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Warfarin Pharmacogenomics for Precision Medicine in Real-Life Clinical Practice in Southern Africa: Harnessing 73 Variants in 29 Pharmacogenes

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Abstract

Pharmacogenomics is universally relevant for worldwide modern therapeutics and yet needs further development in resource-limited countries. While there is an abundance of genetic association studies in controlled medical settings, there is a paucity of studies with a naturalistic design in real-life clinical practice in patients with comorbidities and under multiple drug treatment regimens. African patients are often burdened with communicable and non-communicable comorbidities, yet the application of pharmacogenomics in African clinical settings remains limited. Using warfarin as a model, this study aims at minimizing gaps in precision/personalized medicine research in African clinical practice. We present, therefore, pharmacogenomic profiles of a cohort of 503 black Africans ($n = 252$) and Mixed Ancestry ($n = 251$) patients from Southern Africa, on warfarin and co-prescribed drugs in a naturalized noncontrolled environment. Seventy-three ($n = 73$) single nucleotide polymorphisms (SNPs) in 29 pharmacogenes were characterized using a combination of allelic discrimination, Sanger sequencing, restriction fragment length polymorphism, and Sequenom Mass Array. The common comorbidities were hypertension (43–46%), heart failure (39–45%), diabetes mellitus (18%), arrhythmia (25%), and HIV infection (15%). Accordingly, the most common co-prescribed drugs were antihypertensives, antiarrhythmic drugs, antidiabetics, and antiretroviral therapy. We observed marked variation in major pharmacogenes both at interethnic levels and within African subpopulations. The Mixed Ancestry group presented a profile of genetic variants reflecting their European, Asian, and African admixture. Precision medicine requires that African populations begin to capture their own pharmacogenetic SNPs as they cannot always infer with absolute certainty from Asian and European populations. In the current historical moment of the COVID-19 pandemic, we also underscore that the spectrum of drugs interacting with warfarin will likely increase, given the systemic and cardiovascular effects of COVID-19, and the anticipated influx of COVID-19 medicines in the near future. This observational clinical pharmacogenomics study of warfarin, together with past precision medicine research, collectively, lends strong support for incorporation of pharmacogenetic profiling in clinical settings in African patients for effective and safe administration of therapeutics.

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Introduction

PHARMACOGENOMICS IS UNIVERSALLY RELEVANT for worldwide modern therapeutics and yet needs further development in resource-limited countries and in real-life clinical practice contexts. Moreover, for pharmacogenomics to be implemented in routine medical practice, genetic variation type and frequencies ought to be established not only for a given prescribed drug but also for all other commonly co-administered drugs. To this end, Sub-Saharan Africa (SSA) presents with a high burden of communicable diseases such as HIV/AIDS, TB, and malaria, with a rising incidence of noncommunicable cardiovascular disease (CVD) comorbidities (Stambler and Ngunga, 2015). Managing the colliding pandemic of infectious diseases and a growing burden of CVDs faces the potential of numerous drug-drug interactions (Dandara et al., 2014; Danwang et al., 2017).

Of great significance to SSA are thromboembolic disorders, which are one of the leading causes of CVDs (Danwang et al., 2017). Despite the introduction of direct oral anticoagulants (DOACs), the vitamin K antagonist warfarin remains the mainstay of thromboembolic management in many African countries because of its efficacy, low cost, and familiarity of its use by physicians (Lowery et al., 2005; Manji et al., 2011; Nguyen et al., 2013; Sonuga et al., 2016; Stambler and Ngunga, 2015). Analysis of the clinicodemographic profiles of African patients on warfarin treatment confirms the need to manage comorbidities requiring concomitant medications, as this poses huge risks of drug-drug interactions, which may lead to adverse drug events (ADEs) (Kanyi et al., 2017; Ndadza et al., 2019a).

Genotyping and haplotyping data provide extensive evidence of genomic profiles varying significantly between different ethnic groups, with groups in Africa exhibiting 60% heterogeneity compared to Asians and European groups (Tishkoff et al., 2009). Very few African populations have been studied for the genetics of warfarin response, yet warfarin use now encourages the use of pharmacogenetics algorithms (Asiimwe et al., 2020). It is our contention that such algorithms generated with little or no data from African populations may not be effective in Africans because of quantitative and qualitative differences in important pharmacogenomic variants that affect warfarin response (Ndadza et al., 2019b). In the era of precision medicine, pharmacogenomics has an important role, thus the growing need to genetically characterize a wide range of different African ethnic groups.

Due to African genetic diversity, one African ethnic group cannot be used as representative for all African populations. Understanding African population genetics and issues of admixture will allow pharmacogenetics-guided safe and efficacious warfarin dosing in African populations. This study therefore aimed to decode the pharmacogenomic profiles of Africans in Southern Africa, concentrating on genes/pathways associated with warfarin and comedication disposition, by reporting on 73 single nucleotide polymorphisms (SNPs) in 29 genes. The study seeks to contribute to knowledge on the prevalence of genetic variation in Africans and its implications for rational drug use in a region with colliding epidemics of communicable and noncommunicable diseases.

Materials and Methods

Study participants

The study included an analysis of 503 participants on warfarin treatment recruited from Groote Schuur Hospital, Gugulethu Community Health Centre (GCHC) in the Western Cape, South Africa, and Parirenyatwa Group of Hospitals (PGH) in Harare, Zimbabwe. The South African group comprised 98 black Africans and 251 Mixed Ancestry, while all the 154 Zimbabweans were of black African Ancestry.

Ethical considerations

Ethical approval was obtained from the University of Cape Town Human Research Ethics Committee (UCT-HREC Ref. No. 581/2015), the University of Zimbabwe College of Health Sciences, Joint Research Ethics Committee (JREC; Ref. 160/13), and Medical Research Council of Zimbabwe (MRCZ; Ref. MRCZ/B/1815). All participants provided written informed consent before recruitment.

Genotyping

Genomic DNA was extracted from 5 mL of whole blood drawn from each participant, using a modified salting out DNA purification method (modified from Gustafson et al., 1987) and Qiagen Blood Mini Kit according to manufacturer's instructions (Qiagen, Hilden, Germany). Genotyping of 73 variants in 29 genes (Supplementary Table S1) was done using a combination of allelic discrimination, Sanger sequencing, restriction fragment length polymorphism, and Sequenom Mass Array (full methods are described in Supplementary Data). Genotype and allele frequencies of *CYP1A1*, *CYP2C9* (rs2256871), *microsomal epoxide hydrolase 1* (*EPHX1*), *nuclear receptor subfamily 1 group I member 2* (*NR1I2*), and *nuclear receptor subfamily 1 group I member 3* (*NR1I3*) were obtained from work previously published in our group (Dandara et al., 2002; Masimirembwa et al., 1998; Swart et al., 2012). However, Mixed Ancestry was not characterized in these studies.

Data analysis

Genotype and allele frequencies for the studied SNPs among both the black Africans and Mixed Ancestry were determined using an online software platform, Shesis (Yong and He, 2005). Chi-squared analysis was performed on R (version 4.0.3 [October 10, 2020]) to compare the allele frequency distribution among the studied populations and previously reported data among world populations, which included West Africans, East Africans, African Americans, East Asians, and Europeans. Data from the 1000 genome project phase 3 and NCBI ALFA published on Ensembl

(https://www.ensemb.org/index.html) and NCBI single nucleotide polymorphism Database (dbSNP; https://www.ncbi.nlm.nih.gov/snp) were utilized to obtain the comparative data. All statistical tests were performed taking a 5% significance level.

Results

We present results on the pharmacogenomic profiling of participants from Southern Africa (i.e., black Africans and Mixed Ancestry), focusing on 73 SNPs in 29 genes (Table 1) involved in the pharmacology of drugs prescribed together with warfarin to treat comorbidities in African populations. The findings are primarily important in understanding the determinants of response to warfarin treatment as well as responses to the comorbidities, which could improve treatment outcomes. In addition, the results contribute to knowledge on the prevalence of genetic variation in Africans and further inform on rational drug use in a region with colliding epidemics of infectious and noncommunicable diseases, taking into account pharmacogenomics.

The reasons for warfarin treatment for this cohort have been previously reported (Ndadza et al., 2019a, 2021). Among black Africans, the most common warfarin indication (Table 2) was deep vein thrombosis (31%) and the most common comorbidities were hypertension (46%), heart failure (45%), and arrhythmia (25%), respectively. In contrast, mechanical valve replacement (45%) was the most common reason for warfarin treatment among the Mixed Ancestry, with hypertension (43%), heart failure (39%), and diabetes mellitus (18%) as common comorbidities, respectively. Figure 1 highlights the burden of additional concomitant drug usage in patients already on warfarin treatment among black Africans and the Mixed Ancestry. At least 21% (53/251) of Mixed Ancestry patients on warfarin also had statin coprescription.

However, among the black Africans, 45% took herbal supplements, followed by the diuretic furosemide, statins, and the ACE inhibitor enalapril. Both furosemide and enalapril are used to treat high blood pressure and heart failure. Antihypertensives constituted 8 out of the top 12 (75%) coprescribed drugs among black Africans on warfarin. Of the 38 HIV-positive black African participants on warfarin, 25 (10%) were also on antiretroviral therapy consisting of efavirenz (EFV), nevirapine, and lamivudine.

The genotype and allele frequency distribution of the 73 SNPs involved in the pharmacology of drugs co-prescribed with warfarin are presented in Tables 1 and 3, respectively. Allele combinations (i.e., genotypes) in Table 1 are displayed as RR (homozygous reference allele), RO (heterozygous reference and alternate/variant alleles), and OO (homozygous alternate/variant allele). We report here qualitative and quantitative differences in the distribution of variant allele frequencies among black Africans and the Mixed Ancestry. For example, the Mixed Ancestry group presents with an allele frequency of 18% for opioid receptor Mu1 (*OPRM1*) rs1799971 compared to 0% reported among black Africans.

Although over 90% of the variants reported showed similar allele frequencies when comparing black Africans from Southern, West, and East Africa, *CYP2C9* rs2256871 exhibited a high frequency of 58% among black Africans from Southern Africa compared to black Africans from East Africa (15%) and West African (9%). In contrast, the frequency for *CYP2D6* rs16947 was significantly lower among black Africans in Southern Africa (12%) when compared to their West African (65%) and East African counterparts (56%).

There are differences in the distribution of pharmacogene variants when comparing global populations, with over 60% of the variants having varying allele frequencies when comparing black Africans to Europeans and Asians. This is supported by the varying minor allele frequency profiles for *CYP2C8* rs11572103 among Africans ($\sim$20%), Asians (0%), and Europeans (0%), and by the skewed distribution of solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) rs4149056 minor allele as seen in black Africans in Southern Africa (0%), Asians (13%), and Europeans (16%).

Furthermore, variants involved in warfarin disposition, which include *CYP2C9*2* (*c.430T*, rs1799853), *CYP2C9*3* (*c.1075C*, rs1057910), *VKORC1*2* (*g.-1639A*, rs9923231), Vitamin K epoxide Reductase Complex subunit 1 (*VKORC1*) *g.6484T* (rs9934438), *VKORC1*4* (*g.6009T*, rs17708472), and *CYP4F2*2* (*c.1297C>T*, rs2108622) presented with significantly high frequencies ($p \leq 0.05$) among Europeans, Asians, and the Mixed Ancestry group compared to black Africans from Southern Africa (Table 3).

Variants *CYP2C9*8* (*c.449A*, rs7900194) and *VKORC1* (g.6171T, rs13336384) seem exclusively reported among populations of African ancestry. In addition, we report on actionable drug-gene interaction according to recommendations by the Clinical Pharmacogenetics Implementation Consortium [CPIC (2021)] (Fig. 2). Mixed Ancestry participants had high actionable variants for warfarin compared to the black African participants (47 vs. 16), while black Africans had high actionable variants for tacrolimus and codeine among 94 and 59 participants, respectively, compared to 56 and 37 participants among Mixed Ancestry participants.

Discussion

The place of pharmacogenomics in precision medicine is not contested, thus making it imperative that pharmacogenomics knowledge permeates clinical practice for the health and well-being of patients as we respond to sustainable development goals. Historically, clinical observational pharmacogenomics studies have been missing in the precision medicine literature and this article addresses this knowledge gap in a context of warfarin. Warfarin anticoagulant is a good example to illustrate how the integration of pharmacogenomics is central to the success of precision medicine. At least 14.6 million warfarin scripts (MEPS, 2021) were prescribed in the United States alone in 2019.

The 29 genes evaluated and analyzed in this study metabolize drugs that are used in the treatment of a spectrum of disorders. For example, some of the genes affected by the variants metabolize drugs used in psychiatry (e.g., *CYP2D6* and atomoxetine), hematology (coagulation factor 2/coagulation factor 5 [*F2/F5*] and avatrombopag), cardiology (*CYP2D6* and carvedilol), rheumatology (e.g., *CYP2C19* and Carisoprodol), gastroenterology (*CYP2C9* and dexlansoprazole), cardiology (Prasugrel and *CYP2C9*, *2C19*, *2B6*, and *3A5*), and infectious diseases (EFV and *CYP2B6*).

Although warfarin is administered globally, it is administered in different settings and under different backgrounds of disease burden. In European countries, warfarin is administered

Table 1. Genotype Distribution of the 73 Single Nucleotide Polymorphisms in 29 Genes Among Black Africans and Mixed Ancestry

		Black Africans			*Mixed Ancestry*		
Gene	*dbSNP No.*	*RR* (n)	*RO* (n)	*OO* (n)	*RR* (n)	*RO* (n)	*OO* (n)
ABCB1	rs1045642	0.82 (106)	0.17 (22)	0.01 (1)	0.35 (40)	0.50 (56)	0.15 (17)
APOE	rs429358	0.62 (77)	0.34 (45)	0.04 (6)	0.67 (75)	0.32 (36)	0.01 (1)
APOE	rs7412	0.65 (88)	0.28 (34)	0.08 (6)	0.84 (94)	0.14 (16)	0.02 (2)
CALU	rs1043550	0.86 (132)	0.13 (21)	0.01 (1)	0.68 (72)	0.28 (30)	0.04 (4)
CALU	rs339097	0.62 (94)	0.35 (54)	0.03 (5)	0.82 (87)	0.15 (16)	0.03 (3)
COMT	rs4680	0.41 (48)	0.49 (65)	0.10 (15)	0.44 (49)	0.41 (46)	0.15 (16)
CYP1A1[a]	rs1048943	1.00 (244)	0 (0)	0 (0)	—	—	—
CYP1A2	rs2069514	0.58 (68)	0.32 (46)	0.10 (9)	0.73 (80)	0.25 (27)	0.03 (3)
CYP1A2	rs762551	0.20 (30)	0.48 (61)	0.32 (32)	0.07 (8)	0.58 (64)	0.35 (38)
CYP2B6	rs28399499	0.81 (103)	0.18 (23)	0.01 (2)	0.94 (105)	0.06 (7)	0 (0)
CYP2B6	rs3745274	0.45 (52)	0.42 (53)	0.13 (23)	0.50 (56)	0.37 (41)	0.13 (15)
CYP2C cluster	rs12777823	0.49 (115)	0.43 (110)	0.08 (20)	0.59 (148)	0.33 (83)	0.08 (21)
CYP2C cluster	rs12772169	0.30 (62)	0.57 (115)	0.14 (31)	0.33 (49)	0.57 (85)	0.10 (16)
CYP2C8	rs11572105	0.94 (126)	0.05 (7)	0.01 (1)	0.97 (30)	0.03 (1)	0 (0)
CYP2C8	rs11572103	0.65 (120)	0.31 (58)	0.04 (10)	0.87 (122)	0.13 (19)	0 (0)
CYP2C8	rs1058930	0.99 (190)	0.01 (3)	0	0.98 (146)	0.02 (3)	0 (0)
CYP2C8	rs188934928	1.00 (194)	0.00 (0)	0	1.00 (149)	0.00 (0)	0 (0)
CYP2C8	rs11572101	0.68 (125)	0.27 (51)	0.05 (7)	0.58 (82)	0.36 (51)	0.06 (9)
CYP2C8	rs11572100	0.84 (149)	0.15 (31)	0.01 (2)	0.89 (126)	0.11 (16)	0 (0)
CYP2C8	rs1926705	0.79 (148)	0.20 (34)	0.01 (1)	0.52 (73)	0.39 (55)	0.09 (13)
CYP2C9	rs1799853	0.98 (176)	0.02 (4)	0 (0)	0.92 (220)	0.08 (19)	0 (0)
CYP2C9	rs1057910	1.00 (190)	0 (0)	0 (0)	0.90 (197)	0.10 (23)	0 (0)
CYP2C9	rs28371686	0.98 (113)	0.02 (4)	0 (0)	1.00 (105)	0.00 (0)	0 (0)
CYP2C9	rs9332131	0.995 (116)	0.05 (1)	0 (0)	1.00 (105)	0.00 (0)	0 (0)
CYP2C9	rs7900194	0.81 (146)	0.17 (30)	0.02 (4)	0.96 (228)	0.04 (10)	0 (0)
CYP2C9[b]	rs2256871	0.17[c]	0.49[c]	0.34[c]	—	—	—
CYP2C9	rs28371685	0.98 (175)	0.02 (5)	0 (0)	0.98 (213)	0.02 (5)	0 (0)
CYP2C9	rs9332239	1.00 (117)	0 (0)	0 (0)	0.99 (104)	0.01 (1)	0 (0)
CYP2C19	rs12248560	0.01 (2)	0.27 (34)	0.72 (85)	0.03 (3)	0.21 (22)	0.76 (82)
CYP2C19	rs4244285	0.71 (83)	0.26 (33)	0.03 (5)	0.63 (69)	0.30 (33)	0.06 (7)
CYP2C19	rs4986893	1.00 (121)	0 (0)	0 (0)	0.96 (105)	0.04 (4)	0 (0)
CYP2C19	rs28399504	1.00 (121)	0 (0)	0 (0)	0.99 (108)	0.01 (1)	0 (0)
CYP2D6	rs1065852	0.88 (85)	0.10 (8)	0.02 (1)	0.80 (63)	0.20 (16)	0 (0)
CYP2D6	rs28371706	0.68 (61)	0.27 (28)	0.05 (5)	0.95 (75)	0.03 (2)	0.02 (2)
CYP2D6	rs59421388	0.71 (65)	0.28 (28)	0.01 (1)	0.99 (78)	0.01 (1)	0 (0)
CYP2D6	rs35742686	1.00 (94)	0 (0)	0 (0)	0.97 (22)	0.03 (2)	0 (0)
CYP2D6	rs3892097	0.96 (91)	0.04 (3)	0 (0)	0.79 (62)	0.20 (16)	0.01 (1)
CYP2D6	rs28371725	0.94 (87)	0.06 (7)	0 (0)	0.91 (72)	0.08 (6)	0.01 (1)
CYP2D6	rs5030655	1.00 (94)	0 (0)	0 (0)	1.00 (78)	0 (0)	0 (0)
CYP2D6	rs5030656	1.00 (94)	0 (0)	0 (0)	0.99 (78)	0.01 (1)	0 (0)
CYP2D6	rs16947	0.78 (68)	0.21 (24)	0.01 (2)	0.63 (50)	0.28 (22)	0.09 (7)
CYP2D6	rs72549357	0.94 (91)	0.02 (1)	0.04 (2)	0.97 (77)	0 (0)	0.03 (2)
CYP3A4	rs35599367	1.00 (182)	0 (0)	0 (0)	0.94 (104)	0.06 (7)	0 (0)
CYP3A5	rs776746	0.73 (90)	0.26 (33)	0.01 (2)	0.19 (21)	0.46 (50)	0.35 (38)
CYP3A5	rs10264272	0.58 (75)	0.38 (47)	0.04 (3)	0.91 (99)	0.08 (9)	0.01 (1)
CYP3A5	rs41303343	0.73 (94)	0.26 (29)	0.01 (2)	0.92 (100)	0.08 (9)	0 (0)
CYP4F2	rs2108622	0.86 (230)	0.10 (24)	0.04 (9)	0.48 (127)	0.35 (92)	0.17 (43)
DRD	rs1800497	0.37 (46)	0.49 (60)	0.14 (23)	0.43 (48)	0.50 (56)	0.08 (9)
EPHX1[d]	rs1051740	0.65 (74)	0.33 (38)	0.02 (4)	—	—	—
EPHX1[d]	rs2234922	0.51 (96)	0.42 (77)	0.07 (14)	—	—	—
F2	rs1799963	1.00 (182)	0 (0)	0 (0)	0.97 (106)	0.03 (3)	0 (0)
F5	rs6025	1.00 (182)	0 (0)	0 (0)	0.96 (108)	0.04 (4)	0 (0)
GGCX	rs12714145	0.27 (41)	0.48 (74)	0.25 (39)	0.37 (39)	0.47 (50)	0.14 (15)
GLP1R	rs1042044	0.12 (16)	0.51 (59)	0.37 (50)	0.14 (16)	0.52 (56)	0.34 (36)
GLP1R	rs2300615	0.81 (106)	0.17 (21)	0.02 (2)	0.55 (61)	0.39 (42)	0.06 (7)
GLP1R	rs6923761	0.98 (127)	0.00 (0)	0.02 (2)	0.83 (94)	0.15 (17)	0.02 (2)
MTHFR	rs1801131	0.73 (102)	0.26 (26)	0.01 (1)	0.46 (52)	0.43 (49)	0.11 (12)
MTHFR	rs1801133	0.86 (110)	0.14 (28)	0.01 (1)	0.72 (81)	0.25 (28)	0.03 (3)
NR112[e]	rs3732356	0.61 (95)	0.32 (50)	0.08 (12)	—	—	—

(*continued*)

Table 1. (Continued)

Gene	dbSNP No.	Black Africans			Mixed Ancestry		
		RR (n)	*RO* (n)	*OO* (n)	*RR* (n)	*RO* (n)	*OO* (n)
NR112[e]	rs2472677	0.37 (57)	0.48 (75)	0.15 (23)	—	—	—
NR112[e]	rs6785049	0.85 (128)	0.14 (21)	0.007 (1)	—	—	—
NR113[e]	rs2307424	0.88 (138)	0.12 (19)	0.00 (0)	—	—	—
NR113[e]	rs3003596	0.33 (51)	0.46 (72)	0.22 (34)	—	—	—
NR113[e]	rs2502815	0.56 (88)	0.34 (53)	0.10 (15)	—	—	—
OPRM1	rs1799971	1.00 (129)	0.00 (0)	0.00 (0)	0.66 (75)	0.310 (35)	0.03 (3)
PNPLA5	rs5764010	0.94 (120)	0.05 (8)	0.01 (1)	0.78 (88)	0.20 (23)	0.02 (2)
SLCO1B1	rs4149056	0.995 (180)	0.005 (2)	0 (0)	0.86 (96)	0.13 (15)	0.01 (1)
SULT4A1	rs763120	0.94 (119)	0.06 (10)	0.00 (0)	0.78 (87)	0.20 (22)	0.03 (3)
VKORC1	rs9923231	0.85 (208)	0.14 (32)	0.01 (3)	0.49 (123)[c]	0.41 (102)[c]	0.10 (26)[c]
VKORC1	rs9934438	0.75 (210)	0.20 (51)	0.05 (3)	0.54 (141)	0.36 (95)	0.10 (26)
VKORC1	rs7294	0.34 (87)	0.45 (121)	0.21 (55)	0.28 (73)	0.49 (127)	0.23 (59)
VKORC1	rs17708472	1.00 (154)	0 (0)	0 (0)	—	—	—
VKORC1	rs13336384	0.90 (138)	0.10 (16)	0 (0)	—	—	—

[a]Adapted from Dandara et al. (2002).
[b]Adapted from Soko (PhD thesis, personal communication).
[c]Genotype frequencies inferred from allele frequencies. NB: the following 15 polymorphisms, *CYP1A2*2*, *CYP2C8*14*, *CYP2C9*2*, **3*, **12*, *CYP2C19*3*, **4*, *CYP2D6*3*, **6*, **9*, **15*, *CYP3A4*22*, *VKORC1*4*, *F2* rs1799963, *F5* rs6025, were monomorphic.
[d]Adapted from Masimirembwa et al. (1998).
[e]Adapted from Swart et al. (2012).

ABCB1, ATP binding cassette subfamily B member 1; *APOE*, apolipoprotein E; *CALU*, Calumenin; *COMT*, catechol-O-methyltransferase; *CYP*, cytochrome P450; dbSNP, Single Nucleotide Polymorphism Database; *DRD2*, dopamine receptor D2; *EPHX1*, microsomal epoxide hydrolase 1; *F2*, coagulation factor 2; *F5*, coagulation factor 5; *GGCX*, gamma glutamyl carboxylase; *GLP1R*, glucagon like peptide 1 receptor; *MTHFR*, methylenetetrahydrofolate reductase; *NR1/2*, nuclear receptor subfamily 1 group 1 member 2; *NR1/3*, nuclear receptor subfamily 1 group 1 member 3; *OPRM1*, opiod receptor Mu1; *PNPLA5*, patatin like phospholipase domain containing 5; *SLCO1B1*, solute carrier organic anion transporter family member 1B1; *SULT4A1*, sulfotransferase family 4A member 1; *VKORC1*, Vitamin K epoxide Reductase Complex subunit 1.

in a background of cardiovascular comorbidities, while in developing or poor countries, warfarin is administered in a background of infectious disease pandemics colliding with an increasing burden of CVDs. Thus, across the world, patients on warfarin treatment present with a diverse spectrum of comorbidities and therefore response patterns.

Such comorbidities also require treatment with additional drugs that potentially interact with warfarin, thereby affecting treatment outcomes. For example, in this study among Africans in Southern Africa, it was observed that at least 80% of respondents had at least one other concurrent illness that required treatment with an additional drug, which compares well to the 70% reported in another South African study (Sonuga et al., 2016) and 100% reported respondents in a study among Kenyans (Kamuren et al., 2018), and is substantially higher than the 35–42% reported in a Swedish study (Rydberg et al., 2020).

Besides the number of concurrent illnesses, it is important to note that, the type of illness also differs across population groups, thereby pointing to the different outcomes of warfarin treatment due to different interacting drugs in different geographical areas. An additional important factor is the underlying genomic variation that affects drug response, the pharmacogenomics footprint.

Comorbidities can potentially enhance pathology of conditions indicated for warfarin, as observed with atrial fibrillation (AF), where both valvular heart disease and hypertension have been identified as the most common risk factors (Nguyen et al., 2013). Long-standing hypertension is associated with AF, while AF itself is a major clinical indication for initiation of warfarin therapy (Njovane and Fasinu, 2012). Hypertension was a predominant concurrent illness in our study cohort and is known to be a major illness among Africans, most of the time asymptomatically in males (Mutowo et al., 2015). Similarly, hypertension was a predominant comorbidity in similar studies in Sweden (Rydberg et al., 2020), South Africa (Njovane and Fasinu, 2012; Sonuga et al., 2016), and Brazil (Botton et al., 2020).

As much as there seems to be some similarity with respect to predominant comorbidities among patients on warfarin, outcomes in such cases are often comparatively different due to underlying pharmacogene variant profiles across global populations (Ndadza et al., 2021). For example, the observed differences in the frequencies of *CYP2B6 c.516T*, with nearly 40% among Africans and less than 20% in other geographical populations, would differentially affect the outcomes of patients on warfarin and antiretroviral (ARV) drug EFV, with more Africans being affected, while the *SLCO1B1* rs4149056 of 0% among Africans, but up to 16% among Europeans, would affect warfarin outcomes among patients on statins, and the effects would be felt more among Europeans than Africans.

Comorbidities lead to requirements for concurrent medications, which increases risks of drug-to-drug interactions. Data in this study show that at least 31% of the drugs coprescribed with warfarin are contraindicated for warfarin therapy (FDA, Ref ID: 3022954; US FDA, 2021). Our findings support observations in the United States of at least 70–80% of patients on warfarin being co-administered with potentially interacting drugs (Wittkowsky et al., 2004) and about 13% of these being contraindicated for warfarin therapy. Risk of bleeding increases with concurrent illness owing to drug-to-drug interactions. It is even more complicated with

Table 2. Distribution of Parameters Observed Among Participants on Warfarin

Parameters	*Black Africans (N = 252),* N *(frequencies)*	*Mixed Ancestry (N = 251),* N *(frequencies)*
Warfarin indication**		
Atrial fibrillation	34 (0.14)	65 (0.26)
Deep venous thrombosis	77 (0.31)	44 (0.18)
Mechanical valve replacement	53 (0.21)	114 (0.45)
Pulmonary embolism	14 (0.06)	25 (0.10)
Cardiomyopathy	8 (0.03)	0
Congestive cardiac failure	26 (0.10)	0
Rheumatic heart disease	17 (0.07)	0
Stroke	6 (0.02)	1 (0.004)
Others	17 (0.07)	2 (0.008)
Comorbidity*		
Hypertension	11 (0.46)	107 (0.43)
Diabetes mellitus	16 (0.06)	45 (0.18)
Heart failure	113 (0.45)	97 (0.39)
HIV positive	38 (0.15)	7 (0.03)
Arrhythmia	64 (0.25)	0
Dyslipidemia	6 (0.02)	0
Concomitant drugs**		
ARVs	25 (0.10)	3 (0.01)
Aspirin	3 (0.01)	2 (0.01)
Allopurinol	0	3 (0.01)
Amiodarone	2 (0.01)	2 (0.01)
Amlodipine	7 (0.03)	8 (0.03)
Digoxin	13 (0.05)	2 (0.01)
Enalapril	30 (0.12)	0
Furosemide	61 (0.24)	4 (0.02)
Losartan	16 (0.06)	1 (0.004)
Statins	22 (0.09)	53 (0.21)
Spironolactone	28 (0.11)	1 (0.004)
Herbal medicine	72 (0.29)	0
Other drugs (rifampicin, nifedipine, atenolol, propranolol)	106 (0.42)	57 (0.23)

Adapted with modifications from Ndadza et al. (2019a, 2021).
*Denotes global p-value <0.01.
**Denotes global p-value <0.0001.
ARVs, antiretroviral drugs (i.e., efavirenz, nevirapine and lamivudine).

herbal medicines that are commonly used throughout African populations, as evidenced by the 50% respondents in this study using herbal supplements alongside warfarin therapy (Dandara et al., 2021; Thomford et al., 2016).

The major complication with herbal therapy is the lack of information on their effects when used together with other drugs, thus requiring a new impetus to understand herb-drug interactions (Dandara et al., 2019). However, there is little known about warfarin-herb interactions, and in African populations, this paucity of knowledge is further mystified by the limited knowledge on the actual herbal supplements taken, as well as limited research on common African herbal supplements (Ge et al., 2014; Thomford et al., 2018). Herbal medicines together with food interactions are cited numerously as one of the main causes of unstable International Normalized Ratio (INR) (Dandara et al., 2019; Gohil and Patel, 2007).

Importantly, the lack of knowledge on herbal medicines should not always be viewed as a negative on herbal medicines, but should be viewed as a gap in indigenous knowledge translation (Dandara et al., 2021). Thus, a strong call for research on herbal medicines is made and this has a huge potential to unlock a whole health value chain.

Differences in pharmacogene variants are evident among African populations, and these run along ethnic or geographical lines. African populations show marked heterogeneity (Campbell and Tishkoff, 2008; Dandara et al., 2014; Thomford et al., 2015); thus, the need for pharmacogenetic profiling and testing among African populations are large and should be considered for effective treatment and management of disease, especially in cases of concurrent illnesses (Dandara et al., 2014). For instance, the efflux transporter variant ATP binding cassette subfamily B member 1 (*ABCB1*) rs1045642 (c.3435C>T, p.Ile1145Ile) is associated with reduced tramadol, digoxin (Neuvonen et al., 2011), and nevirapine (Zhu et al., 2013) response.

This variant has a frequency of 40% and 57% in South Africans of Mixed Ancestry and among East/Central Africans, respectively, while it only occurs in about 10% among other African populations. Thus, the use of tramadol and digoxin in the South African population, for example, is bound to result in different outcomes among the Mixed Ancestry and the black African groups, with pronounced effects in the Mixed Ancestry population group. This difference in the profile of pharmacogene variants is likely to differentially affect drug interactions that involve warfarin. Thus, our current study on the pharmacogenetic profiles of Africans on warfarin treatment, with special emphasis on the major concurrent medications prescribed to patients, is a step in setting a stage for precision medicine in Africa.

Evaluating the 73 SNPs in the 22 genes shows that several groups of drugs (e.g., statins, antiretrovirals, antidiabetics, and antihypertensives), which are potentially prescribed together with warfarin, need to be taken into account to avoid drug-drug interactions and improve the health and well-being of patients.

Statin pharmacogenetics

Dyslipidemia was observed at a frequency of 21% among patients on warfarin in this study, which agrees with an average of 25% reported for population-based prevalence for dyslipidemia in African populations (Noubiap et al., 2018), implying at least a quarter of Africans on the continent have elevated cholesterol. Statins are the drugs of choice in the treatment of dyslipidemia and are prescribed in at least 5% of patients on warfarin in this study. Influx transporter *SLCO1B1* encoding organic anion transporter protein (OATPIBI) is an important pharmacogene in statin pharmacokinetics. This transporter is partially responsible for the hepatic uptake of statins.

The liver is the site for both therapeutic effect and elimination of the hydrophilic statins pravastatin and active (acidic) simvastatin and rosuvastatin. The variant *SLCO1B1* rs4149056 c.521T>C, p.Val174Ala has been implicated in statin-induced myopathy (Link et al., 2008). The *SLCO1B1* c.521C allele has been shown to increase the odds of statin-induced myopathy in patients on statins through reduced transport or influx of the statin into the liver (Kameyama

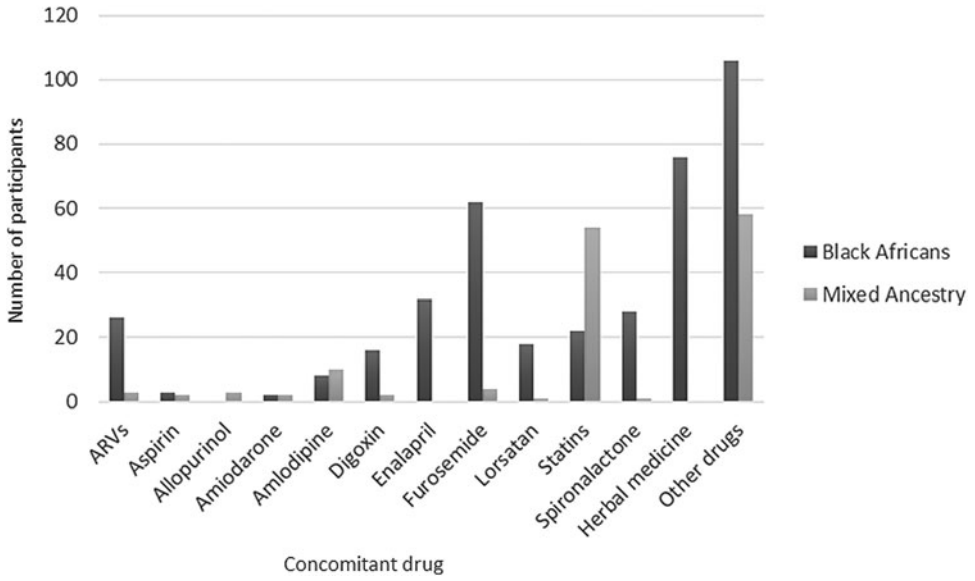

FIG. 1. Burden of various concomitant drug use among Black Africans and Mixed Ancestry.

et al., 2005). Consequently, the CPIC (Ramsey et al., 2014) issued precaution on patients with *SLCO1B1* c.521C and considering statin therapy.

There is marked interethnic variability in the presence of *SLCO1B1* c.521C in global populations. Indeed, in African populations, c.521C allele is significantly lower ($p > 0.0003$) (Soko et al., 2019) when compared to European and Asian populations. Similarly, in our study, *SLCO1B1* c.521C variant occurred at a frequency of 1% in black African populations compared to 13% in the Mixed Ancestry who have significantly higher European admixture (de Wit et al., 2010). This discrepancy strengthens the call for pharmacogenetic profiling among African populations as some subsets of African populations may require different doses of the same drug for effective and safe therapeutic outcome.

Statin therapy is also affected by pharmacodynamic effects and the ability of effective response by the patient. *Apolipoprotein E* (*APOE*) has been associated with varying response to statin therapy. The protein APOE has three isoforms E2, E3, and E4. Of the three isoforms, E2 is associated with better response to statin therapy (Zhang et al., 2019), while E4, defined by the presence of *APOE4* rs429358C and rs7412C polymorphisms, shows the lowest response to statin therapy (Anoop et al., 2010). In patients on warfarin therapy in our study, rs429358C was observed at frequencies of 0.22 and 0.17 in black Africans and the Mixed Ancestry group respectively (Table 3).

These frequencies are much higher than what is observed in both Asian (0.06) and European (0.04) populations. This has bearing on the efficacy of statins in Africans with dyslipidemia with a tendency toward poor outcomes among black Africans compared to their Mixed Ancestry counterparts. *APOE4* may therefore be an important pharmacogene variant in treatment of dyslipidemia in African populations on warfarin therapy.

Antiretroviral pharmacogenetics

The global burden of HIV disproportionately affects Southern Africa. In 2019, 1.4 million and 7.7 million people were living with HIV and AIDS in Zimbabwe and South Africa alone, respectively (UNAIDS, 2020). EFV has been the backbone of first- and second-line regimens in the fight against HIV and AIDS for the past decade. Concurrent administration of antiretroviral therapy in African patients on warfarin is inevitable. Indeed, in this study, the most prescribed ARV drug was EFV. EFV is metabolized predominantly by CYP2B6 and to a lesser extent by CYP1A2 and CYP3A5. *CYP2B6*6*, defined by *c.516G>T* and *c.785A>G* allele, is the most clinically elucidated polymorphism of *CYP2B6* and is associated with decreased expression and function of CYP2B6 enzyme (Hofmann et al., 2008).

Individuals expressing this variant consistently demonstrate significantly reduced levels of the 8-hydroxylation of EFV and increased circulating plasma levels of the parent EFV compound (Xu et al., 2012), which is associated with increased neurotoxicity and central nervous system (CNS) side effects (Nyakutira et al., 2008; Swart et al., 2013). Increased neuropsychiatric events have been reported among Zimbabwean (Nyakutira et al., 2008) and South African patients (Gounden et al., 2010; Pinillos et al., 2015) harboring the *CYP2B6*6* c.516T allele when compared to their counterparts expressing the *CYP2B6*6* c.516C allele.

Frequency of the *CYP2B6*6* c.516T allele is higher in African populations than both Asian and European populations (Dandara et al., 2014; Swart et al., 2013), as is confirmed in our study cohort where frequency of *CYP2B6*6* c.516T ranged from 0.28 to 0.49 in African populations (Table 3), being lowest in South African Mixed Ancestry populations, while frequency was as low as 0.17 in Asians and 0.03 in Europeans, respectively. Thus, *CYP2B6*6 c.516T* pharmacogenetic profiling may be necessary in warfarin patients co-prescribed EFV and any other *CYP2B6* metabolized drugs to reduce potential adverse drug reactions and enhance safe treatment.

CYP1A2 variation is more frequent in African populations with one study done in Ethiopia showing individuals with total absence of CYP1A2 activity (Aklillu et al., 2003). *CYP1A2*1C* was higher in our study cohort among the black Africans when compared to the Mixed Ancestry (Table 3)

Table 3. Comparison of Pharmacogene Variant Distribution Among Major Global Populations

Gene	dbSNP No.	Variant allele	This study		Other African populations		African Americans	East Asians	Europeans
			Black Africans	Mixed Ancestry	West Africans (YRI)	East Africans (LWK)			
ABCB1	rs1045642	T	0.09*,†,‡,§	0.40‡,§,**,††	0.13	0.14	0.23	0.40	0.52
APOE	rs429358	C	0.22†,‡,**	0.17‡,**	0.24	0.38	0.05	0.09	0.16
APOE	rs7412	T	0.22*,†,‡,§,**,††	0.09§	0.11	0.05	0.10	0.10	0.06
CALU	rs1043550	G	0.07*,‡,§	0.18*,‡,§	0.09	0.11	0.17	0.07	0.42
CALU	rs339097	G	0.21*,‡,§	0.10*,‡,§	0.19	0.18	0.14	0.01	0.00
COMT	rs4680	A	0.35	0.35	0.31	0.29	0.31	0.24	0.50
CYP1A1[a]	rs1048943	G	0.00*,†	—	0.00	0.00	0.02	0.25	0.04
CYP1A2	rs2069514	A	0.26*	0.15*,**,††	0.32	0.32	0.21	0.28	0.02
CYP1A2	rs762551	A	0.55	0.64	0.54	0.48	0.60	0.67	0.68
CYP2B6b	rs28399499	C	0.10*,‡,§	0.03§,††	0.12	0.06	0.07	0.00	0.00
CYP2B6	rs3745274	T	0.35	0.32	0.40	0.36	0.35	0.22	0.24
CYP2C cluster	rs12777823	A	0.30*	0.25*	0.29	0.27	0.25	0.31	0.15
CYP2C cluster	rs12772169	T	0.42*	0.39*	0.44	0.41	0.38	0.37	0.22
CYP2C8	rs11572105	T	0.03	0.02	0.04	0.05	0.04	0.00	0.02
CYP2C8	rs11572103	A	0.21*,†,‡,§	0.07*,†,§,††	0.20	0.14	0.07	0.00	0.00
CYP2C8	rs1058930	C	0.005*	0.01*	0.005	0.00	0.02	0.001	0.06
CYP2C8	rs188934928	G	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CYP2C8	rs11572101	G	0.19†	0.24	0.19	0.17	0.19	0.38	0.19
CYP2C8	rs11572100	C	0.09†	0.06†	0.11	0.14	0.09	0.00	0.04
CYP2C8	rs1926705	T	0.89†	0.71†	0.88	0.87	0.80	0.45	0.69
CYP2C9	rs1799853	T	0.01*,‡	0.04*	0.00	0.00	0.07	0.001	0.12
CYP2C9	rs1057910	C	0.00*,§	0.05§	0.00	0.00	0.02	0.03	0.07
CYP2C9	rs28371686	G	0.01	0.00	0.03	0.01	0.006	0.00	0.00
CYP2C9	rs9332131	delA	0.003	0.00	0.02	0.00	0.005	0.00	0.00
CYP2C9	rs7900194	A/T	0.11*,†,‡,§	0.02§	0.05	0.07	0.02	0.00	0.002
CYP2C9[b]	rs2256871	G	0.58*,†,‡,**,††	—	0.09	0.15	0.07	0.00	0.001
CYP2C9	rs28371685	T	0.01	0.01	0.05	0.02	0.02	0.00	0.002
CYP2C9	rs9332239	T	0.00	0.005	0.00	0.00	0.001	0.00	0.003
CYP2C19	rs12248560	T	0.14†	0.13†	0.25	0.18	0.22	0.02	0.22
CYP2C19	rs4244285	A	0.17†	0.22	0.17	0.21	0.18	0.31	0.15
CYP2C19	rs4986893	A	0.00†	0.00†	0.00	0.01	0.00	0.06	0.00
CYP2C19	rs28399504	G	0.00	0.00	0.00	0.00	0.001	0.001	0.001
CYP2D6	rs1065852	T	0.07*,†,‡	0.10*,†	0.11	0.04	0.19	0.57	0.20
CYP2D6	rs72549357	T	0.05	0.03	—	—	—	—	—
CYP2D6	rs28371706	T	0.19*,‡,§	0.04‡,§,**,††	0.26	0.19	0.14	0.00	0.00
CYP2D6	rs59421388	A	0.15*,‡,§	0.006‡,§ **,††	0.11	0.17	0.07	0.00	0.00
CYP2D6	rs35742686	delA	0.00	0.00	0.00	0.00	0.008	0.00	0.02
CYP2D6	rs3892097	A	0.02*,‡,§	0.11†,§ **	0.06	0.03	0.15	0.00	0.19
CYP2D6	rs28371725	A	0.03	0.05††	0.009	0.03	0.09	0.04	0.09
CYP2D6	rs5030655	delA	0.00	0.00	0.00	0.00	0.01	0.00	0.02
CYP2D6	rs5030656	CT	0.00	0.006	0.00	0.00	0.01	0.00	0.03
CYP2D6	rs16947	T	0.12*,‡,**,††	0.23‡,**,††	0.56	0.65	0.46	0.14	0.34
CYP3A4	rs35599367	T	0.00*	0.03	0.00	0.00	0.00	0.00	0.05
CYP3A5	rs776746	G	0.15*,†,‡,§	0.58*,‡,§,**,††	0.17	0.12	0.31	0.71	0.95
CYP3A5	rs10264272	A	0.24*,†,‡,§	0.05*,†,§,**,††	0.17	0.24	0.12	0.00	0.003
CYP3A5	rs41303343	T	0.14*,†,‡,§	0.04§,**,††	0.12	0.12	0.04	0.00	0.00
CYP4F2	rs2108622	T	0.10*,‡,§	0.34‡,§,**,††	0.06	0.11	0.12	0.21	0.29
DRD	rs1800497	A	0.39*	0.33	0.41	0.37	0.34	0.41	0.19
EPHX1[c]	rs1051740	C	0.19†	—	0.10	0.20	0.19	0.48	0.30
EPHX1[c]	rs2234922	G	0.28†	—	0.42	0.33	0.32	0.12	0.16
F2	rs1799963	A	0.00	0.01	0.00	0.00	0.00	0.00	0.008
F5	rs6025	A	0.00	0.02	0.00	0.00	0.005	0.00	0.02
GGCX	rs12714145	T	0.49	0.40	0.52	0.46	0.39	0.38	0.42
GLP1R	rs1042044	C	0.63	0.60	0.61	0.67	0.57	0.55	0.56
GLP1R	rs2300615	G	0.11†,§	0.26‡,§,**,††	0.06	0.09	0.09	0.44	0.18
GLP1R	rs6923761	A	0.03*	0.09*	0.00	0.005	0.09	0.01	0.33
MTHFR	rs1801131	C	0.15*,§	0.32§,††	0.12	0.19	0.17	0.22	0.31

(*continued*)

TABLE 3. (CONTINUED)

			This study		Other African populations			East Asians	Europeans
Gene	*dbSNP No.*	*Variant allele*	*Black Africans*	*Mixed Ancestry*	*West Africans (YRI)*	*East Africans (LWK)*	*African Americans*		
MTHFR	rs1801133	T	0.08*,†	0.15*,†	0.11	0.07	0.13	0.30	0.36
NR1I2[d]	rs3732356	G	0.23*	—	0.31	0.30	0.26	0.12	0.06
NR1I2[d]	rs2472677	T	0.35*,†	—	0.36	0.40	0.40	0.62	0.66
NR1I2[d]	rs6785049	A	0.04*,†,‡	—	0	0.05	0.12	0.40	0.62
NR1I3[d]	rs2307424	T	0.05*,†,‡	—	0.10	0.09	0.16	0.52	0.35
NR1I3[d]	rs3003596	C	0.42	—	0.61	0.52	0.49	0.57	0.44
NR1I3[d]	rs2502815	T	0.23†,**,††	—	0.44	0.38	0.31	0.44	0.25
OPRM1	rs1799971	G	0.00*,†,‡,§	0.18†,‡,§	0.00	0.005	0.05	0.39	0.16
PNPLA5	rs5764010	T	0.03*,‡,§	0.12‡,§ **,††	0.04	0.006	0.04	0.19	0.10
SLCO1B1	rs4149056	C	0.005*,†,‡,§	0.08§,††	0.009	0.02	0.04	0.12	0.16
SULT4A1	rs763120	G	0.03*,‡,§	0.13§,**,††	0.04	0.01	0.05	0.29	0.10
VKORC1	rs9923231	A	0.09*,‡,§	0.31†,‡,§,**,††	0.03	0.04	0.12	0.88	0.39
VKORC1	rs9934438	T	0.12*,‡,§	0.28†,‡,§,**,††	0.03	0.04	0.11	0.88	0.39
VKORC1	rs7294	A	0.44†	0.47†	0.51	0.43	0.46	0.11	0.37
VKORC1	rs17708472	T	0.02*	—	0.02	0.06	0.07	0.00	0.23
VKORC1	rs13336384	T	0.05*,†	—	0.04	0.09	0.05	0.00	0.00

*p-Value ≤0.05 between studied population and Europeans.
†p-Value ≤0.05 between studied population and Asians.
‡p-Value ≤0.05 between studied population and African Americans.
§p-Value ≤0.05 between the studied populations (i.e., Black Africans and Mixed Ancestry).
**p-Value ≤0.05 between studied population and East African.
††p-Value ≤0.05 between studied population and West Africans.
[a]Adapted from Dandara et al. (2002).
[b]Adapted from Soko (2017).
[c]Adapted from Masimirembwa et al. (1998).
[d]Adapted from Swart et al. (2012).
LWK, Luhya in Webuye Kenya; YRI, Yoruba in Ibadan Nigeria.

and was similarly higher than in global populations such as the Europeans and African Americans. Both CYP1A2 and CYP2B6 expression are regulated by the transcription factors pregnane X receptor (PXR) and constitutive androstane receptor (CAR). The genes *NR1I2* and *NR1I3* encode PXR and CAR, respectively. Both PXR and CAR have been implicated in variation in EFV plasma levels in South African patients (Swart et al., 2012). *NR1I3* rs2307424 was associated with increased levels of EFV and hence increased risk of CNS adverse drug response (Swart et al., 2012).

This polymorphism occurs at a significantly lower frequency in African patients (Swart et al., 2012) than in European and Asian patients, likewise this trend was observed among the black African warfarin patients (Table 3). Polymorphisms *NR1I2* rs2472677, rs6785049 and *NR1I3* rs3003596 and rs2502815 were all associated with reduced EFV plasma levels in South African HIV/AIDS patients. The presence of these polymorphisms is postulated to increase expression of CYP2B6 and CYP1A2, hence increasing metabolism of EFV and lowering its concentration in the blood. *NR1I2* rs2472677C>T occurs at a significantly higher frequency in African populations than in both Asian populations, implying the failure of viral suppression maybe higher in African patients with HIV on warfarin treatment coprescribed EFV, than their European and Asian counterparts.

In 2019, the WHO recommended the use of an integrase inhibitor Dolutegravir (DTG) as a first- and second-line regimen to gradually replace EFV therapeutic use in HIV patients. DTG has good tolerability and predictable pharmacokinetics in adults (WHO, 2019) when compared to EFV. However, concerns surround plasma level-linked neuropsychiatric events (Yagura et al., 2017) and weight gain (Norwood et al., 2017) in patients switching from DTG to EFV. DTG is primarily metabolized by UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1), and partly by CYP3A. Variations in the *UGT1A1* gene, in particular *UGT1A1*28* and *6*, have been associated with increased levels of DTG (Yagura et al., 2017). Transporter genes *SLC22A2* (Norwood et al., 2017) and *ABCG2* (Tsuchiya et al., 2017) have also been implicated in increased plasma levels of DTG.

Frequency of both *UGT1A1*28* and *6* in African populations is low; these polymorphisms are likely to play a minor role in the pharmacogenomics of DTG therapy in African HIV patients. Similarly, *ABCG2* rs2231142 (c.421C>A, pGln141Lys), which is associated with increased plasma levels of DTG, also occurs in significantly ($p < 0.001$) lower levels in African populations (1%) than in both European (10%) and Asian (19%) populations (Soko et al., 2019). It may be necessary to investigate the African-specific pharmacogenetic variants that may play a role in interindividual variability in response and toxicity to DTG than rely on polymorphisms identified in Asian or European populations.

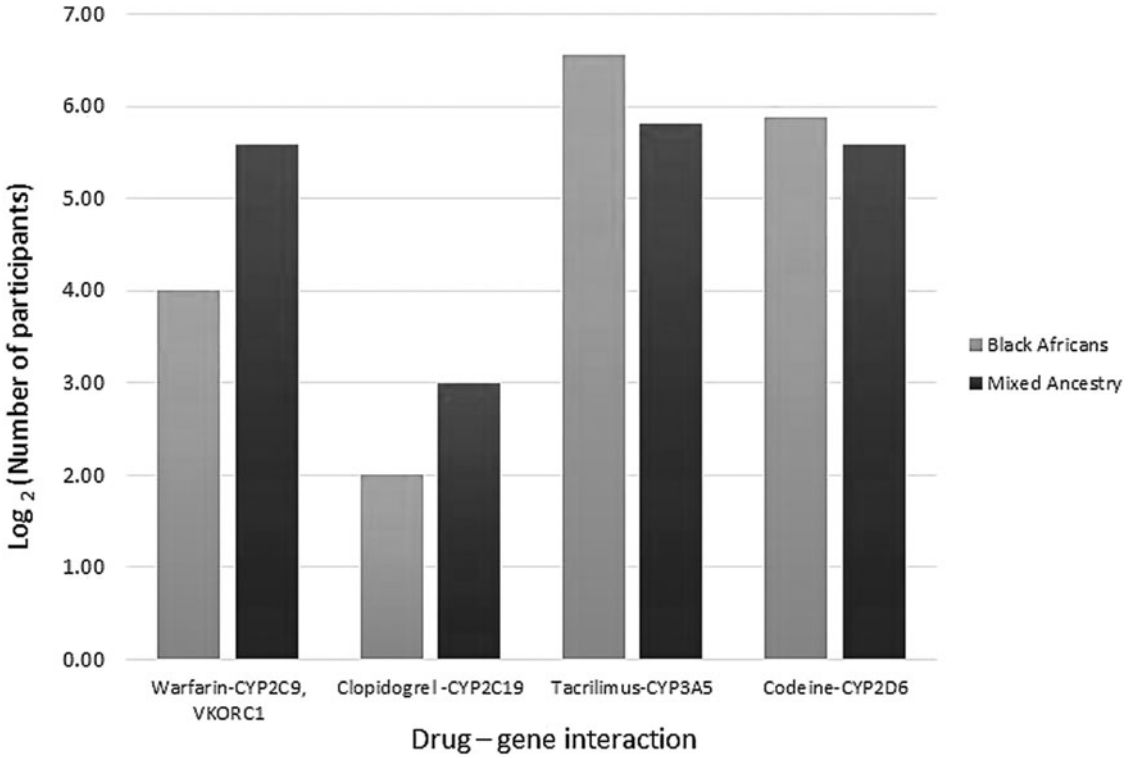

FIG. 2. Clinically actionable drug-genotype interactions among Black Africans and Mixed ancestry. The definition of actionable variants as adapted (with modifications) from Goh et al. (2017) is defined as follows: (1) warfarin, *CYP2C9**2, **3*, **8*, **11* heterozygote or homozygote with *VKORC1* GA or AA genotype and *CYP2C9*1* with *VKORC1* AA genotype; (2) clopidogrel, *CYP2C19**2 and **3*; (3) tacrolimus, *CYP3A5**1 heterozygote or homozygous; and (4) codeine, *CYP2D6* poor and intermediate metabolizers. *VKORC1*, Vitamin K epoxide Reductase Complex subunit 1.

Antihypertensive pharmacogenetics

The pharmacogenetics of antihypertensives has a huge bearing on patients on warfarin therapy in both Zimbabwe and South Africa. Hypertension was the predominant comorbidity in this study, with at least 40% of the respondents co-prescribed the diuretic furosemide, while the antihypertensives enalapril, losartan, carvedilol, atenolol, hydrochlorothiazide spironolactone, and nifedipine were among the most common co-prescribed drugs in this study population (Table 2).

Antihypertensives are themselves a broad class of drugs with differing pharmacokinetics and pharmacodynamics. Little is known of the pharmacokinetics and pharmacodynamics of furosemide, which is the most prescribed antihypertensive in our study cohort; however, it is believed the drug is metabolized predominantly in the kidney (Pichette and du Souich, 1996) by glucuronidation by UGT1A9, UGT2B7, and UGT1A6. However, variants in genes encoding *CYP2C9*, *CYP2D6*, and *CYP3A5* have all been implicated in the pharmacogenetics of antihypertensive drugs (Oliveira-Paula et al., 2019). Transporter genes *SLC4A1* and *SLCO1B1* have also been associated with the pharmacogenetics of antihypertensives (Oliveira-Paula et al., 2019). Indeed, *SLCO1B1* *15/*15 carriers showed a 6.94-fold increased risk (95% confidence interval = 1.30–37.07, $p = 0.020$) of enalapril-induced cough as an ADE to hypertension therapy.

As *SLCO1B1*15/*15* occurs in close to 0% in Southern African populations, it is unlikely this polymorphism can be used for enalapril toxicity, instead, it is possible that other uniquely African variants (Soko et al., 2019) may play a role in enalapril pharmacogenetics. The enzyme CYP2C9 is an important enzyme in warfarin pharmacogenetics. Poor metabolism of warfarin leads to prolonged bleeding in patients. The CPIC in 2017 (CPIC, 2017) provided a guideline for warfarin dosing using a pharmacogenomics algorithm. Due to interethnic variability in warfarin pharmacogenetics, patients are grouped into non-African ancestry and African ancestry. Genotypes with impact on warfarin dosing for non-Africans are *CYP2C9* *2/*3 and *3/*3.

Numerous studies show that *CYP2C9**2 (R144C) and *3 (I359L), which are predominantly European polymorphisms, increase risk of prolonged bleeding in warfarin patients. Similarly, *CYP2C9*5*, **6*, **8*, and **11*, predominantly African variants, are incorporated into the African warfarin pharmacogenetics algorithm (CPIC, 2017; Ndadza et al., 2021) as they too affect warfarin therapy. Indeed, the effects of *CYP2C9*2* and **3* variants are seen in the angiotensin II receptor blocker, losartan. Losartan is metabolized to its active form E-3174 by CYP2C9. Individuals expressing the *CYP2C9*3* variant showed reduced metabolism of oral losartan (Bae et al., 2012), while *CYP2C9**2 showed a slightly better clearance of plasma levels of oral losartan compared to the *CYP2C9**3 variant (Babaoglu et al., 2004).

Losartan was co-prescribed in 10% of participants in this study (Table 2), implying reduced CYP2C9 would affect not only losartan metabolism but also warfarin metabolism. A uniquely African variant *CYP2C9*9 variant*, first reported

by Matimba et al. (2009), occurs at a frequency of 58% in black Africans (Zimbabweans) and is postulated to reduce CYP2C9 activity, and hence increase toxicity to drugs metabolized by CYP2C9.

This implies that at least 10% of Zimbabwean patients could have adverse drug reactions to both losartan and warfarin, thus decreasing the safe and efficacious use of these two drugs in this population. As *CYP2C9* is an important gene in warfarin treatment, pharmacogenetic testing of *CYP2C9* focusing on uniquely African variants should be of paramount importance in African populations where comorbidities, especially hypertension, are high.

CYP3A4 and CYP3A5 are also important genes/enzymes in the drug-drug interaction of warfarin. CYP3A4 activity is more dominant in European populations, while CYP3A5 is more active in individuals of African ancestry (Bains et al., 2013). CYP3A5 and CYP3A4 have overlapping specificities and together are responsible for the metabolism of 50–60% of all clinical drugs, including erythromycin, nevirapine, lopinavir, tamoxifen, EFV, and the statins (lovastatin, simvastatin, and atorvastatin), drugs that are co-prescribed in warfarin patients, as observed in this study.

The CYP3A isoforms are also responsible for the metabolism of antihypertensives amlodipine and nifedipine, which were also reportedly co-prescribed in patients on warfarin in this study. *CYP3A4**1B and **22* are the most characterized SNPs of *CYP3A4*. *CYP3A4**22 is associated with reduced messenger RNA (mRNA) and enzyme expression that resulted in elevated statin plasma levels (Wang et al., 2011). *CYP3A4*22* is rare in global populations; its highest occurrence is 5% in European populations. In our study, *CYP3A4*22* was only observed among Mixed Ancestry (3%), probably owing to European admixture, and was virtually absent in the black population groups. *CYP3A4*22* is unlikely to play a major role in the pharmacogenetics of antihypertensives, or any other CYP3A cluster drug in Southern African populations.

*CYP3A5*3* (rs776746) is the most documented nonfunctional variant of *CYP3A5*. *CYP3A5*3* is defined by the presence of the c.6986A allele. The A allele creates an alternatively spliced form that alters the reading frame, and introduces a premature stop codon resulting in a nonfunctional truncated protein (Kuehl et al., 2001). Thus, carriers of the *CYP3A5*3/*3* genotype do not express CYP3A5. *CYP3A5*3* occurs at a frequency of 95% in Europeans, 71% in Asians, 58% in South Africans of Mixed Ancestry, and 15% black Africans. The total absence of the *1 wild-type allele in European individuals implies there is reduced activity of CYP3A5 in drug disposition; hence, CYP3A5 plays little role in drug pharmacogenetics in European and Asian individuals.

However, variations in CYP3A5 have an effect on interindividual differences among populations of African ancestry; thus, CYP3A5 inhibitors may have a more profound effect on drug metabolism in black Africans than those of Mixed Ancestry. As CYP3A5 metabolizes over 50% of clinical drugs, including the antihypertensives (amlodipine and nifedipine), this enzyme may play a significant role in the pharmacogenetics of drugs co-prescribed with warfarin in African patients.

CYP2D6 metabolizes up to 25% of drugs commonly used in clinical practice. It is the enzyme primarily responsible for metabolizing the β-blocker carvedilol (Parker et al., 2018). Carvedilol, which is used to treat hypertension and heart disease, was co-prescribed to 10% of Zimbabwean participants on warfarin treatment. *CYP2D6* is highly polymorphic, giving rise to four main phenotypes; poor metabolizers (PM) who lack a single functional allele, intermediate metabolizers (IM) who have one null allele, extensive metabolizers who are the normal phenotype, and ultra-metabolizers who possess more than one copy of the *CYP2D6* gene (i.e., gene duplications). PM have been shown to have two to three times higher R-carvedilol plasma levels, which lead to higher risk of drug-related dizziness (Dean, 2017).

PM status occurs at frequencies of 5–10% in Europeans (Lymperopoulos et al., 2016) and is rare in both Asian and African individuals. *CYP2D6*17* (rs28371706, c.1023C>T) occurs in at least 30% of African individuals (Lymperopoulos et al., 2016; Masimirembwa et al., 1996) and is associated with reduced enzyme activity. Individuals carrying the *CYP2D6**17 allele are classified as IM.

CYP2C19 plays a minor role in the metabolism of the beta-blocker propranolol, which was co-prescribed to 2% of the Zimbabwean respondents. Two important SNPs observed in this study, *CYP2C19*17* (c.-806C>T, rs12248650), occurring 13–17% in the three African populations, are rare among Asians (4%) and highest in Europeans (26%). *CYP2C19*17* is associated with increased activity of the enzyme (Sibbing et al., 2010) and therefore increased clearance of CYP2C19 substrates. However, *CYP2C19*17* carriers may have reduced response to propranolol, especially in the presence of reduced activity of CYP2D6, the principal metabolizing enzyme.

The second variant, *CYP2C19*2* (c.618G>A, rs4244285), is the most common loss of function allele known to CYP2C19 (Scott et al., 2011). *CYP2C19*2* creates an aberrant splice variant in exon 5 that alters the mRNA reading frame producing a truncated nonfunctional protein (de Morais et al., 1994). *CYP2C19*2* occurs at a frequency of 17% in black Africans, 22% in South Africans of Mixed Ancestry, and 31% (Asians) and 15% in Europeans. Evidently, the effect of reduced CYP2C19 is expected to be more pronounced in South Africans of Mixed Ancestry than among their black African counterparts.

COVID-19 therapy or interventions

In July of 2021, the WHO (2021) reported that there were 191,773,590 confirmed COVID-19 cases globally; the WHO Africa Region accounted for 4,688,762 of these cases. Within the African region, South Africa bore half of the COVID-19 burden with 2,327,472 confirmed cases, while Zimbabwe had 91,120 cases. Since December 2019, COVID-19 has grown into a global pandemic that has affected every nation on earth. It can, therefore, be expected that patients on warfarin are likely to be treated for COVID-19. Although during our recruitment, COVID-19 was not yet a global threat to the population under study, concurrent exposure to COVID-19 by warfarin patients in Africa cannot be ignored; hence, the pharmacogenomics of COVID-19 therapy is worth considering.

Pharmacogenomics of COVID-19 is critical among warfarin patients in Africa as it may point clinicians to first-line choices and initial dosages, given that the list of approved drugs keeps growing and recommendations are still evolving. This will allow reduced risk of drug efficacy in patients who cannot afford ineffective therapy in the face of a life-

threatening infection. Furthermore, as patients with severe symptoms tend to also have comorbidities (Yang et al., 2020), drug toxicity needs to be reduced. Using a combination of online sources, Takahashi et al. (2020) identified several drug-gene pairs that may play a role in the pharmacogenomics of COVID-19 therapy. These included hydroxychloroquine/chloroquine (*CYP2C8*, *CYP2D6*, *SLCO1A2*, and *SLCO1B1*), azithromycin (*ABCB1*), ribavirin (*SLC29A1*, *SLC28A2*, and *SLC28A3*) and lopinavir/ritonavir (*SLCO1B1*, *ABCC2*, and *CYP3A*).

The macrolide ivermectin is currently under trial in both Zimbabwe and South Africa for possible application in COVID-19 therapy. Being lipophilic, ivermectin is widely distributed in the body, but it is metabolized in liver microsomes into ten different metabolites by CYP3A4 (Canga et al., 2008). Pharmacogenomics of anti-COVID-19 drugs is likely to be African specific as studies have already shown that pharmacogene variants are ethnically biased (Dandara et al., 2014), a variant that may play a significant role in one population may not play a major role in another.

Furthermore, our study confirms the heterogeneity of African populations as seen by the variation among the South African Mixed populations when compared to their black counterparts. It therefore is imperative for African populations to profile pharmacogenetic variants within their populations to achieve safe and efficacious administration of COVID-19 treatment in patients co-prescribed with warfarin.

COVID-19 is a systemic disease whose long-term effects are far reaching and go beyond the lungs. Long-term effects of COVID-19 include damage to the lungs, heart, and brain (Lopez-Leon et al., 2021). The potential of a myriad of therapeutic agents on an already burdened African warfarin patient cannot be ignored. As such, COVID-19 introduces a new drug-drug dilemma for warfarin patients in Africa. Pharmacogenomic biomarkers are vital (Şardaş and Özdemir, 2021) for inclusion in drug development, on-going clinical trials, and drug repurposing as they offer a conceptual and practical steering wheel for therapeutic management of COVID 19.

As African populations display significant interindividual and population differences in drug treatment outcomes, and pharmacogenomic markers in Africans differ from other global populations, it is vital to include African pharmacogenomic biomarkers in emerging clinical trials for COVID-19 medicines and their therapeutic implementation strategies.

Antimalarial and antidiabetic therapy

CYP2C8, which is implicated in the metabolism of hydroxychloroquine and chloroquine, is also involved in the metabolism of antidiabetics (meglitinides and thiazolidinedione), the antimalarials (chloroquine and aminoquinoline), the anticonvulsant carbamazepine, and the anti-inflammatory pain killers diclofenac and ibuprofen (Daily and Aquilante, 2009).

Diabetes was a concurrent illness in 5% of the Zimbabwean patients, while none of the patients was taking antimalarials during recruitment. However, as malaria is endemic in Zimbabwe, it can be expected that during the course of warfarin therapy, some patients will receive concurrent treatment of malaria. A total of 4/154 (3%) Zimbabwean patients were also co-prescribed CYP2C8 substrate, carbamezapine. CYP2C8 comprises 7% of the total hepatic CYP content of the liver (Totah and Rettie, 2005). It shares 74% sequence homology with *CYP2C9* and is therefore regulated by similar transcription factors like PXR.

Therefore, pharmacogenomic variants that affect PXR function will ultimately affect *CYP2C8* transcription and hence overall function in patients. *CYP2C8* variants show ethnic bias with *CYP2C8*2* occurring at a frequency of 21% in black African patients co-prescribed warfarin. However, *2 is rare in individuals of European descent and occurs in 7% of the Mixed Ancestry South Africans. *CYP2C8*2* (rs11572103) is associated with lowered intrinsic clearance of the enzyme's metabolites and may therefore be an important variant in African populations, especially those with less European admixture. Similarly, *CYP2C8*3*, another *CYP2C8* variant with lowered activity, is rare in African populations, but occurs in 20% of European populations (Daily and Aquilante, 2009). *CYP2C8*4* (rs1058930), another variant associated with low enzyme activity, is rare in African populations, but occurs at a frequency of 1% in European individuals.

Both *CYP2C8*3* and *CYP2C8*4* therefore may play a minor role in African populations co-prescribed CYP2C8 substrates when compared to their European counterparts. However, CYP2C8 has an active site like CYP3A4; as a result, CYP3A4 and CYP2C8 have overlapping substrates. The effects of CYP2C8 are therefore unlikely to be detrimental in African populations co-prescribed warfarin as most concurrent drugs will be metabolized by CYP3A4.

Conclusions

African patients on warfarin tend to have a high incidence of comorbidities owing to the high burden of both communicable and noncommunicable diseases on the African continent. We present the pharmacogenetic profiles of 503 Southern Africans on warfarin therapy. Most of these patients were also on concurrent treatment for hypertension, heart failure, dyslipidemia, diabetes, and/or HIV infection. Thus, pharmacogenetic profiles presented in this study were for drug-metabolizing enzymes, receptors, and transporters involved in the pharmacology of drugs co-prescribed with warfarin in our study cohort. Black African patients present with similar pharmacogenetic profiles.

However, their profiles are distinct from both Asian and European populations, especially in pharmacogenetic SNPs predominantly useful among Asian and European populations. Similarly, South Africans of Mixed Ancestry have pharmacogenetic profiles somewhat different from the black Africans, showing the effect of admixture in African subpopulations.

Hence, to ensure safe and efficacious warfarin therapy, pharmacogenetic profiling of African subpopulations should be enhanced. Precision medicine requires African populations begin to capture their own pharmacogenetic SNPs as they cannot infer with absolute certainty from Asian and European populations. African heterogeneity also requires subpopulation investigation as seen by the differences between African blacks when compared to South Africans of Mixed Ancestry.

Authors' Contributions

S.M. and A.N. conceptualized the idea, generated and analyzed data and drafted the article. B.K. generated data. E.C. analyzed data and reviewed the article. G.K., C.M.M. and C.F.B.N. conceptualized the idea, supervised the work,

and reviewed the article. M.N. and C.D. conceptualized the ideas, supervised all components, and reviewed the article drafts. All authors made a significant intellectual contribution to the final revised version.

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Supplementary Material

Supplementary Data
Supplementary Table S1

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Abbreviations Used

ABCB1 = ATP binding cassette subfamily B member 1
ADE = adverse drug event
AF = atrial fibrillation
APOE = *apolipoprotein E*
ARV = antiretroviral drug
CALU = Calumenin
CAR = constitutive androstane receptor
CNS = central nervous system
COMT = catechol-O-methyltransferase
CPIC = Clinical Pharmacogenetics Implementation Consortium
CVD = cardiovascular disease
CYP = cytochrome P450

Abbreviations Used (Cont.)

dbSNP = Single Nucleotide Polymorphism Database
DRD2 = dopamine receptor D2
DTG = dolutegravir
EFV = efavirenz
EPHX1 = microsomal epoxide hydrolase 1
F2 = coagulation factor 2
F5 = coagulation factor 5
GCHC = Gugulethu Community Health Centre
GGCX = gamma glutamyl carboxylase
GLP1R = glucagon-like peptide 1 receptor
IM = intermediate metabolizers
INR = International Normalized Ratio
LWK = Luhya in Webuye Kenya
mRNA = messenger RNA
MTHFR = methylenetetrahydrofolate reductase
NR1I2 = *nuclear receptor subfamily 1 group I member 2*
NR1I3 = *nuclear receptor subfamily 1 group I member 3*
OPRM1 = opioid receptor Mu1
PGH = Parirenyatwa Group of Hospitals
PM = poor metabolizers
PNPLA5 = patatin like phospholipase domain containing 5
PXR = pregnane X receptor
SAMRC = South African Medical Research Council
SLCO1B1 = solute carrier organic anion transporter family member 1B1
SNP = single nucleotide polymorphism
SSA = Sub-Saharan Africa
SULT4A1 = sulfotransferase family 4A member 1
UGT1A1 = UDP glucuronosyltransferase 1 family, polypeptide A1
VKORC1 = Vitamin K epoxide Reductase Complex subunit 1
YRI = Yoruba in Ibadan Nigeria