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ORIGINAL ARTICLE

Profiling of warfarin pharmacokinetics-associated genetic variants: Black Africans portray unique genetic markers important for an African specific warfarin pharmacogenetics-dosing algorithm

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Abstract

Background: Warfarin dose variability observed in patients is attributed to variation in genes involved in the warfarin metabolic pathway. Genetic variation in *CYP2C9* and *VKORC1* has been the traditional focus in evaluating warfarin dose variability, with little focus on other genes.

Objective: We set out to evaluate 27 single nucleotide polymorphisms (SNPs) in the *CYP2C cluster loci* and 8 genes (VKORC1, *ABCB1*, *CYP2C9*, *CYP2C19*, *CYP2C8*, *CYP1A2*, *CYP3A4*, *and CYP3A5*) involved in pharmacokinetics of warfarin.

Patients/Methods: 503 participants were recruited among black Africans and Mixed Ancestry population groups, from South Africa and Zimbabwe, and a blood sample taken for DNA. Clinical parameters were obtained from patient medical records, and these were correlated with genetic variation.

Results: Among black Africans, the SNPs *CYP2C rs12777823G>A*, *CYP2C9 c.449G>A (*8)*, *CYP2C9 c.1003C>T (*11)* and *CYP2C8 c.805A>T (*2)* were significantly associated with warfarin maintenance dose. Conversely, *CYP2C9 c.430C>T (*2)*, *CYP2C8 c.792C>G (*4)* and *VKORC1 g.-1639G>A* were significantly associated with maintenance dose among the Mixed Ancestry. The presence of *CYP2C8*2* and *CYP3A5*6*

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alleles was associated with increased mean warfarin maintenance dose, whereas *CYP2C9*8* allele was associated with reduced warfarin maintenance dose.

Conclusion: African populations present with a diversity of variants that are important in predicting pharmacogenetics-based warfarin dosing in addition to those reported in *CYP2C9* and *VKORC1*. It is therefore important, to include African populations in pharmacogenomics studies to be able to identify all possible biomarkers that are potential predictors for drug response.

KEYWORDS
pharmacogenetics, pharmacokinetics, South Africa, Southern Africans, warfarin, Zimbabwe

1 | BACKGROUND

Warfarin is a widely prescribed anticoagulant for prevention and treatment of thromboembolic disorders and associated complications such as stroke.[1,2] In Africa, warfarin remains the most prescribed anticoagulant despite the availability of alternative anticoagulants, due to its relatively low cost and extensive knowledge of use by physicians.[3] Warfarin use is however complicated by its narrow therapeutic range, and difficulties in establishing standard doses that allow reaching stable international normalized ratio (INR) of 2–3.5, in a relative short space of time.[4,5] Consequently, patients often require different starting doses to reach the same INR range, leading to a proportion of patients presenting with warfarin associated adverse drug reactions (ADRs) and increased risk for hospitalization or death after administration of standard starting doses.[5,6] Differences in warfarin starting dose requirements among patients are due to both genetic and environmental/non-genetic factors.[7] Non-genetics factors include demographic variables (e.g., age and gender), lifestyle (e.g., diet, tobacco smoking and alcohol consumption) and clinical factors (e.g., comorbidities, co-medications, and physiological aspects).[8,9] Whilst genetic factors associated with the warfarin dose variability include variants in genes encoding enzymes involved in the warfarin disposition pathway.[10,11]

Warfarin exerts its effects by targeting the vitamin K epoxide reductase complex 1 (VKORC1) through blocking its recycling of vitamin K, consequently inhibiting the activation of clotting factors,[12] thus, *VKORC1* gene has become an important gene to evaluate. CYP2C9 principally metabolizes the most potent form of warfarin, S-warfarin, whilst CYP3A4/5 and CYP1A2 metabolize the R-warfarin form. Other enzymes involved in the metabolism of warfarin include CYP2C8, CYP2C18 and CYP2C19.[13,14] Polymorphisms in *CYP2C9* and *VKORC1* have been widely studied with respect to warfarin dosing variability. *CYP2C9*2* (rs1799853), *CYP2C9*3* (rs1057910) and *VKORC1 g-1639G>A* (rs9923231) are the most investigated variants and have been widely associated with reduced warfarin dose requirements.[11,15–17] Consequently, *CYP2C9*2*, *CYP2C9*3* and *VKORC1 g.-1639G>A* variants coupled with some non-genetic factors have been incorporated in warfarin pharmacogenetics-based dosing algorithms to assist in predicting the best warfarin starting doses.[18,19] However, these variants show qualitative and quantitative differences in their frequencies across world populations, with generally higher frequencies among European and Asian populations, when compared to African populations.[11,17,20,21]

Recent studies have started to expand on the repertoire of genetic variants important in warfarin dosing, reporting on *CYP2C9*5*, *CYP2C9*8*, *CYP2C9*11* and *CYP2C rs12777823G>A*.[22–27] The additional variants have started unravelling the causes of variability in warfarin doses observed in African populations, even after taking into account the most commonly studied *CYP2C9* and *VKORC1* polymorphisms. Although, several algorithms for estimating warfarin starting doses have been proposed, the effectiveness of the available pharmacogenetics algorithms in African populations remains in doubt due to very little data from Africans.[28–30] Furthermore, most studies focus on the two principal genes, *CYP2C9* and *VKORC1*, excluding other genes involved in warfarin disposition. Thus, we present here the determination among Southern African populations (i.e., black Africans, and the Mixed Ancestry) the combined effects of 27 single nucleotide polymorphisms (SNPs) in 8 genes (i.e., *ABCB1*, *CYP1A2*, *CYP2C8*, *CYP2C9*, *CYP2C19*, *CYP3A4*, *CYP3A5* and *VKORC1*) plus additional loci in the CYP2C cluster, which play a role in the pharmacokinetics of warfarin. We included participants from black African and Mixed ancestry population groups in the study as they are the dominating population groups in Southern Africa and have been poorly characterised with respect to warfarin

ESSENTIALS

- Warfarin is a widely prescribed anticoagulant, that is highly affected by genetic and non-genetic factors.
- Genetic variants affecting warfarin dose were investigated among Southern Africans recruited in Cape Town, South Africa and Harare, Zimbabwe
- Single nucleotide polymorphisms which included *CYP2C8 c.805A>T (*2)*, *CYP2C9 c.449G>A (*8) and CYP2C rs12777823G>A* affected warfarin dose variability among black Africans.
- Age, BMI and *VKORC1 g.-1639G>A* explained 22% of warfarin dose variability among Mixed Ancestry.

pharmacogenes. Furthermore, previous reports have shown that individuals of the Cape Mixed Ancestry (i.e., included here), are an admixed population group with a gene pool comprising of European, African, South Asian and Indonesian ancestry, whilst black Africans are an indigenous African population group.[31–33] Thus, distinct genomic profiles of black Africans and the Mixed ancestry will allow us to compare and determine whether the influence of the studied SNPs on warfarin variability varies between the two population groups. Additionally, we also set out to present variables that could be included in an African-specific pharmacogenetics-based warfarin dosing algorithm.

2 | METHODS AND MATERIALS

2.1 | Patient cohort

Sample size was determined using a method by Naing[34] described as $n = (z/\Delta)^2 p(1-p)$. The sample size for a SNP with a lowest frequency of 2% was determined as follows; the proportion (p) of the sampled patients that will take abnormally long to reach INR was assumed to be 10%, then the required sample size to achieve a 95% confidence interval of width 0.10 (i.e., $\Delta = 0.05$) was calculated as $n = (1.96/0.05)^2$ 0.10 (1–0.10) = 138. The minimum participants required for the study was 138 each for both Black Africans and Mixed Ancestry. Thus, the study included 503 participants recruited between 2016 and 2017 from INR clinics at Groote Schuur Hospital (GSH), Gugulethu Community Health Centre (GCHC) in Western Cape, South Africa, and Parirenyatwa Group of Hospitals (PGH) in Harare, Zimbabwe. Two hundred and fifty-two (252) participants were of Black African (BA) descent and 251 participants were of Mixed Ancestry (MA) descent. The participants provided consent to be enrolled in the study after ethical approval was granted by the University of Cape Town Human Research Ethics Committee (UCT-HREC REF No. 581/2015), Faculty of Medicine, University of Zimbabwe Ethics Committee (JREC/160/13) and the Medical Research Council of Zimbabwe (MRCZ/A/1815). Recruitment of the patients including accessing of demographic variables (e.g., age, gender), clinical parameters (e.g., warfarin indications, comedications, and comorbidities) and biological samples was described before.[27] For anonymity, participant names were replaced by a laboratory generated number.

2.2 | Genetic characterization for pharmacogenetic variants

DNA was extracted from whole blood using the salting-out DNA purification method (modified from Gustafson et al[35]). Fifty (50) µl of 50ng of purified DNA was sent to Inqaba biotec (Pretoria, South Africa) for genotyping using the iPLEX PGx74 Mass Genotyping Array platform (Agena Bioscience, Inc. San Diego, CA). The iPLEX PGx74 panel targets 69 SNPs/INDELs and 5 CNVs in 20 genes of pharmacogenetics importance (Agena Bioscience, Inc. San Diego, CA). However, from the 20 genes targeted, the present paper reports on only 6 genes of pharmacokinetics relevance on warfarin, namely, *ABCB1*, *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP3A4* and *CYP3A5*. In addition to the genes targeted by the iPLEX PGx74 panel, SNPs in the CYP2C cluster non-genomic loci, *CYP2C8* and *VKORC1* have also been included in the study. Genetic characterization of *CYP2C rs12777823G>A* and *VKORC1 g.-1639G>A* has been described before.[27] The *CYP2C rs12772169C>T* SNP and seven *CYP2C8* SNPs were genotyped using PCR-RFLP and Sanger sequencing, respectively.

Oligonucleotides for the *CYP2C8* and *CYP2C rs12772169C>T* SNPs were designed using the Integrated DNA Technologies (IDT) PrimerQuest tool (https://eu.idtdna.com/PrimerQuest/Home/Index) and were blasted on National Center for Biotechnology Information (NCBI) Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastDescAd). A restriction enzyme was identified using the NEBcutter tool (http://nc2.neb.com/NEBcutter2/) for the *CYP2C rs12772169C>T* RFLP. For *CYP2C8* SNPs, PCR was carried out in a 25 µl reaction containing 0.4 µM forward primer: 5′-CCA TCG TTC TCA GCA TAC TAT CAC-3′ and reverse primer: 5′-CTA TGC ATT CTA GCC ATT GGA CAAT-3′ each, 0.4 mM of dNTPs, 0.02 U/µl Go-Taq polymerase, 1X Go-Taq Flexi buffer, 10 ng DNA, and 3 mM $MgCl_2$, made up to 25 µl with 12.37 µl of nuclease free H_2O. PCR cyclic conditions involved an initial denaturation at 95°C for 3 min, followed by 25 cycles of denaturation at 95°C for 30 s, annealing at 65°C for 30 s, and extension at 72°C for 30 s, followed by a final incubation at 72°C for 5 min, to allow extension. PCR products were cleaned up in an enzymatic reaction of Exonuclease I (Exo I) and Shrimp Alkaline Phosphatase, according to the manufacturer's protocol (New England Biolabs, Ipswich, MA), and then sequenced using Big-Dye Terminator sequencing according to the manufacturer's protocol (Applied Biosystems, Foster City, CA).

The sequencing protocol and results analysis were done as described by Ndadza et al.[27] For the *CYP2C rs12772169C>T* SNP, PCR was carried out as described above using the following primer set; forward primer: 5′-AAG ACA GTT CTC TCT ACA GGA GT-3′ and reverse primer: 5′-GTT GCA GTG TTA AAA CTA GCT GGA-3′, with an annealing temperature of 56.9°C. The PCR products were then digested using the *AatII* (GACGTC) restriction enzyme, in a 15 µl reaction containing 3 µl of the PCR product, 0.13 U/µl of the restriction enzyme, and 1X of cutsmart buffer made up to 15 µl with 9.9 µl of nuclease free H_2O. The reaction was then incubated for 2 h at 37°C and then the enzyme was inactivated at 80°C for 20 min. The RFLP results were then validated with Sanger sequencing as described before.[27]

2.3 | Statistical data analysis

Twenty-seven ($n = 27$) SNPs from 8 genes (i.e., *ABCB1*, *CYP1A2*, *CYP2C8*,*CYP2C9*, *CYP2C19*, *CYP3A4*, *CYP3A5* and VKORC1) and

2 non-genomic loci in the CYP2C cluster (Table S1) were successfully characterized and assessed on their contribution to warfarin maintenance dose. The analysis included previously published data on the following 6 SNPs; *CYP2C rs12777823G>A*, *CYP2C9 c.430C>T (*2)*, *CYP2C9 c.1075A>C (*3)*, *CYP2C9 c.449G>A (*8)*, *CYP2C9 c.1003C>T (*11)* and *VKORC1 g.-1639G>A*,[27] allowing re-analysis with increased sample size. Statistical analysis was performed using STATA for windows version 15 (StataCorp, College Station, Texas, USA) and various statistical packages in R (version 4.0.3 [2020–10–10]) as follows; patients' characteristics and clinical data were determined by calculating the frequency (presented as n [freq]) and mean ± standard deviation for the categorical and continuous variables, respectively. The distribution of weekly warfarin maintenance dose was assessed for normality using the Shapiro wilks test. Further comparison of the maintenance dose distribution according to SNPs genotypes, demographic and clinical characteristics groupings were done using the appropriate tests depending on the distribution of the data. To assess the association or the allele occurrence of SNPs located in the CYP2C cluster region to each other and to identify important haplotypes, linkage disequilibrium (LD) was calculated and visualised for 19 SNPs in the CYP2C cluster in both the studied cohorts using the HaploView software (https://www.broadinstitute.org/haploview).

Continuous variables such as age and BMI were grouped according to categories to allow comparison of the maintenance dose distribution. Age was categorized into three groups; <50 years, 50–70 years and >70 years as described by Shendre et al.[36] BMI was grouped into four categories; underweight (<18.5), normal [18.5–24.9], overweight [25–29.9] and obese (≥30), as described by the centres of disease control and prevention (CDC) (https://www.cdc.gov/obesity/adult/defining.html). Box plots were constructed using GraphPad prism (version 6) to compare the maintenance dose distribution according to age categories. The effect size was determined through the Cohen's d, comparing the mean maintenance dose between two groups for each variable, for variables with more than 2 groups the upper and lower groups mean maintenance dose were then compared (e.g., homozygous wild-type and homozygous mutant genotypes were compared for each SNPs). Stepwise regression analysis was done to control for confounding factors and to determine the interaction and cumulative influence of the various studied genetic and non-genetic variables. The variables that were eligible to enter the stepwise regression analysis were those with a $p \leq .20$ for their effect in univariable analysis. To control for missing data and reduce bias, imputation was done on variables that were eligible for the stepwise regression analysis and have missing data using the multivariate imputation by chained equations (MICE) package in R. Table S2 outlines the missing data according to each variable. All statistical tests were performed taking a 5% significance level.

3 | RESULTS

3.1 | Correlation of demographic and clinical characteristics with warfarin maintenance dose

The demographic and clinical characteristics of these cohorts have been previously reported.[27] However, data for black Africans was re-analyzed to accommodate an increased sample size (Table S3). Table 1 presents the correlation between various demographic and clinical characteristics with warfarin maintenance dose. Similar to observations from other studies,[37,38] increasing age was significantly inversely correlated with reduced warfarin weekly maintenance dose ($p < .05$) among both the black and the Mixed Ancestry Southern African patients with an effect size of 0.64 (95% CI: 0.22 to 1.06) and 0.74 (95% CI: 0.38 to 1.09), respectively (Figure 1). For example, patients <50 years of age required mean warfarin maintenances doses of nearly 40 mg/week compared to the much lower 30 mg/week for patients >70 years of age ($p < .05$; Table 1). Deep venous thrombosis (DVT) was associated with higher mean warfarin maintenance dose requirements in the Mixed Ancestry group ($p = .004$), whilst mechanical valve replacement was significantly associated with higher warfarin maintenance dose among Black Africans ($p < .05$). Hypertension and heart failure were significantly correlated ($p = .04$ and .03, respectively) with a reduced warfarin dose requirement among Black Africans only. Other variables such as BMI, gender, concomitant drug use, diabetes mellitus, HIV, tobacco smoking, and alcohol consumption did not significantly affect the required mean weekly warfarin maintenance doses in both Black Africans and Mixed Ancestry Southern Africans.

3.2 | Distribution of pharmacogenes variants among the black Africans and Mixed Ancestry group

We present an analysis on 27 SNPs with pharmacokinetics relevance on the disposition of warfarin (except *VKORC1 g.-1639G>A*, included for its central role in warfarin mechanism of action) on their association with warfarin maintenance dose (Table 2). Data for 6 SNPs has previously been presented by Ndadza et al.[27] For the 27 SNPs characterized, there was quantitative and qualitative differences in the distribution and effects of variant SNPs, when comparing Black Africans to the Mixed Ancestry group. Two SNPs, *CYP2C9 c.1076T>C (*4)*, and *CYP2C8 c.712G>C (*14)* were monomorphic in both the Black Africans and Mixed Ancestry groups, thus, were therefore excluded from further analysis. *CYP2C9 c.1075A>C (*3)* and *CYP3A4 g.15389C>T (*22)* were monomorphic among the Black Africans, and *CYP2C9 c.1080C>G (*5)* was monomorphic among the Mixed Ancestry group. Table S4 shows the variant allele frequencies for the 27 SNPs in the two population groups. The variants that significantly varied according to the distribution between the Black African and Mixed Ancestry group included *ABCB1 c.3435T*, *CYP2C8*2*, *CYP2C9*8*, *CYP3A5*3*, *CYP3A5*6* and *VKORC1 g.-1639A*.

TABLE 1 Effects of demographic and clinical characteristics on warfarin maintenance dose among black Africans and Mixed Ancestry

	Black Africans ($N = 252$)			Mixed Ancestry ($N = 251$)		
Variable	Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)	Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)
Age						
<50 years	39 ± 13 (17.5–72.5)	.03	0.64 (0.22 to 1.06)	38 ± 15 (10–72.5)	<.0001	0.74 (0.38 to 1.09)
50–70 years	39 ± 13 (12.5–75)			33 ± 13 (7.5–72.5)		
>70 years	31 ± 12 (17.5–70)			28 ± 12 (7.5–52.5)		
Gender						
Female	39 ± 13 (17.5–75)	.49	0.15 (−0.14 to 0.45)	32 ± 14 (7.5–72.5)	.25	−0.17 (−0.43 to 0.09)
Male	37 ± 13 (12.5–70)			35 ± 15 (10–72.5)		
BMI						
<18.5	43 ± 18 (17.5–70)	.73	0.38 (−0.26 to 1.02)	31 ± 13 (17.5–52.5)	.05	−0.20 (−0.91 to 0.51)
18.5–24.9	37 ± 14 (17.5–75)			30 ± 13 (7.5–72.5)		
25–29.9	39 ± 13 (12.5–70)			36 ± 16 (12.5–72.5)		
>30	38 ± 12 (12.5–70)			34 ± 14 (10–72.5)		
Warfarin indication						
Atrial fibrillation						
No	39 ± 13 (17.5–75)	.16	0.29 (−0.06 to 0.66)	33 ± 14 (7.5–72.5)	.81	0.002 (−0.28 to 0.28)
Yes	35 ± 13 (12.5–70)			33 ± 15 (10–72.5)		
Deep venous thrombosis						
No	37 ± 13 (12.5–75)	.11	−0.20 (−0.47 to 0.07)	32 ± 14 (7.5–72.5)	.004	−0.41 (−0.74 to −0.09)
Yes	40 ± 12 (17.5–70)			38 ± 12 (12.5–65)		
Mechanical valve replacement						
No	37 ± 12 (12.5–70)	.05	−0.38 (−0.68 to −0.08)	34 ± 15 (7.5–72.5)	.17	0.17 (−0.08 to 0.42)
Yes	42 ± 15 (20–75)			32 ± 14 (10–72.5)		
Pulmonary embolism						
No	38 ± 13 (12.5–75)	.61	0.18 (−0.36 to 0.72)	34 ± 14 (10–72.5)	.33	0.19 (−0.21 to 0.61)
Yes	36 ± 12 (17.5–57.5)			31 ± 17 (7.5–62.5)		
Comorbidities						
Hypertension						
No	40 ± 13 (12.5–72.5)	.04	0.27 (0.02 to 0.52)	34 ± 13 (10–72.5)	.41	0.07 (−0.17 to 0.32)
Yes	36 ± 13 (12.5–75)			33 ± 15 (7.5–72.5)		
Diabetes mellitus						
No	39 ± 13 (12.5–75)	.32	0.32 (−0.18 to 0.83)	33 ± 13 (7.5–72.5)	.38	−0.24 (−0.56 to 0.08)
Yes	34 ± 14 (12.5–55)			36 ± 17 (10–72.5)		
Heart failure						
No	40 ± 12 (17.5–72.5)	.03	0.25 (−0.004 to 0.49)	34 ± 14 (10–72.5)	.16	0.17 (−0.08 to 0.42)
Yes	37 ± 14 (12.5–75)			32 ± 14 (7.5–72.5)		
HIV						
No	38 ± 13 (12.5–75)	.91	−0.04 (−0.38 to 0.31)	33 ± 14 (7.5–72.5)	.36	−0.21 (−0.92 to 0.49)
Yes	39 ± 15 (17.5–70)			36 ± 14 (7.5–52.5)		
Co-treatments						
Statins						
No	39 ± 13 (12.5–72.5)	.06	0.35 (−0.09 to 0.79)	33 ± 13 (7.5–72.5)	.73	−0.15 (−0.45 to 0.15)
Yes	34 ± 15 (12.5–75)			35 ± 17 (10–72.5)		

(Continues)

TABLE 1 (Continued)

Variable	Black Africans (*N* = 252) Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)	Mixed Ancestry (*N* = 251) Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)
Efavirenz						
No	38 ± 13 (12.5 ± 75)	.77	−0.15 (−0.66 to 0.36)	33 ± 14 (7.5–72.5)	.20	−0.61 (−1.75 to 0.53)
Yes	40 ± 15 (17.5–70)			42 ± 6 (35–47.5)		
Alcohol consumption						
No	38 ± 12 (12.5–75)	.23	−0.33 (−0.70 to 0.04)	32 ± 14 (7.5–72.5)	.18	−0.17 (−0.41 to 0.08)
Yes	42 ± 16 (17.5–72.5)			35 ± 14 (7.5–72.5)		
Tobacco smoking						
No	38 ± 13 (12.5–75)	.35	−0.23 (−0.66 to 0.19)	33 ± 14 (7.5–72.5)	.98	<0 (−0.25 to 0.25)
Yes	41 ± 13 (20–70)			33 ± 14 (7.5–72.5)		

Abbreviation: CI, confidence interval.

3.3 | Linkage disequilibrium mapping of the CYP2C cluster including CYP2C8, 9 and 19

The profile of linkage disequilibrium (LD) mapping in the CYP2C cluster region varied between the Black Africans and Mixed Ancestry population groups (Figure S1). The strongest LD was observed among Black Africans between SNPs *CYP2C19 c.681G>A (*2)* (rs4244285) and *CYP2C8 rs11572101T>C* (D' = 0.94, r^2 = 0.80), whilst, *CYP2C rs12777823G>A* had a moderate LD with *CYP2C rs12772169C>T* and *CYP2C8 rs11572101T>C* in *the* Black Africans (D' = 0.91, r^2 = 0.48; D' = 0.79, r^2 = 0.32, respectively) and in the Mixed Ancestry (D' = 0.77, r^2 = 0.30; D' = 0.56, r^2 = 0.31,respectively). However, a moderate LD was observed between SNPs *CYP2C19 g.-806C>T (*17) (rs12248560)* and *CYP2C8 c.805A>T (*2) (rs11572103)* only *among* Black Africans (D' = 0.72, r^2 = 0.45), whilst SNPs *CYP2C rs12772169C>T* and *CYP2C8 rs1926705G>A* had a moderate LD only among the Mixed Ancestry ([D' = 0.61, r^2 = 0.10]). Detailed LD results with complete LD parameters (i.e. LOD score, D' and r^2) for the studied CYP2C cluster SNPs among both Black Africans and Mixed Ancestry is outlined in Table S5.

3.4 | Correlation of SNP genotypes with warfarin weekly maintenance dose

The SNP genotypes were evaluated for their association with mean warfarin weekly maintenance doses required to keep INR in the normal range for the two population groups, Black Africans and the Mixed Ancestry. The comparison of mean weekly warfarin maintenance dose for genotypes were done using additive, dominant and recessive genetic models. Among the Black Africans, the maintenance dose distribution was significantly correlated ($p \leq .05$) with the following SNPs in an additive model: *CYP2C8 c.805A>T (*2)*, *CYP2C9 c.449G>A (*8)*, *CYP2C9 c.1003C>T (*11)* and *CYP2C rs12777823G>A* with the following effect size −1.06 (95% CI: −1.68 to −0.43), 1.09 (95% CI: −0.05 to 2.24), 0.94 (95% CI: 0.05 to 1.84) and 0.98 (95% CI: 0.46 to 1.50), respectively. *CYP2C rs12772169C>T* and *CYP3A5 c.624G>A (*6)*, were borderline correlated (p = .05 and .04, respectively) with the warfarin maintenance dose with the effect size of 0.54 (95% CI: 0.09 to 0.98) and −0.52 (95% CI: −1.68 to 0.64), respectively, among Black Africans.

The variants *CYP2C9 c.449A* (i.e., GG = 40 ± 13 mg/week vs. AA+GA = 31 ± 10 mg/week), *CYP2C rs12777823A* (i.e., GG = 41 ± 14 mg/week vs. AA+GA = 35 ± 12 mg/week), *CYP2C rs12772169T* allele (i.e., CC = 40 ± 13 mg/week vs. TT+CT = 36 ± 13 mg/week) were associated with decreased mean weekly warfarin maintenance doses. In contrast, *CYP2C8 c.805T (*2)* (i.e., AA = 36 ± 12 mg/week vs. TT+AT = 40 ± 14 mg/week), and *CYP3A5 c.624A (*6)* (i.e., GG = 35 ± 13 mg/week vs. AA+GA = 40 ± 12 mg/week) variants were associated with significantly higher mean weekly warfarin maintenance dose (p < .05) requirements. Among the Mixed Ancestry population group, the SNPs that exhibited statistically significant correlation with warfarin maintenance dose in an additive model, included, *CYP2C9 c.430C>T (*2)*, *CYP2C8 c.792C>G (*4)* and *VKORC1 g.-1639G>A* (p = .02, .03 and <.0001, respectively). *CYP2C9 c.430C>T (*2)* had a borderline association with warfarin maintenance dose with the effect size of 0.55 (95% CI:0.06 to 1.03), whilst *CYP2C8 c.792C>G (*4)* and *VKORC1 g.-1639G>A* had a significant association with the warfarin maintenance dose with the effect size of 1.47 (95% CI: −2.63 to −0.31) and 1.25 (95% CI:0.79 to 1.69), respectively.to 1.03), whilst *CYP2C8 c.792C>G (*4)* and *VKORC1 g.-1639G>A* had a significant association with the warfarin maintenance dose with the effect size of 1.47 (95% CI: −2.63 to −0.31) and 1.25 (95% CI:0.79 to 1.69), respectively. There were no important haplotypes identified that had an influence on weekly warfarin maintenance dose requirement, as the SNPs with strong linkage (i.e., *CYP2C19 c.681G>A (*2)* (rs4244285) and *CYP2C8 rs11572101T>C)* did not have any individual statistical significance correlation with mean weekly warfarin maintenance dose among both Black Africans and Mixed ancestry.

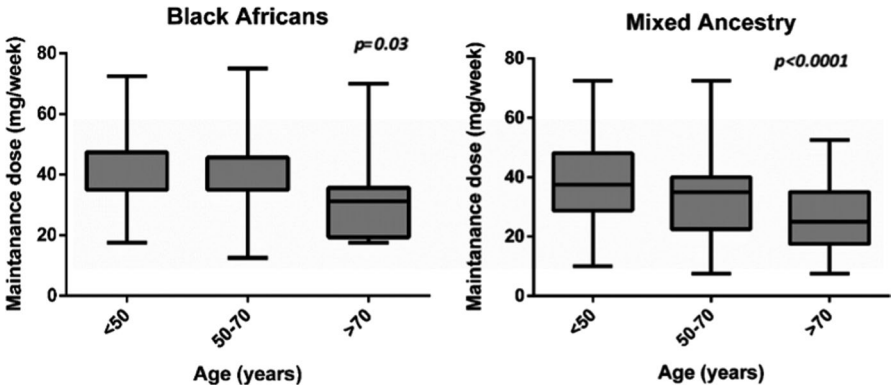

FIGURE 1 Comparison of the warfarin weekly maintenance dose distribution according to age groupings among black Africans (recruited from Cape Town, South Africa and Harare Zimbabwe) and the Mixed Ancestry (recruited from Cape Town)

The interaction and cumulative influence of the various studied genetic and nongenetic variables on the warfarin maintenance dose was analyzed using a stepwise regression model. Table 3 outlines the stepwise regression analysis for the studied population groups, starting with a full model comprising non-genetic variables and SNPs with a $p \leq .20$ for their effect in univariable analysis. Variables were then eliminated in the subsequent models, in a stepwise fashion, starting with variables with the highest *p*-value until variables with $p \leq .05$ remained in the final model. In a univariate analysis, deep venous thrombosis did not have a statistically significant effect on warfarin dose variability ($p = .14$, $R^2 = 0.004$) among Black Africans, however the effect of deep venous thrombosis was increased when factors such as mechanical valve replacement, *CYP2C8 c.805A>T (*2)* and *CYP3A5 c.624G>A (*6)* were entered in the stepwise regression analysis. In contrast, the effect of age and mechanical valve replacement were independent of other variables as they both had a statistical significance influence on warfarin dose variability in a univariate analysis among Black Africans. Thus, age, deep venous thrombosis and mechanical valve replacement were non-genetic factors that were significantly associated ($p \leq .05$) with the mean maintenance dose among Black Africans, together with five SNPs, *CYP2C rs12777823G>A*, *CYP2C8 c.805A>T (*2)*, *CYP2C9 c.449G>A (*8)*, *CYP2C9 c.1003C>T (*11)*, and *CYP3A5 c.624G>A (*6)* which exhibited a consistent significant association ($p \leq .05$).

The maintenance dose variability explained by non-genetic (i.e., age, deep venous thrombosis, mechanical valve replacement) and genetic factors (i.e. *CYP2C8 c.805A>T (*2)*, *CYP2C9 c.449G>A (*8)*, *CYP2C9 c.1003C>T (*11)*, *CYP2C rs12777823G>A* and *CYP3A5 c.624G>A (*6)*), among Black Africans were 9.2% and 18.8%, respectively, thus explaining 28% of the warfarin variability ($p < .0001$). In a univariate analysis for the Mixed Ancestry, non-genetic variables such as deep venous thrombosis, age and BMI had a statistical significance influence on warfarin dose variability, however when entered in a stepwise regression analysis, deep venous thrombosis lost its significance effect. Thus, a stepwise regression analysis for the Mixed Ancestry group resulted in age and BMI being the non-genetics factors remaining in the final model complimented by *VKORC1 g.-1639G>A* as a single genetic factor that was significantly ($p \leq .05$) associated with the weekly maintenance dose in the final model, taken together explaining 22% of warfarin variability ($p < .0001$). Non-genetic factors (i.e. age and BMI) and genetic factors (i.e. *VKORC1 g.-1639G>A*) contributed 10% and 12%, respectively, to the cumulative (22%) maintenance dose variability among the Mixed Ancestry group.

In a model combining the two populations groups and adjusted for ethnicity; age, deep venous thrombosis, *CYP2C8 c.805A>T (*2)*, *CYP2C9 c.449G>A (*8)*, *CYP2C9 c.1003C>T (*11)*, *CYP3A5 c.624G>A (*6)*, and *VKORC1 g.-1639G>A* stayed in the final model explaining 23% of the warfarin maintenance dose variability ($p < .0001$) (Table S6). When interaction terms were included in the stepwise regression model of the combined ethnicities, ethnicity*age and ethnicity*BMI were the only interaction terms that were significant in the final model but they did not the improve the variability ($R^2 = 0.23$) explained by the model. Thus, factors that are of possible importance regardless of ethnic groups were age, deep venous thrombosis, *CYP2C8 c.805A>T (*2)*, *CYP2C9 c.449G>A (*8)*, *CYP2C9 c.1003C>T (*11)*, *CYP3A5 c.624G>A (*6)*, and *VKORC1 g.-1639G>A*. Table 4 outlines factors that we are proposing to be considered for inclusion in warfarin-dosing pharmacogenetic algorithms among African populations. We present here parameters/variables that should be considered in warfarin pharmacogenetics-dosing algorithm among Black Africans and the Mixed Ancestry population group.

4 | DISCUSSION

Warfarin remains a very important therapeutic drug widely used across the world and particularly in poorly resourced healthcare settings including many African countries. However, the variability in the dose required to reach and maintain warfarin international normalized ratio, differs greatly among patients. Whereas several studies have decoded the factors associated with this variability, there is little or no information on genetic variation and its contribution to the variability observed in African populations.[39,40] This study is a contribution in decoding the pharmacogenomics of Africans using Southern African populations. The study investigated genetic variation in the genes that affect the pharmacokinetics of warfarin with respect to their contribution to the variability in warfarin dosing. The study particularly focused on SNPs in *ABCB1*, *CYP1A2*, *CYP2C8*, *CYP2C19*, *CYP3A4* and *CYP3A5*, in addition to the traditionally characterized *VKORC1* and *CYP2C9* genes.

TABLE 2 Effects of genetic variation in warfarin-associated pharmacogenes on warfarin maintenance dose among black Africans and Mixed Ancestry

	Black Africans ($N = 252$)			Mixed Ancestry ($N = 251$)		
SNP genotype	Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)	Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)
ABCB1 c.3435C>T, rs1045642						
CC	37 ± 13 (12.5–70)	.29	0.25 (−0.22 to 0.72)	32 ± 14 (10–65)	.77	0.09 (−0.52 to 0.69)
CT	34 ± 9 (17.5–47.5)			33 ± 14 (10–72.5)		
TT	52.5 (52.5–52.5)			31 ± 14 (12.5–52.5)		
CYP1A2 g.−3860G>A (*1C), rs2069514						
GG	36 ± 12 (12.5–70)	.67	0.20 (−0.49 to 0.89)	33 ± 15 (10–72.5)	.87	−0.28 (−1.69 to 1.13)
GA	39 ± 14 (12.5–70)			33 ± 15 (12.5–65)		
AA	34 ± 15 (17.5–57.5)			36 ± 4 (32.5–40)		
CYP1A2 g.−163C>A (*1F), rs762551						
CC	34 ± 13 (12.5–70)	.28*	−0.16 (−0.59 to 0.27)	26 ± 14 (17.5–52.5)	.38	−0.40 (−1.21 to 0.41)
CA	39 ± 12 (17.5–70)			35 ± 16 (10–72.5)		
AA	38 ± 14 (12.5–70)			31 ± 11 (15–52.5)		
CYP2C8 rs1926705G>A						
GG	41 ± 13 (27.5–52.5)	.83	0.26 (−0.88 to 1.41)	35 ± 17 (17.5–70)	.76	0.03 (−0.66 to 0.74)
GA	37 ± 12 (17.5–70)			32 ± 14 (12.5–72.5)		
AA	37 ± 13 (12.5–72.5)			34 ± 16 (7.5–72.5)		
CYP2C8 rs11572100A>G						
AA	37 ± 13 (12.5–72.5)	.85	0.47 (−0.93 to 1.86)	33 ± 15 (7.5–72.5)	.96	−0.78 (−0.63 to 0.48)
AG	38 ± 11 (17.5–70)			35 ± 17 (15–70)		
GG	31 ± 19 (17.5–45)			-		
CYP2C8 rs11572101T>C						
TT	37 ± 13 (12.5–72.5)	.98	−0.13 (−0.94 to 0.69)	32 ± 14 (7.5–72.5)	.63	−0.11 (0.84 to 0.63)
TC	37 ± 13 (12.5–70)			36 ± 17 (10–72.5)		
CC	39 ± 17 (20–67.5)			34 ± 11 (15–52.5)		
CYP2C8 c.792C>G (*4), rs1058930						
CC	37 ± 13 (12.5–72.5)	.76	0.19 (−1.21 to 1.58)	33 ± 14 (7.5–72.5)	.03	1.47 (−2.63 to −0.31)
CG	35 (35–35)			54 ± 14 (45–70)		
CYP2C8 c.805A>T (*2), rs11572103						

(Continues)

TABLE 2 (Continued)

	Black Africans ($N = 252$)			Mixed Ancestry ($N = 251$)		
SNP genotype	Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)	Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)
AA	36 ± 12 (12.5–70)	.04*	−1.06 (−1.68 to −0.43)	33 ± 14 (7.5–72.5)	.19	−0.38 (−0.90 to 0.15)
AT	38 ± 12 (17.5–70)			38 ± 16 (17.5–72.5)		
TT	49 ± 19 (20–72.5)			-		
CYP2C8 rs11572105C>A						
CC	37 ± 13 (17.5–70)	.41	−0.33 (−1.04 to 0.39)	33 ± 13 (10–62.5)	.16	-
CA	41 ± 12 (35–70)			15 (15–15)		
AA	45 (45–45)					
CYP2C9 c.430C>T (*2), rs1799853						
CC	38 ± 13 (12.5–70)	.52	0.29 (−0.71 to 1.28)	34 ± 14 (7.5–72.5)	.02	0.55 (0.06 to 1.03)
CT	34 ± 9 (27.5–47.5)			27 ± 13 (10–62.5)		
CYP2C9 c.1075 A>C (*3), rs1057910						
AA	38 ± 13 (12.5–70)	-	-	34 ± 14 (7.5–72.5)	.32	0.21 (−0.22 to 0.65)
AC	-			31 ± 15 (10–62.5)		
CYP2C9 c.1080C>G (*5), rs28371686						
CC	38 ± 13 (12.5–70)	.43	−0.33 (−1.32 to 0.67)	34 ± 14 (7.5–72.5)	-	-
CG	42 ± 9 (35–52.5)			-		
CYP2C9 c.449G>A (*8), rs7900194						
GG	40 ± 13 (12.5–70)	.001*	1.09 (−0.05 to 2.24)	35 ± 17 (17.5–72.5)	.88	−0.07 (−0.74 to 0.59)
GA	32 ± 10 (17.5–57.5)			34 ± 14 (7.5–72.5)		
AA	26 ± 9 (17.5–25)			-		
CYP2C9 c.1003C>T (*11), rs28371685						
CC	39 ± 13 (12.5–70)	.03	0.94 (0.05 to 1.84)	34 ± 14 (10–72.5)	.48	0.41 (−0.48 to 1.29)
CT	27 ± 8 (17.5–35)			28 ± 12 (7.5–37.5)		
CYP2C19 c.681G>A (*2), rs4244285						
GG	37 ± 13 (12.5–70)	.52	0.30 (−0.70 to 1.31)	33 ± 15 (10–70)	.78	0.27 (−0.51 to 1.05)
GA	38 ± 12 (17.5–70)			34 ± 15 (15–72.5)		
AA	33 ± 5 (25–35)			29 ± 12 (17.5–47.5)		
CYP2C19 c.636G>A (*3), rs4986893						
GG	37 ± 13 (12.5–70)	-	-	33 ± 14 (10–47.5)	.76	0.19 (−0.82 to 1.19)
GA	-			30 ± 19 (10–47.5)		

(Continues)

TABLE 2 (Continued)

	Black Africans (*N* = 252)			Mixed Ancestry (*N* = 251)		
SNP genotype	Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)	Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)
CYP2C19 c.1A>G (*4), rs28399504						
GG	37 ± 12 (12.5–70)	-	-	33 ± 14 (10–72.5)	.63	-
GA				37.5 (37.5–37.5)		
CYP2C19 g.−806C>T (*17), rs12248560						
CC	36 ± 12 (12.5–70)	.64	0.08 (−1.32 to 1.49)	33 ± 14 (10–70)	.69	0.49 (−0.67 to 1.64)
CT	39 ± 14 (17.5–700)			34 ± 16 (12.5–72.5)		
TT	35 (35–35)			26 ± 13 (15–40)		
CYP2C rs12777823G>A						
GG	41 ± 14 (12.5–75)	.0001*	0.98 (0.46 to 1.50)	33 ± 13 (7.5–72.5)	.78	0.16 (−0.29 to 0.62)
GA	37 ± 12 (17.5–70)			34 ± 15 (7.5–72.5)		
AA	28 ± 8 (12.5–45)			31 ± 12 (15–52.5)		
CYP2C rs12772169C>T						
CC	40 ± 13 (17.5–72.5)	.05*	0.54 (0.09 to 0.98)	33 ± 15 (10–70)	.38	0.36 (−0.24 to 0.95)
CT	37 ± 13 (12.5–70)			34 ± 14 (10–72.5)		
TT	33 ± 12 (12.5–52.5)			28 ± 13 (7.5–47.5)		
CYP3A4 g.15389C>T (*22), rs35599367						
CC	37 ± 13 (12.5–70)	-	-	33 ± 15 (10–72.5)	.91	0.004 (−0.82 to 0.83)
CT	-			33 ± 12 (17.5–52.5)		
CYP3A5 g.6986A>G (*3), rs776746						
AA	37 ± 13 (12.5–70)	.44	−0.79 (−2.18 to 0.62)	29 ± 14 (10–62.5)	.64	−0.06 (−0.63 to 0.52)
AG	40 ± 14 (12.5–70)			34 ± 13 (10–72.5)		
GG	48 ± 18 (35–60)			34 ± 16 (12.5–70)		
CYP3A5 c.624G>A (*6), rs10264272						
GG	35 ± 12 (12.5–70)	.04*	−0.52 (−1.68 to 0.64)	33 ± 15 (10–72.5)	.93	0.008 (−0.82 to 0.83)
GA	40 ± 12 (17.5–70)			31 ± 13 (17.5–52.5)		
AA	41 ± 5 (35–45)			27.5 (27.5– 27.5)		
CYP3A5 g.27131_27132insT (*7), rs41303343						
AA	38 ± 13 (12.5–70)	.63	0.22 (−1.18 to 1.62)	32 ± 15 (10–72.5)	.25	−0.36 (−1.08 to 0.37)
AT	34 ± 12 (12.5–52.5)			37 ± 12 (17.5–50)		
TT	35 (35–35)			-		

(Continues)

TABLE 2 (Continued)

	Black Africans ($N = 252$)			Mixed Ancestry ($N = 251$)		
SNP genotype	Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)	Maintenance dose (mg/week), mean ± SD (range)	*p* value	Cohen's d (95% CI)
VKORC1 g.–1639G>A, rs9923231						
GG	38 ± 13 (12.5–75)	.49	0.52 (−0.87 to 1.91)	37 ± 13 (10–72.5)	.0001*	1.25 (0.79 to 1.69)
GA	38 ± 11 (17.5–70)			31 ± 14 (7.5–72.5)		
AA	31 ± 5 (27.5–35)			21 ± 12 (10–62.5)		

Abbreviation: CI, confidence interval.

*p value ≤.05 for the dominant genetic model.

Evaluating current pharmacogenetics-guided warfarin algorithms and the presented data on African populations here, it is interesting to note that variants such as *CYP2C9*2* and *CYP2C9*3* which are an important inclusion in most warfarin-dosing algorithms appear to play no significant role among Black Africans, because they are rare among Africans.[23,27,41] Some of the common variables included in warfarin dosing algorithms include genetic factors *CYP2C9 c.430C>T (*2)*, *CYP2C9 c.1075A>C (*3)* & *VKORC1 g.–1639G>A* and non-genetics factors age, body surface area, amiodarone, race, targeted INR, smoking, thromboembolism, height, weight, enzyme inducer status, stroke indication, diabetes, and Fluvastatin use, in different combinations (Table 4).[18,42,43] We present variables that are important in African populations and these include deep venous thrombosis, BMI, variants in *CYP2C8*, *CYP2C9* and *CYP3A5*, which have not been included in most of the available warfarin dosing algorithms.

Age is the only non-genetic variable that seems to be important across population or geographical populations as reflected in the various warfarin dosing algorithms (Table 4). What is clear is that African populations present with a wide range of important genetic variants markers that are important for warfarin-dosing, further buttressing the genomic diversity of African populations and the short linkage disequilibrium (LD). The genomic diversity among Africans is further confirmed by the varying LD profiles of the CYP2C cluster SNPs observed between the two population groups included in the present study. Our results further show the important role of gene-environmental interactions on warfarin maintenance dose variability in various population groups, as non-genetic factors such as deep venous thrombosis and mechanical valve replacement contributed to warfarin dose variability among Black Africans only and not in Mixed Ancestry.

The common denominator of existing warfarin-dosing algorithms is their exclusion or lack of consideration of variants that are of pharmacogenomics importance among Africans. We report here on additional variants in *CYP2C9 (CYP2C9*8 & CYP2C9*11)*, and in genes that were peripheral in warfarin disposition, *CYP2C8* and *CYP3A5* on their significant association with mean weekly warfarin maintenance doses among Black Africans. The effect of *CYP2C9 c.449G>A (*8)* and *CYP2C9 c.1003C>T (*11)* on warfarin maintenance dose that we report here, is an extension of our earlier report,[27] which showed a trend towards significant association between these SNPs and mean weekly warfarin maintenance dose. Thus, here we report with a larger sample size for the Black African group ($n = 252$), firmly confirming our earlier report of the influence of *CYP2C9 c.449G>A (*8)* and *CYP2C9 c.1003C>T (*11)* on warfarin maintenance dose and this finding is consistent with other studies that characterized populations with considerable African ancestry such as African Americans.[44]

We further confirm in this study, our earlier report[27] where we showed *CYP2C rs12777823G>A* to be strongly associated with prediction for warfarin maintenance dose among Black Africans. Although the effect of the *CYP2C rs12777823G>A* and *CYP2C9* variants on warfarin maintenance dose has been described before,

TABLE 3 Stepwise regression model of the effect of multiple variables on warfarin weekly maintenance dose among black Africans and Mixed Ancestry

Black Africans				Mixed Ancestry			
Variable	**Coefficient**	**95% CI**	***p* value**	**Variable**	**Coefficient**	**95% CI**	***p* value**
Model including variables with $p \leq .2$ in univariate analysis: $R^2 = 0.30$, $p < .0001$				**Model including variables with $p \leq .2$ in univariate analysis: $R^2 = 0.25$, $p < .0001$**			
Age	−0.14	−0.25 to −0.03	.009	Age	−0.22	−0.33 to −0.11	<.0001
Atrial fibrillation	1.43	−3.32 to 6.18	.56	Gender	2.75	−0.72 to 6.23	.12
Deep venous thrombosis	4.00	−0.39 to 8.39	.07	BMI	0.46	0.13 to 0.78	.006
Mechanical valve replacement	6.62	2.14 to 11.1	.004	Deep venous thrombosis	0.78	−3.97 to 5.54	.74
Hypertension	0.12	−3.17 to 3.42	.94	Mechanical valve replacement	−0.44	4.16 to 3.27	.81
Diabetes mellitus	−1.72	−8.00 to 4.57	.59	Diabetes mellitus	2.93	−1.54 to 7.40	.19
Heart failure	−2.13	−5.71 to 1.44	.24	Heart failure	−2.30	−5.78 to 1.18	.19
Statins	−1.83	−7.48 to 3.82	.52	HIV	0.51	−8.77 to 9.78	.91
Alcohol consumption	−1.49	−6.41 to 3.43	.55	Alcohol consumption	1.74	−1.60 to 5.08	.31
*CYP2C8 c.805A>T (*2), rs11572103*	13.3	6.36 to 20.3	.0002 (.001)*	*CYP2C8 c.805A>T (*2), rs11572103*	0.50	−3.99 to 4.99	.83
*CYP2C9 c.449G>A (*8), rs7900194*	−5.65	−9.99 to −1.31	.01 (.06)*	*CYP2C8 c.792C>G (*4), rs1058930*	4.31	−1.34 to 9.95	.13
*CYP2C9 c.1003C>T (*11), rs28371685*	−7.82	−14.3 to −1.30	.02 (.12)*	*CYP2C9 c.430C>T (*2), rs1799853*	−3.63	−9.28 to 2.02	.23
CYP2C rs12777823G>A	−6.54	−13.8 to 0.71	.07	*VKORC1 g.−1639G>A, rs9923231*	−15.1	−20.7 to −9.41	<.0001 (.0004)*
CYP2C rs12772169C>T	1.21	−5.16 to 7.58	.71	-	-	-	-
*CYP3A5 c.624G>A (*6), rs10264272*	8.88	5.44 to 12.3	<.0001 (.0006)*	-	-	-	-
Model including variables with $p \leq .05$: $R^2 = 0.28$, $p < .0001$				**Model including variables with $p \leq .05$: $R^2 = 0.22$, $p < .0001$**			
Age	−0.14	−0.24 to −0.05	.004	Age	−0.24	−0.35 to −0.14	<.0001
Deep venous thrombosis	4.91	1.52 to 8.28	.005	BMI	0.49	0.23 to 0.77	.0004
Mechanical valve replacement	6.36	2.67 to 10.0	.0008	*VKORC1 g.−1639G>A, rs9923231*	−16.1	−21.5 to −10.7	<.0001
*CYP2C8 c.805A>T (*2), rs11572103*	13.2	6.63 to 19.7	<.0001 (.0005)*	-	-	-	-
*CYP2C9 c.449G>A (*8), rs7900194*	−5.62	−9.82 to −1.42	.009 (.05)*	-	-	-	-
*CYP2C9 c.1003C>T (*11), rs28371685*	−7.97	−14.3 to −1.63	.01 (.05)*	-	-	-	-
CYP2C rs12777823G>A	−6.49	−12.4 to −0.60	.03 (.15)*	-	-	-	-
*CYP3A5 c.624G>A (*6), rs10264272*	8.27	5.15 to 11.4	<.0001 (.0005)*	-	-	-	-

Abbreviation: CI, confidence interval.

*Adjusted *p* value for the Bonferroni correction.

TABLE 4 Proposed variables for possible inclusion in an African-specific warfarin pharmacogenetics-based dosing algorithm compared to other algorithms reported in different world populations

References	Genetics variables	Demographic and clinical variables
This study	*VKORC1 g–1639 G>ACYP2C rs12777823G>ACYP2C8 c.805A>T (*2),CYP2C9 c.449G>A (*8),CYP2C9 c.1003C>T (*11),CYP3A5 c.624GA (*6)*	Age (in years), gender, BMI, deep venous thrombosis, mechanical valve replacement
Gage et al[18]	*VKORC1 3673G>A,CYP2C9*3CYP2C9*2*	BSA (per 0.25 m^2), Age (per decade), target INR (per 0.5 increase), Amiodarone, Current smoker, African American race, Venous thromboembolism
International warfarin pharmacogenetics consortium[42]	*VKORC1 g–1639 G>A,CYP2C9 *2CYP2C9 *3*	Age in decades, height (in cm), weight (in kg) Race, Enzyme inducer status, Amiodarone status
Lenzini et al[43]	*VKORC1 g.–1639G>A,CYP2C9*2,CYP2C9*2*	Natural logarithm (INR), $dose_{-3}$ (per mg), age (per year), BSA (per 0.25 m^2), target INR, African ancestry, stroke indication, $dose_{-4}$ (per mg), $Dose_{-2}$ (per mg), diabetes, amiodarone use, fluvastatin use
www.warfarindosing.org(modified from Gage et al[18])	*VKORC1 g.–1639G>A,CYP2C9*2,CYP2C9*3,CYP2C9*5, CYP2C9*6,CYP4F2 V433 MGGCX rs11676382*	Age, sex, race, ethnicity, weight (kg or lbs.), height (feet and inches or cm), smokes, liver diseases, indication, baseline INR, target INR, amiodarone/cordarone® dose (mg/day), statin/HMG CoA reductase inhibitor, any azole and Sulfamethoxazole/Septra/Bactrim/Cotrim/Sulfatrim

the effect of *CYP2C8* and *CYP3A5* variants among Africans have only being examined on other drugs other than warfarin.[45–48] Furthermore, *CYP2C8* phenotype-genotype correlation is not well characterized in humans as data available has mainly focused on reporting the allelic distribution of the *CYP2C8* variants[49–51] and in vitro studies investigating the CYP2C8 enzyme activity.[45,52,53] The variants *CYP2C8*2* and *CYP3A5*6*, which we are proposing for consideration for inclusion in the warfarin pharmacogenetics-based dosing algorithm for African populations, affect metabolism of the isomer, R-warfarin.[54] The results presented here suggest that there is also an important role for R-warfarin on pharmacodynamics, confirming findings by Maddison et al.[55]

This study shows that both *CYP2C8*2* and *CYP3A5*6*, increase warfarin dose requirements. However, this is in contrast to the expected outcome as these variants are associated with reduced enzyme activity and gene expression. For instance, The *CYP2C8 c.805T* variant (coding for *CYP2C8*2* allele) results in functionally impaired CYP2C8.2 variant enzyme, exhibiting reduced intrinsic clearance of paclitaxel and amodiaquine.[45,52] *CYP3A5*6* is reported to reduce expression of CYP3A5 due to a splicing defect caused by the presence of a G14690A transition in exon 7, resulting in the splicing deletion of exon 7, thereby, affecting the availability of CYP3A5 enzyme.[56,57] Thus, it would be expected that with respect to warfarin pharmacokinetics, both these variants should be associated with reduced warfarin-dose requirements. However, the conflicting findings we report here with other previous studies could be alluded to the fact that *CYP2C8* phenotype-genotype correlation is not well characterized in humans and available data has mainly been from in vitro studies investigating the CYP2C8 enzyme activity, as previously mentioned.[45,52,53] Furthermore, considering the low clearance rate of R-warfarin compared to that of S-warfarin (mean half-life of 58 h vs. 33 h),[58] the binding affinity that the metabolizing enzymes have with the R-warfarin might be different with that of other substrates.

Rettie et al[59] reported that the R-warfarin metabolites were produced from low binding affinity reactions between R-warfarin metabolizing enzymes and the R-warfarin substrate, thus the low R-warfarin clearance could be due to a low binding affinity between the R-warfarin and the metabolizing enzymes produced by the wild-type alleles. Therefore, the CYP2C8.2 variant enzyme produced by the *CYP2C8*2* allele could be having an increased binding affinity with R-warfarin, thereby increasing warfarin clearance consequently resulting in increased warfarin dose requirement. This phenomenon is similar to that reported by Kaminsky and Zhang[14] where variant CYP1A1-Val462 had a high R-warfarin binding affinity and increased metabolic rate compared to the wild type CYP1A1-Ile462. In addition, our results also show that the effect of *CYP2C8*2* is independent of other SNPs in the CYP2C cluster that have been shown before to affect warfarin dose variability, as the LD results displayed a weak linkage between *CYP2C8*2* with *CYP2C9* SNPs and *CYP2C rs12777823G>A*. With regards to *CYP3A5*6*, previous studies have reported that the CYP3A5 expression and metabolic activity is not modulated by *CYP3A5* SNPs alone but by multiple factors which include endogenous molecules (e.g., circulating hormones and drug-drug interactions) and exogenous molecules (e.g., diet and environmental conditions).[60,61] Thus, the influence of *CYP3A5*6* on warfarin maintenance dose could be driven by additional factors not considered in the study.

Considering that R-warfarin is less potent than S-warfarin, the increased warfarin dose associated with variants of genes encoding enzymes metabolizing R-warfarin could also suggest that to increase the R-warfarin potency an increased dose may be required. This is supported by Maddison et al,[55] where they reported that R-warfarin

reached a pharmacodynamic response when they administered a higher warfarin dose of 80 mg of R-warfarin compared to the 12.5 mg for S-warfarin. More pharmacokinetics-pharmacodynamics studies on the influence of variants involved in R-warfarin metabolism are warranted to further confirm our findings and to better elucidate the functional role they play on R-warfarin metabolism. We further report on the lack of significant association between SNPs *ABCB1 c.3435C>T*, *CYP1A2 g.-3860G>A (*1C)*, *CYP2C19 c.681G>A (*2)*, *CYP3A4 g.15389C>T (*22)* and *CYP3A5 g.6986A>G (*3)* with warfarin maintenance dose. However, *CYP1A2*1C*, has been reported to be associated with significantly high warfarin dose requirements among Chinese patients.[62]

The role of *ABCB1 c.3435C>T* SNP on warfarin dose requirements has been contradictory, with various studies reporting a lack of association with warfarin dose,[63–65] while others such as Wadelius et al[66] and Tavares et al[67] have reported its association with low warfarin dose requirements among Swedish and Brazilian patients, respectively. The lack of association between warfarin dose requirements and *CYP3A5 g.6986A>G (*3)* observed in this study, further confirms observations by Wadelius et al.[66] In addition, to SNPs studied here, it is worth noting that there are other SNPs not reported here but have been studied before in other Sub-Saharan African populations to contribute to warfarin dose variability and should be considered for the African-specific warfarin pharmacogenetics-based dosing algorithm. This include, SNPs *VKORC1 Asp36Tyr* and *VKORC1 Val66Met* which have been associated with warfarin resistance, the former was reported among Ethiopians, Kenyans, Sudanese, and Egyptians at a frequency of 15%, 6%, 6%, and 2.5%, respectively, whilst the latter was reported among Tanzanians and Brazilians of African descent[68–71]

Although our study investigated multiple SNPs in various genes of warfarin pharmacokinetic relevance, this candidate gene approach introduces bias and limits the identification of other novel variants not investigated in the study that could be playing a role in warfarin maintenance dose distribution. Hence, the variables that we identified to be of importance among Black Africans and Mixed ancestry explained only 28% and 22% of warfarin dose variability, respectively. Therefore, a high percentage of the warfarin dose variability remain unexplained, and this could be achieved by decoding more variants important for warfarin dose variability through employing high-throughput techniques such as next generation sequencing (NGS). Our study included individuals recruited at specific areas (i.e., Western Cape and Harare) in South Africa and Zimbabwe, thus, the results presented here does not give a full representation of various factors affecting warfarin dose variability in the different Southern African populations or Africa as a whole. However, the results presented here gives a perspective of some of the important genetic and non-genetic predictors of warfarin dose that can be applied in various African populations, especially populations that migrated from the southern West Africa, knows as populations of "Bantu origin". Missing data on some of the SNPs targeted was a limitation in our study, which was however the genetic variant information was imputed to allow us to draw conclusions on the important genetic variants for warfarin dose among the population studied.

Another the limitation is our study design being cross-sectional and not longitudinal, thus it does not allow us to identify the role of genetics in terms of patients reaching the time to therapeutic range. Considering all the limitations that we have outlined, we postulate that 19%, 3%, 52%, 38% and 40% individuals in the Black African population carry the *CYP2C9*8*, *CYP2C9*11*, *CYP2C rs12777283A*, *CYP2C8*2* and *CYP3A5*6* alleles, respectively, either in the homozygous or heterozygous genotypes. Thus, possibly affecting their warfarin maintenance dose. Furthermore, the difference in warfarin maintenance dose requirements between the homozygous wild-type and homozygous mutant genotypes in these SNPs of interest was over 30% except for *CYP3A5 c.624G>A* which was 17%. Thus, outlining the increased variability in warfarin dose distribution according to alleles in the different SNPs. However, to further confirm the measured clinical significance of these SNPs reported here, additional studies which follow a longitudinal design are required.

5 | CONCLUSION

Our study highlights the importance of widening the populations that are investigated for pharmacogenetics tests and the inclusion of a wide array of genes contributing to pathways that involve warfarin disposition. The current approach of focusing on selected genes (e.g., *VKORC1* and *CYP2C9*) is difficult to apply to different populations. Additionally, our study advocates for inclusion of diverse populations in pharmacogenomics research in order to identify population-specific pharmacogenes variants. We identified 2 SNPs that are potential warfarin dose predictors which have not been studied before with regards to warfarin response in an African setting. We propose an African-specific warfarin pharmacogenetics-based dosing algorithm should include, *VKORC1 g.-1639G>A*, *CYP2C rs12777823G>A*, *CYP2C8 c.805A>T (*2)*, *CYP2C9 c.449G>A (*8)*, *CYP2C9 c.1003C>T (*11)* and *CYP3A5 c.624GA (*6)* in addition to the various demographic and clinical variables. In addition to these warfarin-pharmacokinetics associated genetic variants reported here, it is important to characterize the whole spectrum of warfarin-pharmacodynamics associated genes for their contribution to warfarin response.

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CONFLICT OF INTEREST

None of the authors reported any conflict of interest.

AUTHOR CONTRIBUTION

AN and SM conceptualized the idea, generated the data, analyzed the data and drafted the manuscript; PM reviewed the manuscript draft; APK assisted with data analysis and reviewed the manuscript draft, EC assisted with data analysis and reviewed the manuscript; AW and MN co-supervised all components and reviewed the manuscript draft; CD conceptualized the ideas, supervised all components as principal investigator and reviewed the manuscript drafts. All authors contributed to the final version of the article. The authors read and approved the final manuscript.

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