









# Warfarin-Rifampin-Gene (WARIF-G) Interaction: A Retrospective, Genetic, Case–Control Study

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Warfarin is extensively metabolized by cytochrome P450 2C9 (CYP2C9). Concomitant use with the potent CYP2C9 inducer, rifampin, requires close monitoring and dosage adjustments. Although, in theory, warfarin dose increase should overcome this interaction, most reported cases over the last 50 years have not responded even to high warfarin doses, but some have responded to modest doses. To investigate the genetic polymorphisms' impact on this unexplained interpatient variability, we performed genotyping of *CYP2C9*, *VKORC1*, and *CYP4F2* for warfarin and rifampin concomitant receivers from 2016 to 2022 at Hamad Medical Corporation, Doha, Qatar. We identified and included 36 patients: 22 responders and 14 nonresponders. Warfarin-responders were significantly more likely to have one or more warfarin-sensitizing *CYP2C9/VKORC1* alleles than nonresponders (odds ratio = 23.2, 95% confidence interval = 3.2–195.6;  $P = 0.0001$ ). The mean genetic-based pre-interaction calculated dose was significantly lower for responders than for nonresponders ( $P < 0.001$ ); and was negatively correlated with warfarin sensitivity index (WSI) ( $r = -0.58$ ;  $P = 0.0002$ ). The median percentage time in therapeutic range and mean WSI were significantly higher in the warfarin-sensitizing *CYP2C9/VKORC1* alleles carriers than noncarriers ( $P = 0.017$  and  $0.0004$ , respectively). Whereas the warfarin-sensitizing *CYP2C9/VKORC1* genotypes were associated with modest on-rifampin warfarin dose requirements, the noncarriers would have required more than double these doses to respond. Warfarin-sensitizing *CYP2C9/VKORC1* genotypes and low genetic-based warfarin calculated doses were associated with higher warfarin sensitivity and better anticoagulation quality in patients receiving rifampin concomitantly.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Warfarin is predominantly metabolized by CYP2C9, and its sensitivity is up to 50% dependent on multiple genetic variants, mainly *CYP2C9*, *VKORC1*, and *CYP4F2*. Rifampin has been repeatedly shown almost to eradicate the warfarin anticoagulant effect via strong CYP2C9 induction.

### WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Association of *CYP2C9/VKORC1* warfarin-sensitizing polymorphisms and genetic-based calculated doses with warfarin response and sensitivity in patients receiving rifampin concomitantly.

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ *CYP2C9/VKORC1* warfarin-sensitizing polymorphisms and low genetic-based pre-interaction calculated doses are

associated with increased likelihood of warfarin response at modest doses and higher sensitivity in patients receiving rifampin concomitantly.

### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ While using warfarin and rifampin concomitantly, the carriers of warfarin-sensitizing *CYP2C9/VKORC1* genotypes are anticipated to attain target international normalized ratio at modest daily doses. In contrast, the noncarriers may require more extensive dose escalations. Genotyping can facilitate the identification of potential responders to feasible doses, and guide prescribers to larger dose escalations for normal *CYP2C9/VKORC1* patients.

Whereas the use of direct oral anticoagulants is markedly increasing in consistence with the growing evidence and new guidelines,<sup>1–3</sup> vitamin K antagonists, primarily warfarin, remain

the anticoagulation of choice for patients with valvular atrial fibrillation,<sup>2</sup> antiphospholipid syndrome, mechanical valve replacement, and unusually sited venous thromboembolism.<sup>3,4</sup>

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Warfarin decreases the production of functionally active vitamin K-dependent clotting factors by inhibiting vitamin K epoxide reductase enzyme (VKOR), the rate-limiting catalyst for transforming vitamin K-epoxide to vitamin K.<sup>5</sup> It is a mixture of two active enantiomers, *R* and *S*-warfarin. The anticoagulant effect depends on the *S*-stereoisomer, which is five times more potent and extensively metabolized (90%) by cytochrome P450 2C9 (CYP2C9) to the inactive 7-hydroxywarfarin.<sup>5</sup> Because it is the major pharmacokinetic contributor to *S*-warfarin systemic exposure, CYP2C9 inhibitors and inducers have been reported to alter warfarin plasma concentration significantly, requiring extensive dose adjustments and frequent international normalized ratio (INR) monitoring to avoid bleeding or anticoagulation failure.<sup>6</sup>

Both acute and chronic infection-related inflammation have been associated with hypercoagulability status.<sup>7</sup> This may explain the higher prevalence of pulmonary embolism and deep vein thrombosis in patients with active tuberculosis and infective endocarditis than the general population.<sup>8,9</sup> Rifampin is a cornerstone treatment for tuberculosis, as well as coagulase-negative staphylococci prosthetic valve endocarditis.<sup>9,10</sup> The management of rifampin interactions with oral anticoagulants remains exceptionally challenging.<sup>11,12</sup> Rifampin is a potent inducer of multiple CYPs, including the metabolizers of oral anticoagulants, CYP2C9 and 3A4,<sup>5,13,14</sup> as well as P-gp and BCRP, which are responsible for their active excretion.<sup>11,13,14</sup> Because direct oral anticoagulant dosing regimens are fixed, and their effects cannot be objectively monitored, the concomitant use with rifampin is generally avoided due to the liable risk of thromboembolic events.<sup>11,12,15</sup> Contrastingly, warfarin's monitorable INR led to its repeated trials with rifampin.<sup>11,12</sup>

Rifampin binds to the CYP2C9 primary *de novo* synthesis regulatory nuclear receptor, pregnane X receptor, increasing its mRNA expression rate by up to six times.<sup>16</sup> That has repeatedly been shown to almost eradicate warfarin's effect, which needed extensive dose escalation in all reported cases and is commonly associated with the inability to preserve the therapeutic INR range.<sup>12</sup> During the 1970s to 1980s, studies have shown that rifampin reduced warfarin's area under the curve (AUC) and effect by up to 85%, and several reports showed a significant warfarin dose requirements increase.<sup>17–22</sup> After INR was adopted during the 1980s,<sup>23</sup> more than 30 cases have been reported.<sup>24–34</sup> Most could not attain target INR while on the combination despite extensive escalations up to 30 mg per day.<sup>24–30</sup> Interestingly, most patients who maintained goal INR responded to modest warfarin doses, around 10 mg,<sup>28,32–34</sup> implying unexplained variability.

One explanation for warfarin dose variability is genetic polymorphisms. Along with clinical factors, these genetic variants have been shown to predict warfarin dose requirements by up to 50%.<sup>5,35</sup> Those single nucleotide polymorphisms (SNPs) are mainly carried by the genes *CYP2C9*, vitamin K epoxide reductase enzyme complex subunit 1 (*VKORC1*), and *CYP4F2*.<sup>5</sup> For *CYP2C9*, the most studied allelic variants are *CYP2C9*\*2 (rs1799853) and \*3 (rs1057910), which result from missense mutations that lead to *CYP2C9* moderate to severe decreased function, diminished catalytic activity, and decreased warfarin dose requirements.<sup>5,35</sup>

*VKORC1* encodes for warfarin target, VKOR enzyme. Hence, the *VKORC1* decreased expression variant, *c.-1639G>A* (rs9923231), can amplify warfarin inhibition of vitamin k-dependent proteins production, increasing warfarin sensitivity.<sup>5</sup> Oppositely, *CYP4F2*\*3 (rs2108622), a missense variant of the gene coding for the primary liver vitamin K oxidase, CYP4F2, has been associated with a modest increase in warfarin dose needs (8–11%).<sup>5</sup> Since 2010, the US Food and Drug Administration (FDA) has introduced a genotype-based dosing table on the warfarin label, accounting for *CYP2C9*\*2 and \*3, as well as *VKORC1* genotypes.<sup>36</sup> Additionally, the Gage *et al.* algorithm, which is available at the website [www.warfarindosing.org](http://www.warfarindosing.org), and the International Warfarin Pharmacogenetics Consortium (IWPC) algorithm, are two of the most widely validated warfarin genetic dosing algorithms, which also account for multiple clinical factors, the use of amiodarone, and enzyme inducers. Gage's algorithm additionally adjusts for smoking, *CYP2C9* (\*5 and \*6), *CYP4F2*\*3, and gamma-glutamyl carboxylase genotypes, as well as azoles and sulfamethoxazole/trimethoprim use, but does not account for enzyme inducers.<sup>5,37</sup> Both algorithms are recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines.<sup>5</sup>

As a CYP2C9 inducer, rifampin can generate phenoconversion from a poor to a rapid metabolizing status.<sup>38–41</sup> Because almost all the previously reported warfarin-rifampin interaction cases had not been genotyped,<sup>24–34</sup> carrying warfarin-sensitizing *CYP2C9*/*VKORC1* genotypes might stand as a hidden explanation for adequate responses to modest warfarin doses while concomitantly used with rifampin; due to minimal baseline dose requirements, as shown in the only genotyped case reported by the present authors.<sup>34</sup>

Patients who require rifampin concurrently with anticoagulation are usually either anticoagulated with low molecular weight heparin or tried warfarin with sporadic adequate responses. As a result, patients require frequent INR monitoring and excessive dose escalations. Performing genetic testing for patients who have been on rifampin and warfarin may explain their interaction outcomes.

This study aimed to investigate the association of warfarin-sensitizing *CYP2C9*/*VKORC1* genotypes, as well as pre-interaction genetic-based calculated doses, with positive warfarin response, time in therapeutic range, and warfarin sensitivity in patients that had been concomitantly receiving rifampin.

## METHODS

### Data source and population

We performed a retrospective review of the electronic profiles of patients prescribed warfarin and rifampin in Hamad Medical Corporation, Doha, Qatar, starting from May 2016 to January 2022. Patients were included if they were 18 years old or above, had been receiving warfarin and rifampin concomitantly for 14 days or more, and were able to provide saliva samples for genotyping. Demographics, clinical data, warfarin and rifampin doses, start and end dates, documented administration or dispensing, anticoagulation clinic and infectious diseases notes, and INRs were collected. In addition, dates and doses of amiodarone, sulfamethoxazole/trimethoprim, statins, and azole antifungals were specifically recorded. Ethical approval for this study was provided by the Medical Research Committee (MRC) of Hamad Medical Corporation (HMC) (#MRC-01-22-016).

## Genotyping

A study investigator approached each eligible patient during routine pharmacist-led anticoagulation clinic visits or through phone calls, explained the research, and obtained a signed institutional review board approved informed consent for genetic analysis and publication. Subjects were asked to provide saliva samples using Oragene•DNA (OG-500) self-collection kit (DNA Genotek, Ontario, Canada). Coded samples were sent to Qatar University (QU) for genotyping. Each kit was kept overnight in a 50°C water bath. The prepIT•L2P standard protocol for the purification and extraction of DNA was used.<sup>42</sup> The purified DNA's quality and quantity were evaluated by Nanodrop 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Samples were then genotyped using Taqman assay for allelic discrimination. The assay was performed using the QuantStudio 5 Real-Time Polymerase Chain Reaction system for Human Identification, 96-well, 0.2 mL, desktop (Applied Biosystems, Waltham, MA) to detect the following SNPs: *CYP2C9* \*2 (rs1799853), \*3 (rs1057910), \*8 (rs7900194), and \*11 (rs28371685), as well as *VKORC1* c.-1639G>A (rs9923231), and *CYP4F2*\*3 (rs2108622).

## Study design

This is a retrospective, genetic, case-control study. We investigated the association of warfarin-sensitizing genotypes, defined as carrying at least one warfarin-sensitizing allele: *CYP2C9* \*2, \*3, \*8, or \*11, or one *A* allele of *VKORC1* c.-1639G>A, with warfarin response during rifampin concomitant use. Warfarin-responders (cases) were defined as patients who attained at least two therapeutic INRs at the same average warfarin dose without evidence of rapid decline to subtherapeutic levels. These 2 INR readings should be separated by at least 3 days and occurred after more than 14 days from rifampin initiation. *CYP4F2*\*3 association with response was also investigated. We also compared the mean genetic-based calculated daily doses, estimated by the Gage algorithm with an additional 30% dose reduction for each *CYP2C9* \*8 or \*11 allele,<sup>5</sup> in the warfarin-responders and nonresponders. Additionally, we compared the percentage time in therapeutic range (TTR), calculated by the Rosendaal method,<sup>43</sup> as well as the warfarin sensitivity index (WSI),<sup>44,45</sup> defined as the average INR divided by the corresponding maintenance dose or maximum-tried dose, in the carriers and the noncarriers of warfarin-sensitizing *CYP2C9/VKORC1* genotypes. In addition, we investigated the WSI correlation with the genetic-based calculated dose requirements. Finally, we estimated the warfarin on-rifampin dose requirements for all patients by dividing different average INR targets by the observed WSI.

## Statistical analysis

Descriptive statistics were used to summarize demographics, indications for warfarin and rifampin, doses, and other patient characteristics. Categorical data were expressed as frequencies (percentages). Continuous data, such as concomitant duration, time to reach target INR, doses, TTR, and WSI, were presented as mean ( $\pm$  standard deviation (SD)), or median (interquartile range (IQR)) as appropriate. The Hardy-Weinberg equilibrium was tested using the goodness-of-fit  $\chi^2$  test and minor allele frequencies (MAFs) were presented as frequencies (percentages). For the genotype association with target INR attainment;  $\chi^2$  test was used to compare warfarin-sensitizing *CYP2C9/VKORC1* genotypes distribution among the warfarin-responders vs. the nonresponders. Results were presented and reported in odds ratio (OR), associated 95% confidence interval (CI), and a 2-tailed *P* value < 0.05 was considered statistically significant. The same test was used for *CYP4F2*\*3 association with response. In addition, we compared the mean genetic-based calculated daily dose in warfarin responders and nonresponders via unpaired *t*-test. Last, mean TTR and WSI were compared in the carriers vs. noncarriers of the warfarin-sensitizing *CYP2C9/VKORC1* genotypes using unpaired *t*-test for data showing normal distribution, while Wilcoxon rank-sum test was used for data not normally distributed. Finally, the correlation between the mean genetic-based calculated daily dose and WSI

was tested by Pearson's correlation coefficient (*r*). All statistical analyses were conducted using the statistical package Stata version 17 software.

## RESULTS

### Patients' characteristics and response

Out of 122 patients who have been prescribed warfarin and rifampin during the study period, we identified 60 concomitant receivers for  $\geq 14$  days. From which, we excluded 24 patients; 14 traveled out of the country, 6 died before enrollment, and 4 refused to participate. A total of 36 patients, one of which was previously published by the same authors,<sup>34</sup> were identified as eligible and included in the study. Subjects' mean age was  $42 \pm 12$  years, and 5 of 36 (14%) were women. Warfarin-responders (cases) were 22, whereas the remaining 14 were identified as nonresponders (controls). The gender, mean age, weight, height, smoking status, liver function, warfarin indication, target INR, and concomitant medications were not statistically different between the two groups. The majority of races were Asian/Indian ( $n = 21$ ), White/Caucasian/Middle-Eastern ( $n = 12$ ), then African ( $n = 3$ ). Warfarin-responders reached therapeutic INR at a median (IQR) of 31 (14–55) days and had a significantly longer duration of concomitant warfarin-rifampin use than nonresponders, 98 (45–179) vs. 35 (25–78) days ( $P = 0.014$ ). The mean warfarin target-attaining daily dose in the responders group was significantly lower than the maximum-tried daily dose in the nonresponders,  $10.4 \pm 3.2$  mg vs.  $18.7 \pm 6.1$  mg (mean difference (MD) =  $-8.3$ , 95% CI =  $-11.5$  to  $-5.2$ ;  $P < 0.001$ ). The median TTR (IQR) and mean  $\pm$  SD WSI were significantly higher in the responders than the nonresponders (40 (30–49)% vs. 11 (0–17)%,  $P < 0.001$ ) and ( $0.25 \pm 0.12$  vs.  $0.09 \pm 0.04$ , MD = 0.16, 95% CI =  $0.09$ – $0.23$ ;  $P < 0.001$ ), respectively. Table 1 summarizes baseline demographic and clinical data.

As shown in Figure 1, among the nonresponders group, the mean estimated required on-rifampin warfarin daily dose, based on each patient's target INR divided by the observed WSI, was  $32.4 \pm 12.9$  mg, significantly higher than their maximum-tried dose of  $18.7 \pm 6.1$  mg (MD = 13.7, 95% CI = 5.9–21.5;  $P = 0.0013$ ). Whereas no significant difference was observed among the responders,  $11.8 \pm 4.1$  mg vs.  $10.4 \pm 3.2$  mg (MD = 1.4, 95% CI =  $-0.8$  to 3.6;  $P = 0.2137$ ).

### Genotypes and genetic-based dose association with warfarin response

None of the genotypes deviated from the Hardy-Weinberg equilibrium. For *CYP2C9*, a heterozygous \*2 allele was detected in 4 patients (MAF = 5.56%), \*3 in 5 patients (one of which was homozygous; MAF = 8.33%), and \*11 in 2 patients (MAF 2.78%). *CYP2C9*\*8 was not detected in any subject. For *VKORC1* c.-1639G>A, the *A* allele was detected in 12 heterozygous and 3 homozygous patients (MAF = 25%). *CYP4F2*\*3 variant was detected in 16 heterozygous and 4 homozygous patients (MAF = 33.33%). Genotypes distribution among responders and nonresponders and MAFs are presented in Table 2 and Table S1, respectively.

Out of the warfarin-responders group, 19 of 22 (86%) were carriers of one or more warfarin-sensitizing *CYP2C9/VKORC1* alleles, vs. 3/14 (21%) of the nonresponders (OR = 23.2, 95%

**Table 1** Demographics and clinical characteristics of concomitant warfarin and rifampin receivers between 2016 and 2022 (*n* = 36)

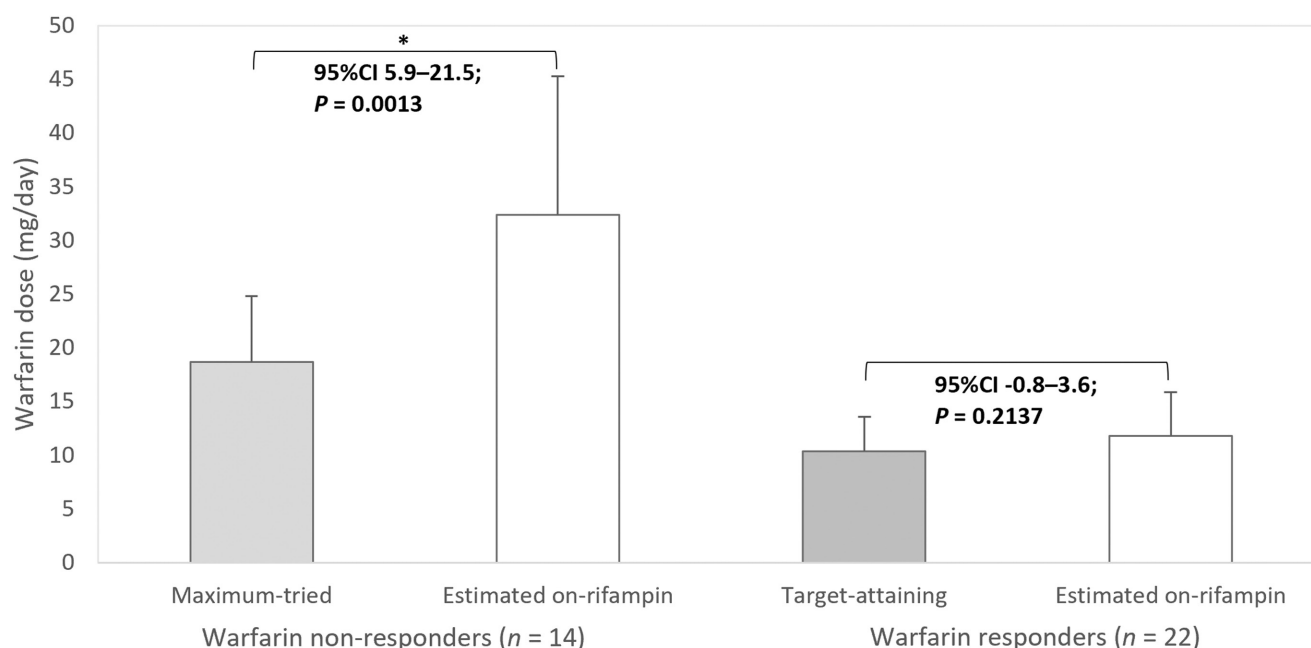
Parameters	Cases (warfarin-responders) ( <i>n</i> = 22)	Controls (warfarin nonresponders) ( <i>n</i> = 14)	<i>P</i> value
Gender, <i>n</i> (%)			0.35
Female	4 (18)	1 (7)	
Male	18 (82)	13 (93)	
Age (years), mean ± SD	42 ± 13	43 ± 11	0.94
Weight (kg), mean ± SD	64 ± 14	72 ± 17	0.18
Height (cm), mean ± SD	167 ± 9	168 ± 5	0.80
Race, <i>n</i> (%)			0.28
African/Black	3 (14)	0 (0)	
Asian/Indian subcontinent	13 (59)	8 (57)	
White/Caucasian/Middle-Eastern	6 (27)	6 (43)	
Smoking, <i>n</i> (%)	0 (0)	1 (7)	0.39
Liver disease, <sup>a</sup> <i>n</i> (%)	11 (50)	4 (29)	0.20
Warfarin indication, <i>n</i> (%)			0.39
Atrial fibrillation	4 (18)	1 (7)	
Deep vein thrombosis	7 (32)	2 (14)	
Heart failure/cardiomyopathy	0 (0)	1 (7)	
Heart valve replacement	6 (27)	4 (29)	
Pulmonary embolism	5 (23)	5 (36)	
Other	0 (0)	1 (7)	
Target INR, <i>n</i> (%)			0.96
2.0–3.0 (2.5)	19 (86)	12 (86)	
2.5–3.5 (3.0)	3 (14)	2 (14)	
Rifampin indication, <i>n</i> (%)			0.057
Active tuberculosis	18 (82)	7 (50)	
Inactive tuberculosis	1 (5)	0 (0)	
Infective endocarditis	3 (14)	4 (29)	
Staphylococcal infection	0 (0)	2 (14)	
Other	0 (0)	1 (7)	
Warfarin initiation sequence, <i>n</i> (%)			0.50
Initiated after or at the same time with rifampin	15 (68)	8 (57)	
Stabilized on warfarin before rifampin initiation	7 (32)	6 (43)	
Concomitant duration (days), median (IQR)	98 (45–179)	35 (25–78)	0.014
Number of INR checks during follow-up, median (IQR)	20 (10–43)	16 (13–21)	0.54
Statins, <i>n</i> (%)			0.49
Atorvastatin	2 (9)	3 (21)	
Rosuvastatin	4 (18)	1 (7)	
Amiodarone, <i>n</i> (%)	4 (18)	1 (7)	0.63
Sulfamethoxazole/trimethoprim, <i>n</i> (%)	1 (5)	0 (0)	1.00
Time to reach target INR (days), median (IQR)	31 (14–55)	Never reached	–
TTR <sup>b</sup> (%), median (IQR)	40 (30–49)	11 (0–17)	<0.001
Target-attaining <sup>c</sup> or maximum-tried <sup>d</sup> warfarin dose (mg/day), mean ± SD	10.4 ± 3.2	18.7 ± 6.1	<0.001
WSI, <sup>e</sup> mean ± SD	0.25 ± 0.12	0.09 ± 0.04	<0.001

INR, international normalized ratio; IQR, interquartile range; TTR, time in therapeutic range; WSI, warfarin sensitivity index.

Means were compared via unpaired *t*-test; medians via Wilcoxon rank-sum test; and categorical variables via chi-square or Fisher's exact as appropriate.

<sup>a</sup>Elevated (2-folds) liver enzymes and/or albumin <3.6 g/dL. <sup>b</sup>Rosendaal method. <sup>c</sup>Warfarin-responders. <sup>d</sup>Warfarin nonresponders. <sup>e</sup>Average INR/target-attaining or maximum-tried dose.





**Figure 1** Maximum-trying and target-attaining dose vs. estimated on-rifampin dose comparison among warfarin nonresponders and responders, respectively. Estimated on-rifampin dose was calculated as: target international normalized ratio (INR; 2.5 or 3.0)/warfarin sensitivity index (WSI). Bars present mean  $\pm$  SD and unpaired *t*-test was used to compare the means. \*Represents *P* value < 0.05. CI, confidence interval.

CI = 3.2–195.6; *P* = 0.0001) which indicates a significant association of warfarin-sensitizing *CYP2C9/VKORC1* genotypes with increased warfarin response during concomitant rifampin use.

On the other hand, *CYP4F2\*3* distribution was not significantly different among the warfarin-responders and nonresponders groups (OR = 0.56, 95% CI = 0.11–2.65; *P* = 0.4). In the non-warfarin-sensitizing *CYP2C9/VKORC1* carriers subgroup, *CYP4F2\*3*

**Table 2** Genotypes distribution for warfarin-responders vs. nonresponders

Parameters	Genotypes			Cases (warfarin-responders) (n = 22), n (%)	Controls (warfarin nonresponders) (n = 14), n (%)	Total (n = 36), n (%)
	<i>CYP2C9</i>	<i>VKORC1</i>	<i>CYP4F2</i>			
Noncarriers of warfarin-sensitizing <i>CYP2C9/VKORC1</i> alleles (n = 14)	*1/*1	G/G	C/C	2 (9)	4 (29)	6 (17)
	*1/*1	G/G	C/T	1 (5)	5 (36)	6 (17)
	*1/*1	G/G	T/T	0 (0)	2 (14)	2 (6)
Carriers of $\geq 1$ warfarin-sensitizing <i>CYP2C9/VKORC1</i> alleles (n = 22)	*1/*1	G/A	C/C	3 (14)	0 (0)	3 (8)
	*1/*1	G/A	C/T	4 (18)	1 (7)	5 (14)
	*1/*1	G/A	T/T	1 (5)	0 (0)	1 (3)
	*1/*1	A/A	C/C	1 (5)	0 (0)	1 (3)
	*1/*1	A/A	C/T	2 (9)	0 (0)	2 (6)
	*1/*2	G/G	C/T	1 (5)	0 (0)	1 (3)
	*1/*2	G/G	T/T	0 (0)	1 (7)	1 (3)
	*1/*2	G/A	C/T	1 (5)	0 (0)	1 (3)
	*1/*3	G/G	C/C	3 (14)	1 (7)	4 (11)
	*1/*11	G/G	C/C	1 (5)	0 (0)	1 (3)
	*2/*11	G/A	C/T	1 (5)	0 (0)	1 (3)
	*3/*3	G/A	C/C	1 (5)	0 (0)	1 (3)
Total				19 (86)	3 (21)	OR <sup>a</sup> = 23.2, 95% CI = 3.2–195.6; <i>P</i> = 0.0001

CI, confidence interval; INR, international normalized ratio; OR, odds ratio.

<sup>a</sup>The odds of carrying  $\geq 1$  warfarin-sensitizing *CYP2C9/VKORC1* alleles in the warfarin-responders vs. the nonresponders group; tested using chi-square test.

prevalence was numerically lower in warfarin-responders 1 of 3 (33%) than nonresponders 7 of 11 (64%) but did not reach statistical significance (OR = 0.29, 95% CI = 0.004–7.86;  $P = 0.35$ ).

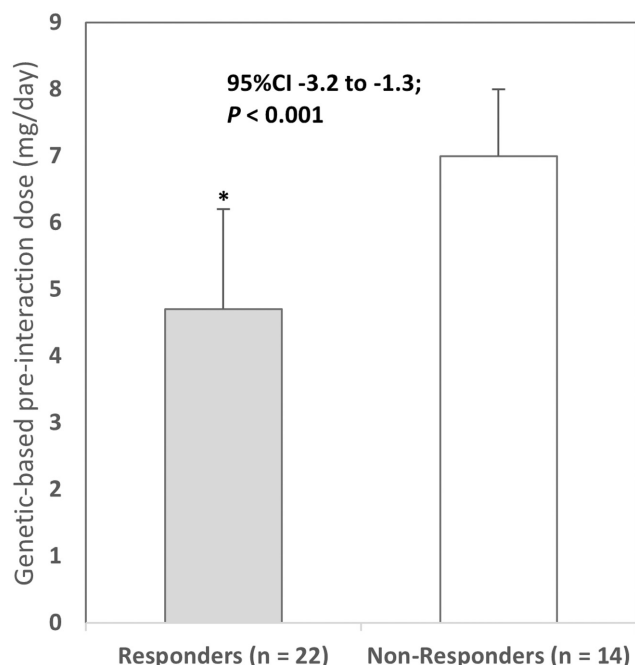
Warfarin-responders had significantly lower mean genetic-based pre-interaction dose than the non-responders,  $4.7 \pm 1.5$  vs.  $7 \pm 1$  mg (MD = -2.3, 95% CI = -3.2 to -1.3;  $P < 0.001$ ; **Figure 2**). The actual median (IQR) dose increase from the pre-interaction genetic-based dose was not different among the responders and nonresponders, 121% (80–189) vs. 151% (82–227;  $P = 0.3$ ).

### Warfarin-sensitizing *CYP2C9/VKORC1* genotypes and genetic-based dose association with TTR and warfarin sensitivity index

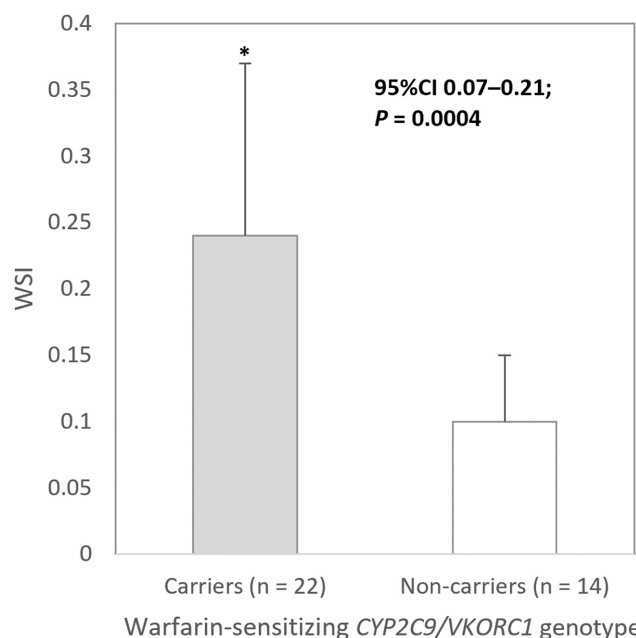
The carriers of warfarin-sensitizing *CYP2C9/VKORC1* genotypes had a significantly higher median TTR than non-carriers (36.3 (23.8–46.2) vs. 11.5 (0–20)%,  $P = 0.017$ ; **Figure S1**). The mean WSI was also significantly higher in the carriers of warfarin-sensitizing *CYP2C9/VKORC1* genotypes than the non-carriers ( $0.24 \pm 0.13$  vs.  $0.1 \pm 0.05$ , MD = 0.14, 95% CI = 0.07–0.21;  $P = 0.0004$ ; **Figure 3**). The genetic-based calculated dose showed a significant negative correlation with WSI ( $r = -0.58$ ;  $P = 0.0002$ ; **Figure 4**). The mean estimated on-rifampin warfarin dose requirements for different INR targets for warfarin-sensitizing *CYP2C9/VKORC1* genotypes carriers and noncarriers are presented in **Table 3**.

### DISCUSSION

In this genetic case-control drug interaction study, we explored the association of the warfarin-sensitizing *CYP2C9/VKORC1*



**Figure 2** Genetic-based pre-interaction warfarin dose among warfarin-responders and nonresponders. Bars present mean  $\pm$  SD and unpaired  $t$ -test was used to compare between the two groups. \*Represents  $P$  value  $< 0.05$ . CI, confidence interval.

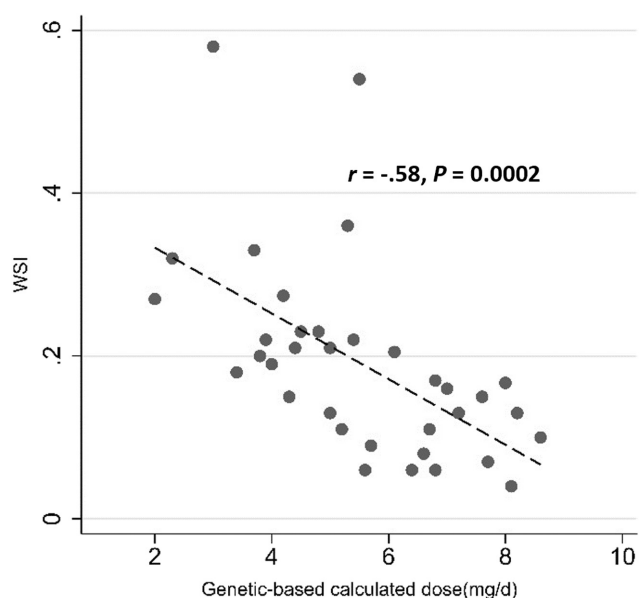


**Figure 3** Warfarin sensitivity index (WSI) in *CYP2C9/VKORC1* warfarin-sensitizing genotypes carriers and noncarriers. Bars present mean  $\pm$  SD and unpaired  $t$ -test was used to compare between the two groups. \*Represents  $P$  value  $< 0.05$ . CI, confidence interval.

genotypes with warfarin response in patients receiving rifampin concomitantly. Our findings showed that most warfarin-responders carry at least one warfarin-sensitizing *CYP2C9/VKORC1* allele, compared with warfarin nonresponders; indicating that those genotypes were associated with higher likelihood of attaining target INR during concomitant rifampin use.

Although warfarin-rifampin interaction has been repeatedly described over the last 50 years, the wide interpatient variable responses to the interaction remained unexplained. The majority of reported cases failing to reach the target INR had been tried on extensive warfarin daily doses up to 30 mg.<sup>24–27,29,30</sup> Inversely, most reported responders attained the target on modest dosing levels of 10 to 15 mg.<sup>30,31,33,34</sup> In a case series in Western Kenya, 5 out of 10 patients who had been receiving warfarin and rifampin concomitantly reached the target INR with perfect warfarin adherence. Two of those were on 27 mg, and the other 3 were on 6.5, 9.5, and 11.8 mg.<sup>28</sup> Another recent case series showed successful target attainment at a median of 30 days following rifampin addition to warfarin by 6 out of 7 patients at daily doses of 5.5, 9.5, 12.5, 16, 20, and 33 mg; which were increased from baseline requirements of 3.5, 3, 3, 8.5, 10.7, and 8.4 mg, respectively.<sup>32</sup> The first patient was also initiated on amiodarone before adding rifampin,<sup>32</sup> which is known to inhibit warfarin metabolism and reduce warfarin dose requirement.<sup>45</sup> Interestingly, in consistence with most of the previously reported cases, our data showed that the mean required warfarin dose to attain the target INR in the responders group was 10.4 mg.

Our study demonstrated that genetic polymorphisms can represent an unrevealed factor of the repeatedly reported wide variable patients' responses to the warfarin-rifampin combination. This is an example of phenoconversion where genotype–phenotype mismatch



**Figure 4** Correlation between genetic-based warfarin pre-interaction calculated dose and warfarin sensitivity index (WSI). X axis presents genetic-based warfarin pre-interaction calculated dose (mg/day) and Y axis presents WSI. Correlation between both groups was tested using Pearson correlation coefficient ( $r$ ).

occurs as a result of drug–drug–gene interaction.<sup>38,39</sup> Rifampin had induced phenoconversion of our patients from warfarin highly or normally sensitive to resistant phenotypes. Nevertheless, as most warfarin-responders were carriers of warfarin-sensitizing *CYP2C9/VKORC1* genotypes, their baseline warfarin dose requirements were elevated from a mean genetic-based calculated daily dose of 4.7 mg to a mean daily dose of 10.4 mg, a relatively modest and rapidly achievable dose, after a median time of 1 month from the combined use with rifampin. That implies that the lower the warfarin baseline estimated requirements, based on pharmacogenomic profile, other interacting medications, and patient parameters, the more likely to attain the target INR at feasible doses while receiving rifampin. Interestingly, the actual median dose increase from the genetic-based dose was not different among the responders and nonresponders; implying that warfarin nonresponders might have required a higher relative dose increase to attain target INR.

By excluding the concomitant carriers of warfarin-sensitizing *CYP2C9/VKORC1* alleles, the warfarin nonresponders were twice as likely to have at least one *CYP4F2*\*3 variant *T* allele than the

responders, indicating that *CYP4F2*\*3 genotypes would require higher warfarin doses. However, the genotype association failed to show statistical significance, likely due to the small sample size.

Another finding in this study is that the carriers of warfarin-sensitizing *CYP2C9/VKORC1* alleles had better TTR than the noncarriers, yet sub-optimum, anticoagulation control. Whereas the generally accepted TTR for optimal anticoagulation is  $\geq 65\%$ , only 5 warfarin-responders, 3 carriers, and 2 noncarriers of warfarin-sensitizing *CYP2C9/VKORC1* genotypes, achieved that target. Multiple reasons can explain the low observed TTRs, even in the warfarin-responders group. For instance, some clinicians prefer initial low doses with slow titration by 10% increments, which might have resulted in a prolonged time to reach sufficient target-attaining doses. Others tried intensive initial doses to overcome the interaction, which may have led to initial supratherapeutic INRs then subsequent extra-conservative titration. Additionally, spaced and infrequent INR monitoring after discharge may have triggered small in-clinic dose increments to avoid the risk of undetected high INRs. Last, early warfarin discontinuation at insufficient doses or short concomitant use with rifampin might have resulted in minimal TTR.

Further, the mean WSI at the target-attaining or maximum-tried dose was significantly higher in the carriers of warfarin-sensitizing *CYP2C9/VKORC1* alleles than in the noncarriers; confirming those genotypes had a significant association with higher warfarin sensitivity while using rifampin, and explaining the lower dose requirements for most warfarin-responders. In addition, the mean low WSI in the nonresponders group can explain their failure to attain target INR, as their estimated mean warfarin requirements after rifampin were much higher than their mean maximum-tried doses. Because the required warfarin dosing level with rifampin was unknown, it was either stopped for nonresponders before sufficient escalation or remained subtherapeutic until rifampin was completed.

Rifampin has been shown, using tolbutamide and phenytoin as probe substrates, to induce *CYP2C9* of various polymorphisms significantly and with the same ratio regardless of the genotype.<sup>40,41</sup> However, the *CYP2C9* pre- and post-induction enzyme activity would be genotype-dependent. Additionally, warfarin sensitivity is not only dependent on the quantitative increase in *CYP2C9* gene expression. Our data showed that the variants of other non-rifampin-affected genes, *VKORC1* and *CYP4F2*, can also impact warfarin dose requirements, sensitivity, and INR attainment potential. Moreover, along with patients' pharmacogenomic profiles, the basic clinical data and

**Table 3** Estimated warfarin dose requirement for different INRs based on observed WSI

Genotype	Estimated on-rifampin required warfarin dose <sup>a</sup> (mg/day), mean $\pm$ SD	
	Target INR range	
	2.0–3.0	2.5–3.5
Noncarriers of warfarin-sensitizing <i>CYP2C9/VKORC1</i> alleles ( $n = 14$ )	22.8 $\pm$ 10.6–34.2 $\pm$ 16	28.5 $\pm$ 13.3–39.9 $\pm$ 18.6
Carriers of $\geq 1$ warfarin-sensitizing <i>CYP2C9/VKORC1</i> alleles ( $n = 22$ )	10.6 $\pm$ 6.3–15.9 $\pm$ 9.5	13.2 $\pm$ 7.9–18.5 $\pm$ 11.1

INR, international normalized ratio; WSI, warfarin sensitivity index.

<sup>a</sup>Anticipated dosing levels to reach INR ranges (2.0–3.0 or 2.5–3.5) = INR/WSI.

the concomitant use of strong metabolism inhibitors, such as amiodarone and sulfamethoxazole/trimethoprim, especially within the first phase of rifampin initiation, may contribute to increasing warfarin sensitivity.

To the best of our knowledge, this is the first real-world study to specifically investigate the genetic polymorphisms association with the warfarin-rifampin interaction outcomes and interpatient variability. Nevertheless, Agrawal *et al.*<sup>46</sup> retrospectively studied the collective effect of CYP2C9 inhibitors and inducers on pre- and post-interaction INR variability and TTR among 302 warfarin receivers of different combined *CYP2C9/VKORC1* genotypes. Although only wild-type *CYP2C9*\*1/\*1 showed significant drug interactions-induced phenoconversion, the combined warfarin-sensitizing genotypes might have masked the CYP2C9 inhibitors' impact on INR variability. Additionally, the genotypes of the nine patients who received rifampin were not distinctly reported.<sup>46</sup> A recent model-based analysis by Cheng *et al.*<sup>47</sup> of 29 healthy volunteers showed that after 7 days of rifampin, *S*-warfarin clearance was increased by 193%, 198%, 119%, and 115% in the carriers of *CYP2C9* \*3/\*3 ( $n = 4$ ), \*2/\*3 ( $n = 3$ ), \*1/\*3 ( $n = 9$ ), and \*1/\*1 ( $n = 8$ ), respectively; which suggests that larger warfarin dose increase fractions might be required for *CYP2C9* double mutant in comparison with single or non-mutant genotypes. That is consistent with our previously published case of genotype *CYP2C9*\*3/\*3,<sup>34</sup> included in this analysis, which required a 400% warfarin dose increase, the highest in our study, from a genetic-based calculated dose of 2–10 mg, for target INR attainment during rifampin use. Interestingly, the second highest dose increase, 280%, from 2.3 to 8.8 mg, was observed in a responder case of genotype *CYP2C9*\*2/\*11. However, because the carriers of decreased function *CYP2C9* diplotypes would require extremely minimal baseline warfarin doses, the target-attaining average dose with rifampin would remain close to the requirements of the other warfarin-sensitizing *CYP2C9/VKORC1* carriers.

Our study findings can guide prescribers to identify warfarin responders at modest dosing levels based on their pharmacogenomic profiles. Whereas the noncarriers of warfarin-sensitizing *CYP2C9/VKORC1* alleles would require much more intensive warfarin doses and low molecular weight heparin may be a more feasible option; the estimated mean warfarin required on-rifampin doses may guide the titration up to a genotype-guided anticipated dose level. Because the onset of rifampin's interacting effect on warfarin appears in most cases after more than 14 days,<sup>24,26,29,34</sup> it is crucial to consider the time course of rifampin *CYP2C9* induction to avoid initial supratherapeutic INRs.<sup>29,34,48,49</sup> Using genetic-based dose as the starting point, frequent monitoring and conservative dose escalation of 10–20% increments should be utilized if warfarin is initiated before or during the first 2 weeks of rifampin. As *CYP2C9* half-life has been reported to reach up to 25 days,<sup>30</sup> warfarin can be initiated at, and moderately escalated by, 20–40% higher doses between rifampin weeks 2 and 4 with careful monitoring. After 4 weeks of rifampin, an intensive initial dose and escalation, ~40% more than the genetic-based dose with twice-weekly escalations, can be used until reaching at least 2 to 3 therapeutic INRs to avoid decline to subtherapeutic levels, especially within the initial 4–6 weeks of rifampin. The anticipated average dose requirements

for the noncarriers of warfarin-sensitizing *CYP2C9/VKORC1* to attain different target INR ranges, based on the observed WSIs in this study, were more than double those for the carriers, as shown in Table 3. This suggested approach can lead to avoiding frequent clinic visits, protracted bridging, and therapeutic failures. It is important to emphasize that after rifampin cessation, frequent monitoring, not less than twice-weekly, and careful dose de-escalations are necessary to avoid bleeding risk if not timely re-adjusted.<sup>32</sup>

The main limitation of this study, apart from its retrospective nature and limited sample size, is that we did not account for the untested SNPs, such as *CYP2C9* \*5 (rs28371686) and \*6 (rs9332131). These variants are associated with lower warfarin dose requirements, and might (if present) have explained some patients' higher-than-expected warfarin sensitivity. For example, 3 Asian/Indian warfarin-responders achieved high TTRs and moderate WSIs of 40–79.9% and 0.15–0.17, respectively, despite the lack of any warfarin-sensitizing *CYP2C9* or *VKORC1* alleles. In addition, the highest WSI (0.58) was observed in an Asian patient with *VKORC1* *c.-1639G>A* (*A/A*), yet no *CYP2C9* variant was detected. Although one of the 3 African patients was of *CYP2C9*\*3/\*3 genotype, the other 2 were detected as \*1/\*1 and \*1/\*11 and had WSIs of 0.36 and 0.54, respectively. Because *CYP2C9* \*5 and \*6 are most prevalent in Africans,<sup>5</sup> they might have explained their observed high-on-rifampin warfarin sensitivity.

In conclusion, while using rifampin, *CYP2C9* and *VKORC1* warfarin-sensitizing genotypes, as well as low genetic-based pre-interaction warfarin doses, were associated with better warfarin response at modest dosing levels, longer TTR, and higher warfarin sensitivity. The noncarriers of warfarin-sensitizing *CYP2C9/VKORC1* alleles would require frequent and protracted monitoring, as well as extensive warfarin dose escalation to more than double the carriers' dosing levels for target INR attainment. Future prospective studies are warranted to determine the optimal warfarin genotype-guided dosing to overcome warfarin-rifampin interaction, which may provide a reasonable and practical solution for anticoagulation for patients who are in need to use this anti-infective agent.

## SUPPORTING INFORMATION

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

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## CONFLICT OF INTEREST

The authors declared no competing interests for this work.

## AUTHOR CONTRIBUTIONS

M.Sa. wrote the manuscript. M.Sa., A.E., M.N.E., and A.K. designed the research. M.Sa., A.E., M.N.E., A.K., H.M., M.K., M.I.E., W.A., and F.H. performed the research. M.Sh., M.Sa., and H.E. analyzed the data. H.E., A.M.F., and L.B. contributed new reagents/analytical tools.

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[Correction added on 14 November 2023, after first online publication: The copyright line was changed.]

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