



## ARTICLE

# Genomewide association analysis of warfarin dose requirements in Middle Eastern and North African populations

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## Abstract

To date, there has been no genomewide association study (GWAS) from the Middle East and North African (MENA) region to identify genetic variants associated with warfarin dose variability using this approach. In this study, we aimed to conduct the first GWAS of warfarin dose requirements in patients from the MENA region. A total of 132 Qatari (discovery) and 50 Egyptians (replication) were genotyped using Illumina Multi-Ethnic Global BeadChip Array. A GWAS was performed on log-transformed weekly warfarin dose in the studied population, adjusting for clinical characteristics and ancestry. The genomewide signals from the discovery cohort were tested in the Egyptian cohort. A GWAS meta-analysis, including the Qatari and Egyptian cohorts, was also performed and the output from this analysis was used in a gene-based analysis. The discovery analysis in Qatari identified five genomewide single-nucleotide polymorphisms (SNPs) in chromosome 16. These signals were replicated in the Egyptian cohort. Combining the two data through a GWAS meta-analysis strengthened the association in chromosome 16 with *VKORC1* rs9934438 being the lead genomewide signal ( $\beta = -0.17, 6 \times 10^{-15}$ ). Other SNPs were identified in chromosome 10 at a  $p$  value less than  $1 \times 10^{-5}$ . The genetic variants within *VKORC1* rs9934438 and *CYP2C9* rs4086116 explained 39% and 27% of the variability in the weekly warfarin dose requirement in the Qatari and Egyptians, respectively. This is the first GWAS of warfarin dose variability in the MENA region. It confirms the importance of *VKORC1* and *CYP2C9* variants in warfarin dose variability among patients from the MENA region.

## Study Highlights

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

Genomewide association study (GWAS) from different populations have shown that genetic polymorphisms in *VKORC1* and *CYP2C9* genes, both of which are

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involved in the pharmacodynamic and kinetic pathway of warfarin, are associated with warfarin dose variability. Variants in the *CYP4F2*, a gene coding for the vitamin K metabolizing enzyme, were also shown to provide a minor contribution to warfarin dose variability.

#### **WHAT QUESTION DID THIS STUDY ADDRESS?**

Which genetic mutations are associated with warfarin dose requirements in populations of Middle East and North African (MENA)? Are there any novel variants associated with warfarin dose in these populations?

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

This is the first GWAS in the MENA region to confirm the importance of *VKORC1* (−1639G>A) and *CYP2C9* rs4086116 variants in predicting warfarin dose in Arabs.

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

This study is an important step to confirm the importance of population specific pharmacogenetic role in guiding warfarin dosing in the Arab population and support the initiation of clinical implementation precision medicine programs in the MENA region.

## **INTRODUCTION**

For more than 6 decades, warfarin served as a cornerstone therapy for the treatment and prevention of a wide variety of thromboembolic conditions.<sup>1</sup> For many patients worldwide, warfarin remains a viable and effective option, given its low cost and generic availability.<sup>2</sup> Whereas direct oral anticoagulants use has increased over the past few years, warfarin is a widely used oral anticoagulant, especially in the Middle East and North Africa (MENA) region.<sup>3</sup> For example, in Qatar, warfarin use comprised 77% of all oral anticoagulants in 2015 compared to direct oral anticoagulants.<sup>4</sup> Even with its widespread use as a primary therapeutic option for many patients, warfarin's narrow therapeutic index remains one of its disadvantages leading to an altered level of coagulation, resulting in bleeding and thrombotic adverse events, which may, in some situations, lead to death.<sup>5</sup> Additionally, warfarin regimens are usually associated with inter- and inpatient variability, which makes it challenging to achieve optimal anticoagulation. For instance, warfarin maintenance dose requirements can vary from 0.5 to 20 mg per day.<sup>6,7</sup> Many factors may contribute to this variability, including age, body surface area, ethnicity, vitamin K intake, concomitant disease conditions, alcohol intake, diet, smoking, and genetics.<sup>8</sup>

Studies have shown that pharmacogenetic algorithms can differ by race, with an improvement in anticoagulation control in certain populations versus others. Furthermore, genomewide association studies (GWAS) conducted in patients of different races have identified different genetic polymorphisms associated with warfarin dose variability.<sup>9–14</sup> However, to date, there have been no studies from

the MENA region to identify genetic variants associated with warfarin dose variability using a GWAS approach. Thus, we aimed in this study to conduct the first GWAS to identify genetic variants associated with warfarin dose requirement and the extent to which genetic and nongenetic factors contribute to warfarin dose variability in patients from the MENA region, mainly from Qatar and Egypt.

## **METHODS**

### **Study population**

This study included a total of 182 warfarin-treated participants (132 Qataris and 50 Egyptians), which were included in the discovery and replication phases, as explained below.

### **Discovery**

The discovery cohort consisted of 132 self-identified Qatari national patients recruited from the anticoagulation clinics at Al-Wakra Hospital, Heart Hospital, and Hamad General Hospital. The three hospitals are part of the Hamad Medical Corporation, the main governmental healthcare entity in Qatar. Recruitment started in September 2016 and was completed in March 2017. DNA samples were processed and analyzed at the laboratories of Qatar University (QU) between November 2016 and June 2017. Detailed inclusion and exclusion criteria have been previously explained by our group.<sup>15</sup>

## Replication cohort

The replication cohort consisted of 50 Egyptian patients recruited from four different clinical sites in Cairo, including Nasser Institute Hospital, Cardiology Clinic at Al Azhar University, Demerdash Hospital, and an Egyptian Cardiology Clinic. Eligible patients were defined as those taking stable weekly doses of warfarin for three consecutive clinic visits, occurring over a minimum of 2 months. We defined a stable weekly dose of warfarin as a dose that did not change by more than 10% across clinic visits, with international normalized ratio (INR) values at each of the three visits being within the patient's INR target range. We excluded patients with liver cirrhosis, advanced malignancy, a recent hospitalization within the previous 4 weeks, and patients who had diarrhea and any febrile illness within the past 2 weeks from the visit.

The research ethics committee approved the protocols for each study at each institution. For the Qatari study, approvals were obtained from the Institutional Review Board of Hamad Medical Corporation and QU. For the Egyptian study, approval was obtained from the Research Ethics Committee at the Faculty of Medicine, Ain Shams University, Cairo, Egypt.

## Sample collection and DNA extraction

For the Qatari samples, genomic DNA was extracted from either whole blood or saliva using the PureLink Genomic DNA mini kits, Invitrogen or the prepIT L2P manual protocol, respectively.<sup>15</sup> For the Egyptian samples, genomic DNA was collected from peripheral blood using QIAamp DNA Blood Mini Kit or automated QIAcube device (QIAGEN), following the manufacturer's protocols and guidelines.<sup>16</sup>

## Genotyping and quality control procedures

Both Qatari and Egyptian samples were genotyped together using the Illumina Multi-Ethnic Global BeadChip Array platform. Quality control procedures were performed in PLINK version 1.9. Single-nucleotide polymorphisms (SNPs) were included if they had a genotyping call rate greater than 95%. Sample missingness rate was set at 95%, and samples were removed if they had a missingness rate higher than this threshold. Samples with a mismatch between genetic and demographic sex were removed. Deviation from Hardy-Weinberg Equilibrium was tested using Fisher's exact test. We conducted Identity By Descent/Identity By Sample, Heterozygosity, and Principal Component of Ancestry (PCA) analyses using

linkage disequilibrium (LD)-pruned, high-quality SNPs with minor allele frequency greater than 10%.

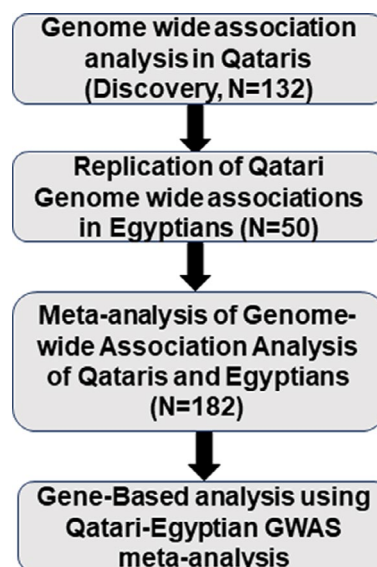
## Assessed phenotype

The study phenotype was defined as a stable, total weekly, therapeutic warfarin dose. Weekly warfarin doses were log-transformed to limit heteroscedasticity and improve model fit.

## Statistical analysis

### Genomewide association analysis

First, we performed a linear regression analysis in PLINK for a total of 1,132,000 genotyped SNPs, using an additive genetic model (Figure 1). This analysis was performed separately in both discovery (Qatari samples,  $N = 132$ ) and replication (Egyptian samples,  $N = 50$ ), adjusting for age, body surface area, smoking status, first four PCAs for ancestry, and clinical comorbidities, such as hypertension and diabetes. Genomewide significant signals were defined as those with association  $p$  value less than  $5 \times 10^{-8}$ . Using Haploview,<sup>17</sup> we assessed the LD between signals in the top associated regions from the GWAS. The GWAS significant SNPs, at  $p < 5 \times 10^{-8}$ , in Qatari (discovery cohort)



**FIGURE 1** Overall description of the study analysis steps. A total of 1,132,000 SNPs were genotyped using an additive genetic model. This analysis was performed separately in both discovery (Qatari samples,  $N = 132$ ) and replication (Egyptian samples,  $N = 50$ ). This was followed by gene-based analysis using a Qatari-Egyptian combined cohort. GWAS, genomewide association study; SNPs, single-nucleotide polymorphisms

were tested in Egyptians (replication cohort). SNPs were considered replicated if the direction of association in the replication cohort was similar to that observed in the discovery cohort, with a statistically significant Bonferroni corrected  $p$  value ( $0.05/\text{number of independent SNPs tested for replication}$ ). Furthermore, we performed a conditional analysis adjusting for the top GWAS-associated signal to identify additional independent signals that may be masked by the effect of the top GWAS signal.

In an attempt to detect novel warfarin associations that were not discovered in either of the cohorts alone, we meta-analyzed the summary statistics from both the discovery and replication cohorts (Qatari and Egyptian samples) to increase the sample size and power of our analysis. The meta-analysis was performed in METAL using fixed effect, inverse-variance method.<sup>18</sup>

## Gene-based analysis

Previous studies have shown that running GWAS using a gene-based approach rather than an SNP-based approach provides more power due to the aggregate effect of multiple SNPs being larger than that of individual SNPs.<sup>19</sup> Thus, we performed a gene-based analysis relying on the input from the Qatari-Egyptians meta-analysis results. This analysis was performed using MAGMA (Multi-marker Analysis of GenoMic Annotation),<sup>19</sup> a tool that is broadly used to run gene-based analysis. For this analysis, SNPs were mapped to genes based on their physical distances (within 10 kb) from protein-coding genes in the human reference assembly (hg 19). Based on this gene mapping strategy, a total of 14,075 genes were defined and served as the basis for the gene-based analysis in MAGMA. A Bonferroni corrected  $p$  value was used to adjust for multiple testing, and according to the number of tested genes, the level of gene-based GWAS significance was set at  $0.05/14,075 = 3.55 \times 10^{-6}$ .

## Contribution of associated SNPs to warfarin dose variability

A stepwise linear regression analysis was performed, using R (version 3.6.2), to evaluate the percent of variability in warfarin dose requirements that could be explained by significant covariates. We performed the regression analysis in the derivation cohort (Qatari) and variable selection was executed using a forward selection to include variables with  $p$  value less than 0.20, and backward elimination to only retain those with  $p$  values less than 0.05 in the model. The `My.stepwise.lm` function from the “My.stepwise” library was used to perform both forward selection and backward elimination method. Independent Qatari genetic signals

that were replicated in the Egyptian cohort were included in the final regression model in addition to the lead SNPs within the top genes that passed the gene-based significance threshold. Partial  $r^2$  of the clinical and genetic factors and adjusted  $r^2$  were reported in the final model.

## RESULTS

A total of 182 warfarin-treated patients from Qatar ( $N = 132$ ) and Egypt ( $N = 50$ ) were included in this study. The average age of patients in years was  $62 \pm 13$  and  $41 \pm 12$  in the Qatari and Egyptian cohorts, respectively. The median and interquartile range for the weekly warfarin dose in Qataris and Egyptians was 32 mg (24.5) and 37 mg (42.0), respectively (Table 1). The indication for warfarin varied between the two cohorts, with atrial fibrillation being the most common indication for warfarin in Qatari ( $n = 87$ , 66%) and heart valve replacement in Egyptians ( $n = 33$ , 66%).

## Warfarin association results in Qatari with validation in Egyptians

A total of five SNPs on chromosome 16 reached genomewide significance at  $p < 5 \times 10^{-8}$ , with the top signal being the *VKORC1* rs9934438 ( $\beta = -0.16$ ,  $p = 2.1 \times 10^{-12}$ ; Table 2 and Figure S1). Qatari patients carrying the rs9934438 A-allele had lower weekly warfarin dose (AA genotypes = 27 mg/week, AG = 39 mg/week, and GG = 52 mg/week; Figure S2). Consistent with this association, Egyptian patients carrying two copies of the A-allele of SNP rs9934438 had lower warfarin dose requirements, 19 mg/week, compared to heterozygous carriers, 41 mg/week, and noncarriers, 46 mg/week. The association of the other four genomewide significant SNPs was evaluated in Egyptians, which showed a consistent association with warfarin dose as in Qatari at a corrected Bonferroni  $p$  value (Table 2). A conditional analysis adjusting for the *VKORC1* rs9934438 SNP led to nonsignificant associations of the other genomewide significant SNPs, suggesting that *VKORC1* rs9934438 SNP is the main driver of the association observed in this region. Evaluating LD for the five genomewide significant signals in Qatari and Egyptians, separately, confirmed that these five SNPs are in high LD (Figure 2).

## Warfarin association results in the Qatari and Egyptian GWAS meta-analysis

The meta-analysis of the Qatari-Egyptian cohort strengthened the observed association in chromosome



Clinical characteristics or demographics	Qataris N = 132	Egyptians N = 50	p value
Age, years, mean ± SD	6262 ± 13	41 ± 12	$2 \times 10^{-16}$
Female, n (%)	75 (57%)	50 (50%)	0.4
BSA mean ± SD	1.9 ± 0.2	1.9 ± 0.2	0.5
Smoker, n (%)	11 (8%)	10 (20%)	0.03
Mean weekly, mg (SD)	35 (16)	44 (28)	0.04
Median weekly dose, mg (IQR)	32 (24.5)	37 (42.0)	0.29
Indication for warfarin			
Atrial Fibrillation, n (%)	87 (66%)	0	$1 \times 10^{-15}$
Venous thromboembolism, n (%)	18 (14%)	11 (22%)	0.2
Valve replacement, n (%)	28 (21%)	33 (66%)	$1 \times 10^{-8}$
Cardiovascular/metabolic comorbidities			
Hypertension, n (%)	89 (67%)	6 (12%)	$2.3 \times 10^{-11}$
Diabetes, n (%)	75 (57%)	2 (4%)	$1.2 \times 10^{-10}$

Note: Categorical variables were expressed as frequency (%). Numerical variables were expressed as mean and standard deviation (SD) as in age and BSA, or median and IQR as in weekly warfarin doses.

Abbreviations: BSA, body surface area; IQR, interquartile range.

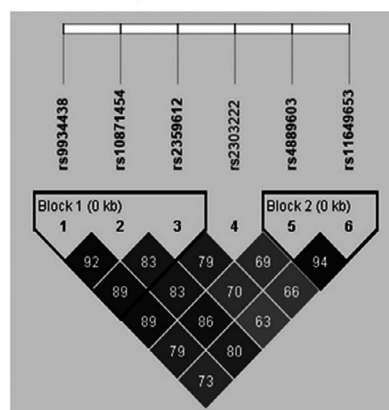
**TABLE 1** Clinical characteristics of Egyptian and Qatari cohorts (N = 182)

**TABLE 2** Top signals in the Qatari cohort (N = 132) that were replicated in Egyptian cohort (N = 50)

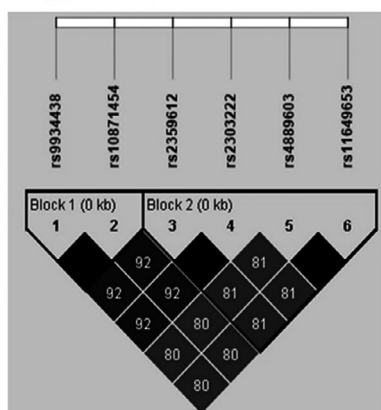
Top associated signals				Qatari, N = 132		Egyptians, N = 50		Meta-analysis (N = 182)	
SNP	Chr	Gene	A1	β	p value	β	p value	β	p value
rs9934438	16	VKORC1	A	-0.16	2.1E-12	-0.21	0.0006	-0.16	7.6E-15
rs10871454	16	STX4	T	-0.16	9.1E-12	-0.21	0.0006	-0.16	4.76E-14
rs2359612	16	VKORC1	A	-0.15	1.1E-10	-0.22	0.0005	-0.16	2.0E-14
rs2303222	16	5' UTR- ZNF668	C	-0.15	4.1E-10	-0.21	0.0003	-0.16	2.33E-14
rs4889603	16	SETDIA	G	-0.15	9.5E-10	-0.19	0.001	-0.15	3.45E-11

Abbreviation: SNP, single-nucleotide polymorphism.

**(a) Qatari Population**



**(b) Egyptian Population**



**FIGURE 2** Linkage disequilibrium for top genomewide association study signals on chromosome 16 in the Qatari population (a) and the Egyptian population (b)

16, with *VKORC1* rs9934438 significantly impacting warfarin dose requirements and reaching the highest association in this region ( $p = 7.6 \times 10^{-15}$ ; Table 2, Table S1,

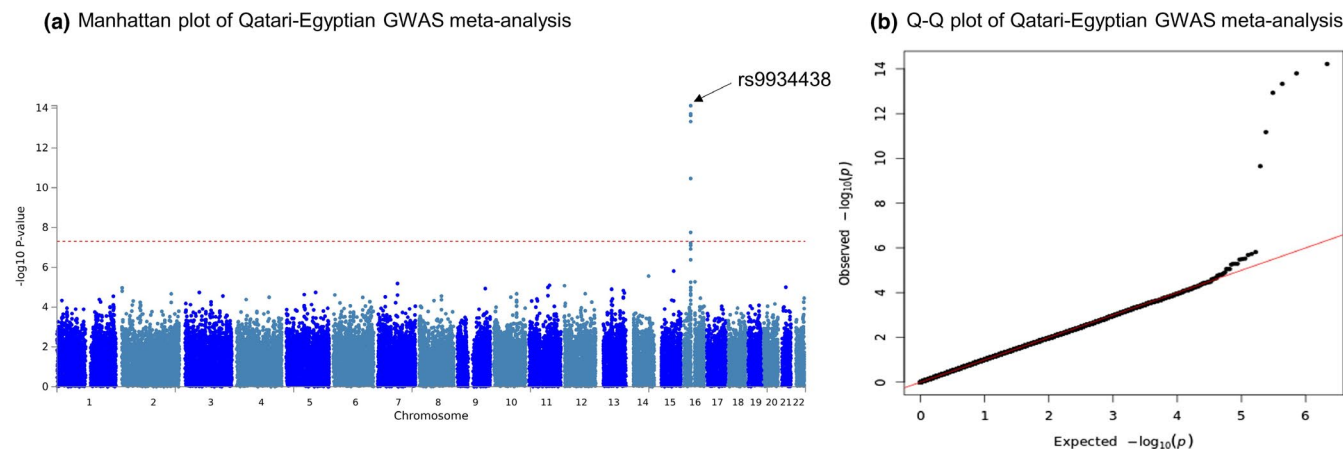
Figure 3). Besides chromosome 16 signals, no other genetic signals passed the genomewide significance level ( $p < 5 \times 10^{-8}$ ).

## Gene-based analysis

The results of the gene-based GWAS identified six significantly associated genes that passed the Bonferroni corrected significance threshold, as explained in the methods. Of these six genes, five are located on chromosome 16, and one is located on chromosome 10, with *VKORC1* and *CYP2C9* being among the top associated genes. The rs9934438 and rs4086116 SNPs were the lead signals in chromosomes 16 and 10, respectively (Figure 4 and Table S2). The rs4086116 T-allele was significantly associated with lower warfarin weekly dose requirement in Qatari's ( $\beta = -0.11$ ,  $p = 3.6 \times 10^{-4}$ ) and Egyptians ( $\beta = -0.18$ ,  $p = 0.02$ ; Figure S3). Patients carrying the rs4086116 T-allele had lower weekly warfarin dose compared to patients with homozygous C-allele in Qataris and Egyptians (Qataris: [TC/TT] = 34 mg, CC = 43 mg; Egyptians: [TC/TT] = 33 mg, CC = 51 mg; Figure S3). Of note, according to the 1000 Genomes Project data, the rs4086116 SNP is in moderate LD ( $r^2 = 0.62$ ,  $D' = 1$ ) with *CYP2C9*\*2 SNP (rs1799853).

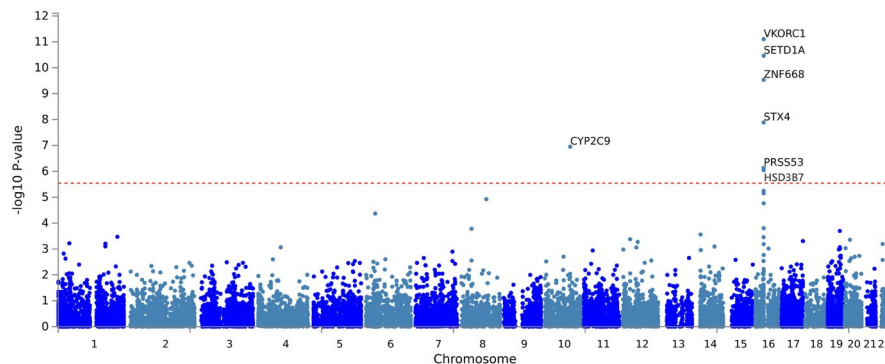
## Contribution of identified genetic variants on warfarin dose variability

A linear regression model was performed in Qatari (derivation cohort) to identify the percent of variability explained by the replicated genetic polymorphisms. This model included the replicated independent signals from Qatari GWAS analysis (*VKORC1* rs9934438) and the lead SNP within the *CYP2C9* gene (rs4086116), which passed the significant threshold of the gene-based analysis. As explained in the methods section, several clinical variables were also included to evaluate the percent of variability in warfarin dose requirements that can be explained using both genetic and clinical variables in Qatari. Collectively, the clinical and genetic predictors explained a total of 53% of the variability in weekly warfarin dose requirement (Table 3). Whereas the clinical predictors, including age, smoking, valve replacement, diabetes, and hypertension explained ~12% of warfarin dose variability, the genetic signals alone (*CYP2C9* rs4086116 and *VKORC1* rs9934438) explained ~39% variability in Qatari's and ~27% in Egyptians.



**FIGURE 3** Association with log-transformed weekly warfarin dose in Qatari-Egyptian GWAS meta-analysis. Panel a: The Manhattan plot of the GWAS association shows the SNPs on chromosome 16 that reached genome-wide significance at  $p < 5 \times 10^{-8}$ , for association with warfarin dose. Top signal included the *VKORC1* rs9934438 as well as rs10871454, rs2359612, rs2303222, rs4889603, and rs11649653. Panel b: Quantile-quantile(Q-Q) plot of the data shown in the Manhattan plot. GWAS, genomewide association study; SNPs, single-nucleotide polymorphisms

**FIGURE 4** Manhattan plot showing association results of gene-based Qatari-Egyptian GWAS meta-analysis. Significance level was set at  $p = 3.55 \times 10^{-6}$  ( $0.05/\#genes$ ). Top signal being *VKORC1*, *SETD1A*, *ZNF668*, *STX4*, *CYP2C9*, *PRSS53*, and *HSD3B7*. GWAS, genomewide association study



Variables	$\beta$ (SE)	<i>p</i> value	Partial $r^2$
Age, years	-0.002 (0.001)	0.02	0.05
Diabetes	0.06 (0.03)	0.04	0.04
Hypertension	-0.08 (0.03)	0.008	0.05
Smoking	0.13 (0.05)	0.006	0.06
<i>VKORC1</i> -rs9934438	-0.17 (0.02)	$<2 \times 10^{-16}$	0.42
<i>CYP2C9</i> -rs4086116 <sup>a</sup>	-0.10 (0.02)	$1.1 \times 10^{-5}$	0.14
Adjusted $R^2$	53%		

<sup>a</sup>According to 1000 Genomes Project data, the rs4086116 SNP is in moderate linkage disequilibrium ( $r^2 = 0.62$ ,  $D' = 1$ ) with *CYP2C9*\*2 (rs1799853).

## DISCUSSION

The geographical location of the Middle East at the crossroads of Africa, Europe, and Asia has enabled populations within this region to be the first to migrate out of Africa, which makes them particularly interesting for genomics research.<sup>20</sup> Qatar is a peninsula in the Persian/Arabian Gulf. It resides at the eastern edge of the Arabian Peninsula, making it the center of migration patterns that took part in the region over the era of human history. Various migrations and historical events have created a genomic admixed Qatari population consisting mainly of Arabic (mainly Bedouins), Persian (Iran, Pakistan, and Afghanistan), and African (Sub-Saharan Africa) ancestry. A high rate of consanguinity still exists among Qatari (35% in 2010), with first cousins' marriages being widely accepted and predominant.<sup>21</sup> Such high levels of consanguinity may have had a profound influence on the genetic make-up of this population.<sup>22</sup> Egyptians are also characterized by having strong Arabic (mainly Bedouins) and African (Sub-Saharan Africa) ancestry.<sup>16</sup> There has been limited genetic research in Arabs despite the unique genetic admixture of that population. Additionally, warfarin is still widely used in the region.<sup>4</sup> Thus, it was imperative to conduct GWAS for warfarin dosing in Arabs, which, to the best of our knowledge, is the first in this population.

Our GWAS indicates that *VKORC1* rs9934438 is the main SNP associated with warfarin dose variability in a cohort of Qatari and was replicated in an independent cohort of Egyptians (Figures S1 and S2). There were four more genomewide significant SNPs on chromosome 16 (Figure 3a), but the association turned to be nonsignificant after the conditional adjustment for the *VKORC1* rs9934438 genotype (data not shown). This suggests that the association in this region is driven primarily by the *VKORC1* rs9934438 signal. *VKORC1* is the gene coding for the vitamin K epoxide reductase (VKOR), an essential enzyme for the vitamin K cycle activity and the activation of the coagulation factors. By inhibiting this

**TABLE 3** Contribution of clinical characteristics and *VKORC1* and *CYP2C9* SNPs to warfarin dose requirement in Qatari ( $N = 132$ )

enzyme, warfarin prevents the activation of the vitamin K-dependent coagulation factors and mediates its anticoagulant activity.<sup>23,24</sup>

Apart from *VKORC1*, there were associated signals in *CYP2C9* when the Qatari and Egyptian cohorts were combined together in the gene-based analysis. *CYP2C9* was one of the signals that passed the gene-based significant threshold, with SNP rs4086116 being the lead SNP in this gene. The lead SNP, rs4086116, was an intronic SNP, which, according to data from the 1000 Genomes Project, it is in moderate LD ( $r^2 = 0.62$ ,  $D' = 1$ ) with *CYP2C9*\*2 variant (rs1799853). Warfarin S isomer is mainly metabolized by the cytochrome P450 2C9 (*CYP2C9*) enzyme and is 3–5 times more potent than the R isomer, but has faster clearance.<sup>25</sup> Findings from this work are in alignment with previous results from warfarin GWAS in other ethnicities, which identified *VKORC1* and *CYP2C9* to be the strongest genetic determinants of warfarin dosing variability.<sup>9–14</sup> SNPs with the following genomic regions/genes, *STX4*, *ZNF668*, *SETD1A*, and *CTF1* also showed GWAS significant association with warfarin dose requirement, but these signals are likely mediated by the *VKORC1*.

In an effort to demonstrate the clinical utility of genetic-guided dosing of warfarin, multiple randomized controlled trials (RCTs) have been conducted over the past decade. In 2013, the first two landmark trials—the Clarification of Optimal Anticoagulation through Genetics (COAG) and the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) were published.<sup>7,26</sup> Whereas the EU-PACT trial showed benefit of genetic-guided dosing of warfarin, results from COAG were negative. Furthermore, COAG found that the percent time in therapeutic range (PTTR) was significantly lower in Black patients in the genetic-guided arm compared to the clinical dosing arm.<sup>26</sup> This is possibly due to the fact that Black patients may have other less common variants affecting warfarin dose that were not well-represented in the genetic algorithm used in the COAG trial.<sup>27</sup> This point also highlights the importance of examining the genetic variation affecting the warfarin

dose across populations instead of relying on a generic warfarin dosing algorithm predominantly derived in patients with European ancestry. Recently, a third landmark trial—Genetics-InFormatics Trial (GIFT) also tested the utility of warfarin pharmacogenetic-guided dosing.<sup>28</sup> The pharmacogenetic dosing algorithm used included genotypes for *CYP2C9*\*2 and \*3, *CYP4F2*\*3, and *VKORC1*-1639. The primary end point was composite of major bleeding, INR greater than or equal to 4, venous thromboembolism, or death. GIFT indicated that genotype-guided dosing could improve the composite outcome of efficacy and safety. Almost 11% of the participants had at least one composite end point in the genotype-guided arm, compared to 14.7% in the clinical arm, resulting in an absolute risk difference of 3.9% (95% confidence interval, 0.7% to 7.2%,  $p = 0.02$ ). There was an improvement in the mean PTTR (54.7% vs. 51.3%,  $p = 0.003$ ) in the genotype-guided group compared to the clinical group. Based on the results from these RCTs, it is still not recommended to perform genetic testing prior to warfarin initiation, as per the evidence-based guidelines. In this paper, we documented that variants in *VKORC1* (rs9934438) and *CYP2C9* (rs4086116) were associated with warfarin dose requirements in the population from the MENA region and, together with age, presence of valve replacement, diabetes, hypertension, and smoking, 53% of the variability in warfarin dose requirements could be explained in Qatari. The use of genetic information alone (*CYP2C9* rs4086116 and *VKORC1* rs9934438) explained ~39% and ~27% of the variability in Qatari and Egyptians, respectively, suggesting that these genetic variants are important warfarin dose determinants in this population.

With the advancement in whole genome sequencing and the tremendous drop in its cost, genetic data are expected to be present in all healthcare records. In the state of Qatar, initiatives from the Qatar Genome and Qatar Biobank already started in 2015 with a primary goal to use the latest DNA sequencing technologies to establish a genomic map of the Qatari population.<sup>29,30</sup> One of the long-term goals of these projects is to integrate this genetic data into the electronic health record system used by government hospitals. Therefore, evidence from our GWAS and other studies may provide some guidance on how to use this genetic data once available. This could be through the use of an exact algorithm to predict the warfarin maintenance dose of the patient or to guide the use of direct oral anticoagulants versus warfarin in patients carrying risk variants, such as *CYP2C9*\*2, \*3, or other variation leading to a very high or very low warfarin dose. Having confirmed the effect of *VKORC1* (rs9934438) and *CYP2C9* (rs4086116) on warfarin dose and their prevalence in the Arab population, it would be important as a

next step to compare the performance of internationally validated warfarin dosing algorithms to those developed specifically in Arabs. This would be followed by a clinical utility study to compare the effectiveness of genetically guided dosing approach using the best algorithm to the conventional dosing before proceeding toward clinical implementation.

Although this study provides the first GWAS evidence on warfarin dosing in the Arab population, it was limited by the small sample size. This is likely the reason why *CYP2C9* genetic polymorphisms did not show a genomewide significant association in GWAS discovery analysis. Nevertheless, with the sample size used, we were able to confirm the importance of *VKORC1* rs9934438 SNP on warfarin dose requirements in the Qatari and Egyptian populations. Additionally, using gene-based analysis, both *VKORC1* and *CYP2C9* were among the top genes influencing warfarin dose requirements, consistent with GWAS results previously reported in other global populations. *CYP2C9*\*2 has been shown to be an important predictor of warfarin dose variability in previously conducted warfarin GWAS; however, *CYP2C9*\*2 was not present within the GWAS chip used in this study. Despite the absence of *CYP2C9*\*2 genotyping data, the top intronic *CYP2C9* SNP identified in the Qatari-Egyptians gene-based analysis was in moderate LD ( $r^2 = 0.62$ ,  $D' = 1$ ) with *CYP2C9*\*2, according to data from the 1000 Genomes Project, which indicates that this association may be mediated via the *CYP2C9*\*2 genetic polymorphism.

In conclusion, the results of this study identified *VKORC1* rs9934438 and *CYP2C9* rs4086116 variants as important genetic variants that impact warfarin dose requirements in populations from the MENA region, which emphasize the substantial role of these variants in guiding warfarin dosing in these populations.

With the limited resources in many countries in the MENA region, warfarin remains a cornerstone treatment for many patients who cannot afford direct oral anticoagulants. Warfarin dose prediction based on the variants identified in *VKORC1* and *CYP2C9* could be valuable, especially if confirmed in other patients from the same region. This study is an important step to confirm the importance of population specific pharmacogenetic role in guiding warfarin dosing in the Arab population and support the initiation of clinical implementation precision medicine programs in the MENA region.

## CONFLICTS OF INTEREST

M.H.S. is an employee of Pfizer, Inc. All other authors have no competing interests for this work.

## AUTHORS CONTRIBUTIONS

All authors wrote the manuscript. M.H.S., S.I.K., and H.E. designed the research. N.E.R., M.H.S., L.B., and H.E.



performed the research. N.E.R., M.H.S., L.B., and H.E. analyzed the data.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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