacokinetic parameters across the three genotype groups included in the present study was performed by the nonparametric Kruskal-Wallis statistical test due to the small number of participants carrying the *CYP2C9*2/*11* genotype. Comparison between carriers of *CYP2C9*1/*11* and *CYP2C9*2/*11* with carriers of the wild-type genotype (i.e., *CYP2C9*1/*1*) was performed using the Mann-Whitney *U* test. Statistical analysis was performed using the SPSS software package (IBM SPSS Statistics, version 23, Chicago, IL). *P* values were adjusted for multiple testing by using false discovery rate (FDR).[34] We first ranked the *P* values in ascending order and then calculated FDR *q* values by dividing each *P* value by its rank number and multiplying by the total number of comparisons.

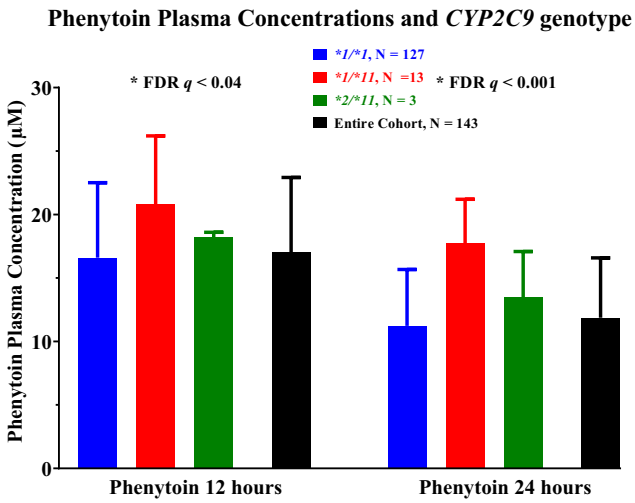


Figure 1 Phenytoin plasma concentration 12 and 24 hours post dosing in carriers of *CYP2C9*1/*1* (blue bars), *CYP2C9*1/*11* (red bars), *CYP2C9*2/*11* (green bars), and the entire cohort (black bars). *FDR *q* value represents the comparison between all three genotypes using the Kruskal-Wallis test adjusted for multiple testing. FDR, false discovery rate.

RESULTS

The demographic details of the participants carrying *CYP2C9*1/*1*, *CYP2C9*1/*11*, and *CYP2C9*2/*11* were comparable (**Table S1**). Plasma phenytoin concentration 12 and 24 hours post dosing varied significantly among carriers of different *CYP2C9* genotypes (**Figure 1**) (FDR $q < 0.04$ and FDR $q < 0.001$, respectively). Thus, plasma phenytoin concentrations 12 and 24 hours post dosing were higher by an average of 25.5% and 57.9% among carriers of *CYP2C9*1/*11* as compared with carriers of *CYP2C9*1/*1* genotypes (FDR $q < 0.016$ and FDR $q < 0.001$, respectively) (**Figure 1**). Total urinary excretion of p-HPPH over 24 hours was significantly correlated with the *CYP2C9* genotype (FDR $q < 0.001$) so that carriers of *CYP2C9*1/*11* and *CYP2C9*2/*11* excreted on average 31.2% and 57.7% less p-HPPH as compared with carriers of *CYP2C9*1/*1* genotypes (FDR $q < 0.002$ and FDR $q < 0.008$, respectively) (**Figure 2**). PMR was also significantly associated with the *CYP2C9* genotype (**Figure 3**). Thus, PMR 24/12 and PMR 24/24 were significantly reduced by an average of 50.0% and 62.2% among carriers of *CYP2C9*1/*11* as compared with carriers of *CYP2C9*1/*1* genotypes, respectively (FDR $q < 0.001$). The respective average decrease in PMR 24/12 and PMR 24/24 among carriers of *CYP2C9*2/*11* as compared with carriers of *CYP2C9*1/*1* was 66.4% and 68.8%, respectively (FDR $q < 0.01$).

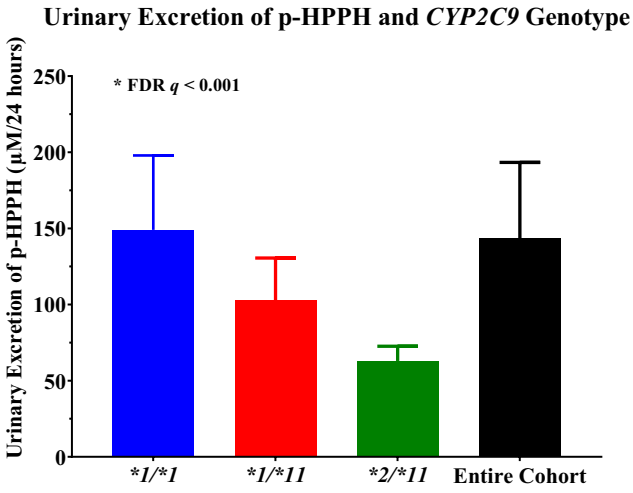


Figure 2 Urinary excretion of p-HPPH in 24-hour urine collection in carriers of *CYP2C9*1/*1* (blue bar), *CYP2C9*1/*11* (red bar), *CYP2C9*2/*11* (green bar), and the entire cohort (black bar). *FDR *q* value represents the comparison between all three genotypes using the Kruskal-Wallis test adjusted for multiple testing. FDR, false discovery rate; p-HPPH, 5-(4-hydroxyphenyl)-5-phenylhydantoin.

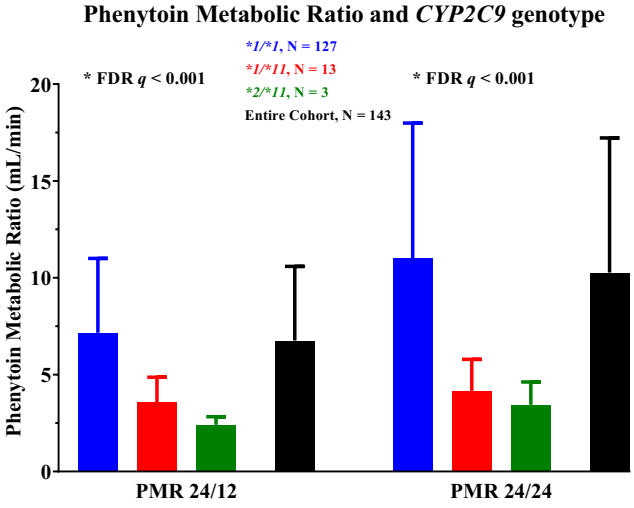
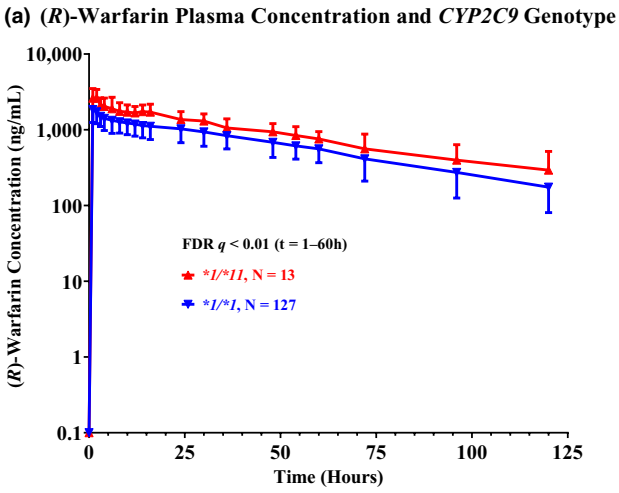


Figure 3 PMR 24/12 and PMR 24/24 in carriers of *CYP2C9*1/*1* (blue bars), *CYP2C9*1/*11* (red bars), *CYP2C9*2/*11* (green bars), and the entire cohort (black bars). *FDR *q* values represent the comparison between all three genotypes using the Kruskal-Wallis test adjusted for multiple testing. FDR, false discovery rate; PMR, phenytoin metabolic ratio.



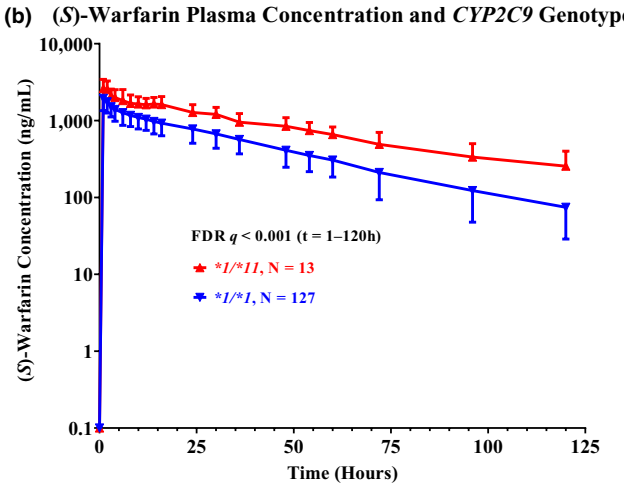


Figure 4 Plasma concentration of (**a**) (*R*)-Warfarin and (**b**) (S)-warfarin over time in carriers of *CYP2C9*1/*1* (blue down-pointing triangles), *CYP2C9*1/*11* (red up-pointing triangles). FDR *q* values represent the comparison between *CYP2C9*1/*1* and *CYP2C9*1/*11* using the Mann-Whitney *U* test adjusted for multiple testing. FDR, false discovery rate.

The plasma concentrations of (*S*)-warfarin and (*R*)-warfarin varied significantly among carriers of different *CYP2C9* genotypes during the entire study period for (*S*)-warfarin (i.e., up to 120 hours post dosing) and up to 60 hours post dosing for (*R*)-warfarin (Kruskal-Wallis, FDR $q < 0.001$ and FDR $q < 0.01$, respectively) (**Figure 4a,b**). The pharmacokinetic parameters of (*S*)-warfarin and (*R*)-warfarin are described in **Table S2**. The oral clearance of (*S*)-warfarin and its elimination half-life varied significantly across carriers of different *CYP2C9* genotypes (**Figure 5** and **Figure 6**) (FDR $q < 0.001$). As compared with carriers of the *CYP2C9*1/*1* genotype, the oral clearance of (*S*)-warfarin was reduced by an average of 52.6% and 55.4% among carriers of *CYP2C9*1/*11* and *CYP2C9*2/*11* genotypes, respectively (FDR $q < 0.001$ and FDR $q < 0.013$, respectively). In addition, (*S*)-warfarin elimination half-life was significantly longer by an average of 70.4% among carriers of *CYP2C9*1/*11* and by 99.0% among carriers of *CYP2C9*2/*11* as compared with carriers of the *CYP2C9*1/*1* genotype (FDR $q < 0.002$ and FDR $q < 0.008$ respectively). Interestingly, the oral clearance of (*R*)-warfarin was also significantly decreased by 33.7% among carriers of *CYP2C9*1/*11* as compared with carriers of *CYP2C9*1/*1* genotypes (FDR $q < 0.004$).

The extent of anticoagulation varied between carriers of the three different *CYP2C9* genotypes included in this analysis. Thus, INR values from 36 hours and until 96 hours post dosing, the area under the INR-time curve ($INR_{0\rightarrow120}$) and maximal measured INR value (INR_{MAX}) correlated with *CYP2C9* genotype (Kruskal-Wallis, FDR $q < 0.02$, FDR $q < 0.04$, FDR $q < 0.05$, respectively) (**Figure 7**). The area under the INR-time curve and

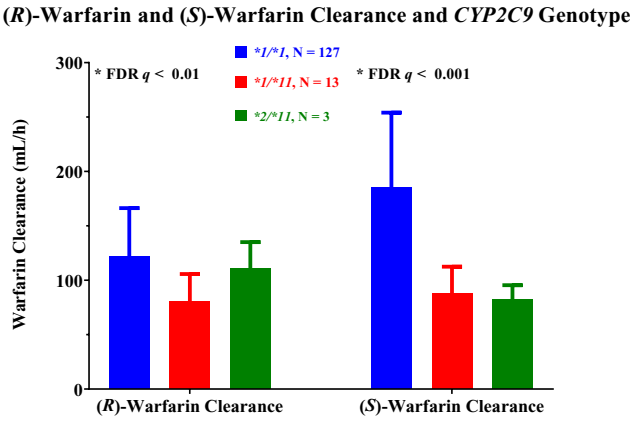


Figure 5 (*R*)-warfarin and (S)-warfarin oral clearance in carriers of *CYP2C9*1/*1* (blue bars), *CYP2C9*1/*11* (red bars), and *CYP2C9*2/*11* (green bars). *FDR *q* values represent the comparison between all three genotypes using the Kruskal-Wallis test adjusted for multiple testing. FDR, false discovery rate.

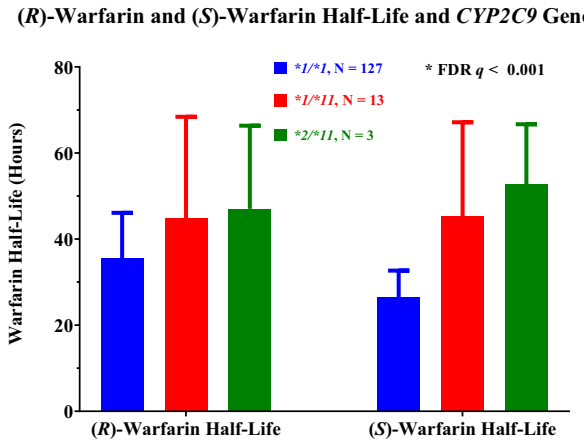


Figure 6 (*R*)-warfarin and (S)-warfarin elimination half-life in carriers of *CYP2C9*1/*1* (blue bars), *CYP2C9*1/*11* (red bars), and *CYP2C9*2/*11* (green bars). *FDR *q* value represents the comparison between all three genotypes using the Kruskal-Wallis test adjusted for multiple testing. FDR, false discovery rate.

the maximal measured INR value (INR_{MAX}) were significantly increased by an average of 12.6% and 16.5% among carriers of *CYP2C9*1/*11* as compared with the carriers of *CYP2C9*1/*1* genotypes, respectively ($INR_{0\to120}$: 189.9 vs. 168.7 hours, respectively, FDR $q < 0.015$; INR_{MAX}: 2.12 vs. 1.82, respectively, FDR $q < 0.015$). The distribution of *VKORC1* genotypes among carriers of *CYP2C9*1/*1* and *CYP2C9*1/*11* was similar (**Table S3**).

DISCUSSION

The systemic exposure of drugs metabolized predominantly by CYP2C9 has been shown to vary among carriers of variant alleles.[35,36] Such differences may be of clinical importance, especially for drugs with narrow therapeutic index such as warfarin, phenytoin, and nonsteroidal anti-inflammatory drugs. The data comparing pharmacokinetics and pharmacodynamics of CYP2C9 substrates between carriers of the wild-type genotype and those

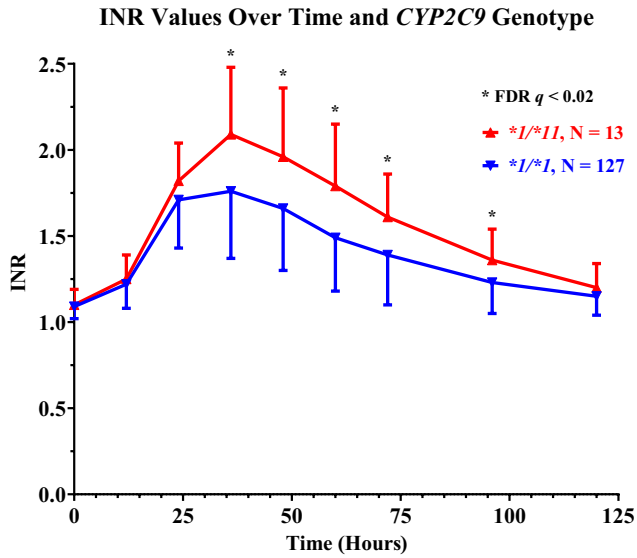


Figure 7 INR values over time in carriers of *CYP2C9*1/*1* (blue down-pointing triangles) and *CYP2C9*1/*11* (red up-pointing triangles). *FDR *q* value represents the comparison between *CYP2C9*1/*1* and *CYP2C9*1/*11* using the Mann-Whitney *U* test adjusted for multiple testing. FDR, false discovery rate; INR, international normalized ratio.

carrying mutated alleles is derived mainly from studies conducted among White patients where *CYP2C9*2* and *CYP2C9*3* are the two most prevalent variant alleles. On the other hand, the data regarding the impact of non-*CYP2C9*2* or non-*CYP2C9*3* variant alleles on the metabolism and pharmacological effect of CYP2C9 substrates is often limited or even nonexistent.

*CYP2C9*11* genetic polymorphism contains substitution of arginine for tryptophan at position 335, a highly conserved region. Previous *in vitro* studies have shown that the change in amino acid composition is associated with decreased thermal stability, which translates into a shorter half-life and decreased metabolic activity of the mutated protein.[10,37] Although *CYP2C9*11* is the fourth most common *CYP2C9* variant allele in White patients, it is a rare allele with estimated allele frequency of 0.0016 and 0.0028 among European and American White patients, respectively, and therefore *in vivo* data are scarce.[2,3] The findings in the present *in vivo* study imply that *CYP2C9*11* is associated with significant reduction in the metabolic capacity toward two prototype substrates of CYP2C9, phenytoin and S-warfarin. Specifically, PMR and the oral clearance of S-warfarin were reduced by more than 50% among carriers of the *CYP2C9*1/*11* genotype as compared with carriers of the wild type genotype.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published in recent years several guidelines with evidence-based recommendations for dosage adjustment of CYP2C9 substrates in participants carrying *CYP2C9* variants alleles.[38–40] Each *CYP2C9* allele is assigned an activity value ranging from 0 for "no activity" to 1, which represents "normal activity" (*CYP2C9*1*), whereas alleles with reduced activity are assigned an activity value of 0.5. The allele activity values are summed to calculate the Activity Score (AS) for each diplotype which is translated into the phenotype classification system

consisting of poor metabolizers (AS equal 0 or 0.5), intermediate metabolizer (AS equal 1 or 1.5), and normal metabolizer (AS equal 2). Using this paradigm, *CYP2C9*3* is assigned a nonfunctional status and (activity value of 0), whereas *CYP2C9*11* is considered a reduced activity allele with activity value of 0.5.[3] Thus, carriers of the *CYP2C9*1/*3* genotype are advised to lower the phenytoin maintenance dose by 25%, whereas no dosage adjustment is recommended for carriers of the *CYP2C9*1/*11* genotype.[39] Subtle difference in the recommended starting doses of nonsteroidal anti-inflammatory drugs between participants carrying *CYP2C9*1/*3* and *CYP2C9*1/*11* is also noted where it is recommended that members of the former group use the lowest recommended starting dose, whereas the guidelines suggest using the recommended starting dose in the latter group.[38] These recommendations are misaligned with the findings in the present study, suggesting that the magnitude of decrease in the enzymatic activity of CYP2C9*11 is comparable to the estimated decrease noted in relation to CYP2C9*3 and probably reflects the lack of reliable pharmacokinetic data of CYP2C9 substrates in participants carrying the *CYP2C9*11* allele at the time these guidelines were constructed.

During the last two decades intensive research efforts have been made to construct an algorithm that could reliably predict warfarin dose requirement based on both clinical and pharmacogenetic information.[8] The International Warfarin Pharmacogenetic Consortium (IWPC) and "Gage" (WarfarinDosing.org) are the two most widely used algorithms.[41,42] Gage and IWPC algorithms could explain a significant portion of the variability in warfarin dose requirement among patients of European ancestry (57% and 45%, respectively) but their success in patients of African origin is substantially less (31% and 26% of the variability, respectively).[8] The genetic component in IWPC algorithm consists of *CYP2C9* and *VKORC1* polymorphisms, whereas in the Gage algorithm *GGCX* (gamma-glutamyl carboxylase) and *CYP4F2* polymorphisms are also taken into account. However, the presence of genetic polymorphisms that are specific for African populations (i.e., *CYP2C9*5, CYP2C9*6, CYP2C9*8, CYP2C9*11*, and the *CYP2C* cluster gene polymorphism *rs12777823*) is not taken into account except for *CYP2C9*5* and *CYP2C9*6,* which are considered by the Gage algorithm. The significant decrease noted in the present study in the oral clearance of (*S*)-warfarin in patients carrying the *CYP2C9*1/*11* genotype advocates the incorporation of *CYP2C9*11* in the warfarin dosing algorithm especially if intended to be used in populations of African ancestry. Indeed, the inclusion of *CYP2C9* African single-nucleotide variants improved in one study the ability to explain variability in warfarin dose requirement from 30% to 36%.[43] It should be noted that despite the rapid spread of direct oral anticoagulants that have become the dominant anticoagulant in the Western world, warfarin is still highly used in less resourceful regions such as Africa and among patients with specific indications.[29] Furthermore, a recent survey done in the United Kingdom revealed that warfarin accounts for 26% of prescribed anticoagulation medications in 2019.[44]

One of the most recent drugs added to the divergent list of CYP2C9 substrates is siponimod, a sphingosine-1-phosphate receptor modulator indicated for the treatment of relapsing forms of multiple sclerosis.[45] Carriers of a single or two *CYP2C9*3* allele(s) exhibit reduced oral clearance of siponimod resulting in a 61% and 285% increase in the area under the plasma concentration-time curve as compared with carriers of the *CYP2C9*1/*1* genotype.[46] *CYP2C9* genotyping is mandatory prior to the initiation of siponimod treatment and carriers of *CYP2C9*2/*3* or *CYP2C9*1/*3* are administered a modified titration regimen and 50% reduced maintenance dose.[47] Furthermore, siponimod is contraindicated in homozygotes for the *CYP2C9*3* allele. The possibility that similar dosage adjustments may be required for carriers of non-*CYP2C9*3* alleles including *CYP2C9*11* is not included in siponimod labeling. The findings in the present study suggest that carriers of *CYP2C9*11* may be at increased risk to experience siponimod-associated adverse effects (i.e., bradycardia, risk of infection, and elevated liver enzymes) if treated with the standard dose. This is of clinical importance especially if one takes into account the fact that the incidence of multiple sclerosis is highest among African American patients as compared with several other ethnic groups, including Hispanic, Asian, and White patients.[48,49]

Our study has several limitations. The decrease in phenytoin and (*S*)-warfarin metabolism was noted after a single-dose administration. It is possible that during chronic administration other non-CYP2C9 isoforms may take a more significant part in the clearance of both drugs, compensating for the decreased activity of CYP2C9 among carriers of the variant allele. However, prediction of (*S*)-warfarin pharmacokinetics is most helpful clinically during the initial phase of treatment when the individual's response to warfarin is unknown. Furthermore, we have previously shown that among patients on chronic warfarin therapy, (*S*)-warfarin oral clearance is reduced on average by 27% and 76% in carriers of a single or two variant alleles respectively, implying that CYP2C9 genetic polymorphism remains a significant determinant of (*S*)-warfarin pharmacokinetics during chronic administration.[50] Similar findings were noted in another study involving patients of different ethnic backgrounds, including African American patients.[51]

Our findings suggest that following a single dose of warfarin the extent of anticoagulation is significantly greater among carriers of *CYP2C9*1/*11* as compared with carriers of *CYP2C9*1/*1*. However, CYP2C9 genetic polymorphisms are known to account for a small fraction of the variability in the anticoagulant effect, whereas the contribution of *VKORC1* genetic polymorphism is significantly more pronounced even among patients of African ethnic origin.[8] Chance imbalance in the distribution of VKORC1 −1639G>A (rs9923231) between carriers of *CYP2C9*1/*1* and *CYP2C9*1/*11* genotypes could have introduced a bias in the extent of anticoagulation. However, genetic analysis of this polymorphism revealed that the distribution of different genotypes of VKORC1 −1639G>A polymorphism was similar (**Table S3**). In addition, the findings in the present study were obtained in a cohort consisting of Ethiopian Jewish participants. African populations are genetically heterogenous and therefore extrapolation to other non-Ethiopian African ethnic groups cannot be simply performed without additional research. Finally, the deleterious effect of specific genetic polymorphisms on CYP2C9 activity may be substrate specific and therefore caution should be exercised when applying the findings in the present study to other CYP2C9

substrates. The proportion of females was higher among carriers of the *CYP2C9*1/*1* genotype as compared with carriers of *CYP2C9*11* alleles (**Table S1**). Such imbalance is almost inevitable in studies concerning participants carrying rare alleles since matching is impossible. Previous studies have failed to demonstrate significant difference in CYP2C9 activity between males and females.[52] No difference was noted in phenytoin plasma concentration 12 and 24 hours post dosing between males and females. The total amount of p-HPPH excreted over 24 hours, PMR 24/12, and PMR 24/24 were 15%, 15%, and 22% lower in females as compared with males but no significant differences were noted in (*S*)-warfarin or (*R*)-warfarin oral clearance and elimination half-life. The reason for the modest decrease in phenytoin metabolism noted in females is currently unclear. It is well known that oral contraceptives inhibit CYP2C9,[53] but the use of oral contraceptive was an exclusion criterion in the current study. The fact that S-warfarin metabolism was not reduced among females raises the possibility of chance finding. Finally, enrollment of a higher proportion of females into the *CYP2C91/*1* group might have attenuated the difference at least regarding phenytoin metabolism between carriers of the *CYP2C9*1/*1* genotype and those carrying the *CYP2C9*11* variant allele.

The strength of the present study is derived from the fact that it was done under controlled conditions among healthy participants not treated by any medications on a chronic basis. In addition, the fact that the extent of decrease in CYP2C9 activity was similar for two different CYP2C9 substrates further supports the validity of our findings.

In conclusion, the presence of *CYP2C9*11* genetic polymorphism is associated with a more than 50% reduction in the *in vivo* metabolic activity of CYP2C9 toward two prototype substrates of CYP2C9, phenytoin and (*S*)-warfarin. The administration of the "normal" therapeutic doses of these drugs and possibly other CYP2C9 substrates to carriers of the *CYP2C9*11* variant allele may results in clinically significant toxicity. Failure to account for African *CYP2C9*-specific alleles resulted in overestimation of the warfarin recommended dose among African American patients treated by pharmacogenetic algorithm in the Clarification of Optimal Anticoagulation through Genetics (COAG) study.[54] Genetic analysis aimed at identifying carriers of this variant allele prior to initiation of CYP2C9 substrates characterized by a narrow therapeutic window is warranted, especially among African populations.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICTS OF INTEREST

The authors declared no competing interests for this work.

AUTHORS CONTRIBUTIONS

M.W., C.S., Z.A.G., S.A., and Y.C. wrote the manuscript. M.W. and Y.C. designed the research. M.W., C.S., Z.A.G., and S.A. performed the research. M.W. and C.S. analyzed the data.

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