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Pharmacogenomics of warfarin in populations of African descent

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Warfarin is the most commonly prescribed oral anticoagulant worldwide despite its narrow therapeutic index and the notorious inter- and intra-individual variability in dose required for the target clinical effect. Pharmacogenetic polymorphisms are major determinants of warfarin pharmacokinetic and dynamics and included in several warfarin dosing algorithms. This review focuses on warfarin pharmacogenomics in sub-Saharan peoples, African Americans and admixed Brazilians. These 'Black' populations differ in several aspects, notably their extent of recent admixture with Europeans, a factor which impacts on the frequency distribution of pharmacogenomic polymorphisms relevant to warfarin dose requirement for the target clinical effect. Whereas a small number of polymorphisms in *VKORC1* (3673G > A, rs9923231), *CYP2C9* (alleles **2* and **3*, rs1799853 and rs1057910, respectively) and arguably *CYP4F2* (rs2108622), may capture most of the pharmacogenomic influence on warfarin dose variance in White populations, additional polymorphisms in these, and in other, genes (e.g. *CALU* rs339097) increase the predictive power of pharmacogenetic warfarin dosing algorithms in the Black populations examined. A personalized strategy for initiation of warfarin therapy, allowing for improved safety and cost-effectiveness for populations of African descent must take into account their pharmacogenomic diversity, as well as socio-economical, cultural and medical factors. Accounting for this heterogeneity in algorithms that are 'friendly' enough to be adopted by warfarin prescribers worldwide requires gathering information from trials at different population levels, but demands also a critical appraisal of racial/ethnic labels that are commonly used in the clinical pharmacology literature but do not accurately reflect genetic ancestry and population diversity.

Introduction

'Pharmacogenetics deals with pharmacological responses and their modification by hereditary influences'. This definition, offered by Werner Kalow in the first book dedicated to pharmacogenetics [1], highlights the three pillars of this discipline: pharmacology, genetics and human diversity. Pharmacogenetics has evolved greatly over the 50 years elapsed since Kalow's book was published, was re-christened as pharmacogenomics in the fashion of the 'omics' revolution, but its conceptual development and praxis remain contingent upon a better understanding of human genomic diversity and its impact on drug pharmacokinetics and pharmacodynamics. The human evolutionary history is remarkably short. There is powerful evidence that the anatomically modern man emerged in Africa around 150 000–200 000 years ago and spread to other continents probably within the last 70 000 years. Thus, from a genomic perspective, we are all African-descendants, whether living in Africa or in a quite recent exile outside of Africa [2]. However, the present review on pharmacogenomics of warfarin in populations of African descent adopts a less inclusive approach, and examines sub-Saharan Africans, African-Americans and self-identified Black or Brown ('pardo' in Portuguese) Brazilians. These 'Black' populations differ in several aspects, notably their extent of recent admixture (within the last 500 years) with non-Africans, primarily Europeans. Estimates of European biogeographical ancestry based on autosomal genetic markers reveal that the *average* proportion of European ancestry ranges between 44–73% in Brown Brazilians, 29–54% in Black Brazilians and 4–35%

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in African Americans, depending on the geographical regions of Brazil [3, 4] and the United States [5] in which the studies were conducted. It is noteworthy that the *individual* proportions of African ancestry vary over wide ranges, and most importantly, in a continuous manner within each of these admixed populations from the Americas [3, 4, 6, 7], a pattern that impacts on the frequency distribution of pharmacogenomic polymorphisms in these peoples [8, 9]. Recent European admixture may also be extensive in some sub-Saharan peoples, such as 'Cape Mixed Ancestry' (CMA) from Cape Town, South Africa, and similarly to admixed American populations, the mean European proportion in CMA (19%) is a poor representation of the large inter-individual range of variation (0–86%; [10]). Beyond European gene flow, high levels of admixture of distinct ancestral African clusters prevail in most sub-Saharan African populations, reflecting historical migration events across the continent [10]. Collectively, the findings presented above indicate the diversity and heterogeneity of the populations of African descent considered in this review, and should caution against lumping them together as an ethnic, racial or continental group and extrapolating pharmacogenetic data across these peoples. Finally, we would like to refer to important studies on the pharmacogenomics of warfarin in populations from Africa, such as Ethiopians [11] and Egyptians [12], or having biogeographical African ancestry, such as Hispanics in the United States [13], which are not included in this review.

Warfarin, a 'global' anticoagulant

Warfarin remains the most commonly prescribed oral anticoagulant worldwide for the prevention and treatment of thromboembolic events, despite the notorious inter- and intra-individual variability in the dose required for the target clinical effect, which is commonly assessed by the international normalized ratio (INR). INR values between 2 and 3–3.5 are recommended for most warfarin therapeutic indications. Warfarin is a racemic mixture of R- and S-enantiomers, which differ in anticoagulant activity and are substrates of different cytochrome P-450 enzymes (CYPs). S-warfarin, the more potent enantiomer is metabolized by CYP2C9, whereas various CYPs contribute to the inactivation of R-warfarin. Both R- and S-warfarin affect the coagulation cascade by inhibiting the vitamin K epoxide reductase complex 1 (VKORC1) thereby preventing the carboxylation of clotting factors II, VII, IX and X (Figure 1). There is convincing evidence that genetic polymorphisms contribute to inter-individual differences in warfarin dose requirements, but the fraction of variability explained by pharmacogenetic polymorphisms varies considerably

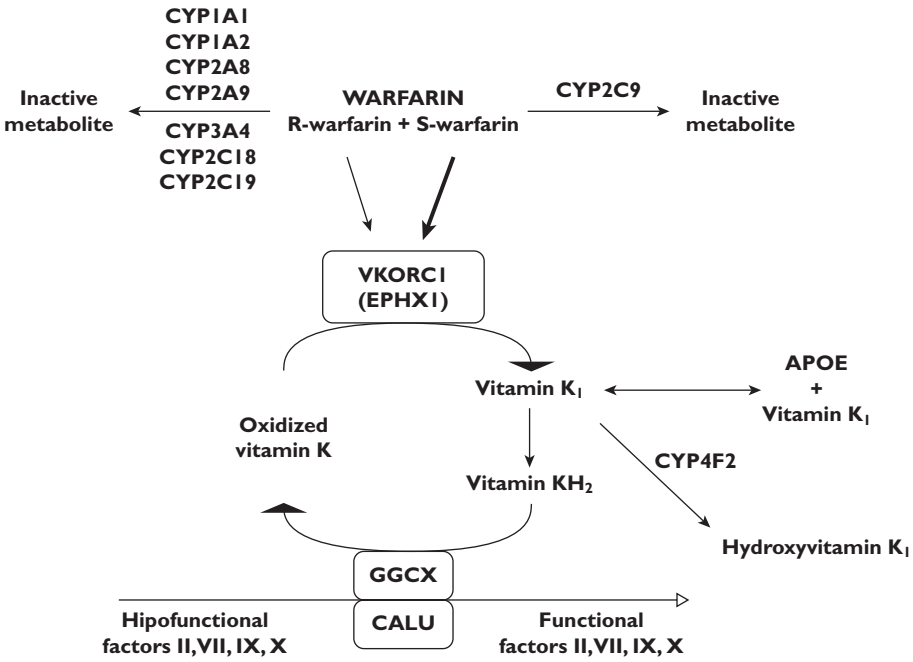

Figure 1

Warfarin interactive pathways. Warfarin R- and S- enantiomers are metabolized by different CYP enzymes and affect the coagulation cascade by inhibiting the vitamin K epoxide reductase complex 1 (VKORC1), preventing the carboxylation of clotting factors II, VII, IX and X. S-warfarin is the more potent enantiomer, as indicated by the thicker arrow. The influence of polymorphisms in the genes encoding CYP2C9, VKORC1, EPXH1, APOE, GGCX, CALU, CYP4F2 and factor VII on warfarin dose requirements is discussed in the text

across populations. Figure 1 presents a schematic view of warfarin pharmacokinetics and pharmacodynamics and Tables 1–3 show the frequency distribution of genetic polymorphisms affecting these warfarin interactive pathways in the African populations examined in the present review.

CYP2C9

The distribution of *CYP2C9* polymorphisms among sub-Saharan populations is characterized by the absence or rarity (<1%) of the defective alleles **2* and **3*, which are relatively common in Europeans. The frequency of these alleles in admixed African populations reflects their relative extent of European ancestry and increases progressively from African Americans and Brazilians with >80% African ancestry, to Black Brazilians and then to Brown Brazilians (Table 1). The low frequency of *CYP2C9 *2* and **3* contributes to the poor performance of warfarin dosing algorithms which rely exclusively on these alleles as predictors of CYP2C9 phenotype in Africans [14–18]. Nevertheless, when present, *CYP2C9 *2* and **3* associate with reduced warfarin requirements in populations of African descent: for example, Voora *et al*. [18] reported 20% and 34% reductions in warfarin dose per copy of alleles **2* and **3*, respectively, in African American, whereas in Brown and Black Brazilians, the combined effect size of *CYP2C9 *2* and **3* amounted to average reductions of 5.1 and 9.4 mg week^{-1} per variant allele, respectively [19]. A number of additional *CYP2C9* variants, listed in Table 1, have been associated with warfarin dose requirements in different African cohorts. *CYP2C9 *8,* a 449G > A transition which leads to an arginine to histidine conversion [20] and lower S-warfarin clearance [21] accounts for significant reductions in warfarin dose in South-Africans [22], African Americans [23, 24] and Mozambicans (Damasceno & Suarez-Kurtz, unpublished observations). Notably, genotyping of *CYP2C9 *8* alone prompted reclassification of the predicted metabolic phenotype in almost 10% of African-Americans, or when combined with *CYP2C9 *5, *6* and **11*, more than 15% [23]. *CYP2C9 *5* and **11* are described in the *CYP2C9* allele database nomenclature [25] as conferring decreased phenotypic activity, whereas *CYP2C9 *6* has no description. However, *CYP2C9 *6* was associated with decreased enzyme activity towards phenytoin, another CYP2C9 substrate [20]. A retrospective study of South African women associated warfarin dose requirement with *CYP2C9 *11* and with a novel high frequency SNP, namely g.46028A > G, described as a possible splice site in intron 7. The combined effect of all *CYP2C9* polymorphisms in this South African cohort was reported to explain 23.2% of the inter-individual variance in warfarin dose [22]. Resequencing of regions of evolutionary conservation and transcriptional binding prediction in *CYP2C9* identified three novel, high frequency SNPs (rs2860905, rs7089580 and rs35511771) in African Americans, which on univariate analyses associated with warfarin requirements [26]. One of these SNPs, rs7089580, was retained in a multivariate model with other genetic and non-genetic covariates, accounting for a 3.7 mg week^{-1} increase in warfarin dose for each allele copy. This effect, which is in the opposite direction of the star *CYP2C9* alleles, was not verified in another African American cohort [27].

VKORC1

VKORC1 encodes the vitamin K epoxide reductase subunit 1 (VKORC1), a key enzyme of the vitamin K cycle and molecular target of coumarin anticoagulants. The influence of *VKORC1* polymorphisms on warfarin dose requirements provides a remarkable example of pharmacogenomic diversity worldwide. This is best documented by the original and the expanded International Warfarin Pharmacogenomic Consortium (IWPC) datasets, comprising 5700–6200 patients recruited from four continents and ascribed to three 'racial' groups, namely Asians, Blacks (predominantly African Americans) and Whites [17]. Univariate analyses showed that possession of the minor rs9923231 allele (3673A, also known as −1639A), which reduces hepatic *VKORC1* expression, was associated with reduced warfarin dose requirements in the three groups. However, the percentage of inter-individual dose variation explained by this polymorphism was considerably lower in Blacks (4.2%) than in Whites (22.5%), a result which was largely explained by the lower frequency of rs9923231 in the Black study group (10.1%), compared with Whites (37.8%). A simulation exercise across the study populations showed that as the minor rs9923231 allele frequency increases, the percentage of variation in warfarin dose explained by this SNP also increases, with the highest variance explained at a frequency of 60–70% [17]. Accordingly, the low frequency of rs9923231 in sub-Saharan Africans (2.2–3.5%, Table 2) suggests that this SNP is a poor predictor of warfarin requirements in these populations. This inference may be extended to rs9934438, which is in extensive linkage disequilibrium (LD) with rs9923231 in sub-Saharan Africans [9]. European admixture coupled with the high frequency of these two SNPs in Europeans, account for rs9923231 and rs9934438 being considerably more common in African Americans (and consequently in the expanded IWPC Black cohort), Brown and Black Brazilians, than in sub-Saharan Africans. In Brazilians, the frequency distribution of rs9923231 is best described as a continuous function of the individual proportions of African ancestry, irrespective of self-reported colour or 'race' [9].

In addition to the tighly linked rs9923231 and rs9934438 SNPs, other *VKORC1* polymorphisms have been associated with warfarin dose in patients of African descent. Lower warfarin requirements have been associated with rs8050894 and rs2884737 in an admixed Brazilian [19] and in some African American cohorts [24, 26], but these SNPs were not included in warfarin dosing algorithms derived for these groups. The rs8050894 was associated with warfarin dose in a multivariate model for

Table 1

CYP2C9 polymorphisms in populations of African descent

Polymorphisms			Populations								
			African			African-American			Brazilian		
rs#	Nucleotide change (allele)	Effect	Cohort (*n*)	Frequency (%)	Reference	*n*	Freqency (%)	Reference	Cohort (*n*)	Frequency (%)	Reference
rs1799853	430C > T (*2)	R144C	Beninese (103)	0	[20]	1889	0–3.6	[23, 26, 27, 29, 30, 40, 54–56]	>80% African ancestry (65)	3.1	[57]
			Mozambican (103)	0	[58]				Black (756)	6.6	[19, 59]
			South African (923)	0	[60]				Brown (816)	8.2, 9.3	[19, 59]
rs1057910	1075A > C (*3)	I359L	Beninese (103)	0	[20]	1938	0.3–2.0	[23, 26, 27, 29, 30, 40, 54–56, 61]	>80% African ancestry (65)	2.3	[57]
			Mozambican (103)	1.0	[58]				Black (756)	2.6, 3.3	[19, 59]
			South African (213)	0.5	[22]				Brown (816)	5.1, 5.9	[19, 59]
rs28371686	1080C > G (*5)	D360E	Beninese (103)	1.8	[20]	1464	0.7–1.5	[23, 26, 27, 29, 30, 55, 56]	>80% African ancestry (65)	2.3	[57]
			Mozambican (103)	1.9	[58]				Black (756)	0, 0.9	[19, 59]
			South African (213)	0	[22]				Brown (816)	0.5, 0.8	[19, 59]
rs9332131	818delA (*6)	273 frameshift	Beninese (103)	2.7	[20]	1051	0.4–1.7	[23, 24, 29, 40, 55, 56]			
			Mozambican (103)	0	[58]						
			South African (213)	0.2	[22]						
rs7900194	449G > A (*8)	R150H	Beninese (103)	8.6	[20]	1111	4.7–7.2	[23, 26, 27, 40]	Black (76)	4.7	[58]
			Mozambican (103)	10.8	[58]				Brown (118)	0.4	[58]
			South African (213)	13.3	[22]						
rs2256871	752A > G (*9)	H251R	Beninese (103)	15.7	[20]						
			South African (213)	15.1	[22]						
rs28371685	1003A > G (*11)	R335W	Beninese (103)	2.7	[20]	1541	1.0–4.0	[23, 26, 27, 29, 40, 55, 56, 61]	Black (756)	0.7, 0.9	[19, 59]
			Mozambican (103)	2.4	[58]				Brown (816)	0, 0.5	[19, 59]
			South African (213)	3.9	[22]						
rs2860905	3858G > A	intron 3	YRI (111)	26.6	[61]	378	22.0, 24.5	[26, 61]			
rs7089580	6787A > T	intron 3	YRI (213)	21.2	[61]	612	19.4–23.0	[26, 27, 61]			
	7880T > C	intron 3				329	2.0	[26]			
rs35511771	13706T > C	intron 5				329	24.0	[26]			
	46028A > G	intron 7	South African (213)	28.8	[22]						

n, number of individuals.

Table 2

VKORC1 polymorphisms in populations of African descent

Polymorphisms			Populations								
			African			African-American			Brazilian		
rs#	Nucleotide change	Effect	Cohort (*n*)	Frequency (%)	Reference	*n*	Frequency (%)	Reference	Cohort (*n*)	Frequency (%)	Reference
rs9923231	3673G > A	5′UTR	Mozambican (143)	3.5	[9]	1754	8.8–22.0	[23, 26, 27, 31, 33, 40, 42, 56, 61]	Black (756)	22.0, 23.8	[19, 59]
			Angolan (73)	2.7	[9]				Brown (816)	30.6, 33.0	[19, 59]
			Black, 4 continents (368)	10.1	[17]				>80% African ancestry (68)	21.1	[9]
rs9934438	6484C > T	intron 1	South African (993)	4.0	[60]	1267	8.0–12.0	[26, 29, 31, 40, 42, 49, 55, 61]			
			Beninese (51)	2.0	[62]						
			Black, 4 continents (368)	10.2	[17]						
rs8050894	6853G > C	intron 2	Mozambican (143)	27.3	[9]	849	20.4–30.0	[26, 29–32]	Black (756)	35.7, 36.0	[19, 59]
			Black, 4 continents (368)	26.4	[17]				Brown (816)	38.0, 38.6	[19, 59]
			Angolan (73)	19.2	[9]				>80% African ancestry (68)	34.4	[9]
rs2884737	5808T > G	intron 1	Mozambican (143)	1.1	[9]	698	3.5–7.1	[24, 33, 55, 61]	Black (756)	10.0, 12.8	[19, 59]
			Angolan (73)	0	[9]				Brown (816)	13.5, 20.0	[19, 59]
									>80% African ancestry (68)	10.7	[9]
rs7294	9041G > A	3′UTR	South African (991)	40.0	[60]	973	44.0–52.3	[24, 26, 29, 32, 55, 61]	Black (756)	37.9, 41.0	[19, 59]
			Mozambican (143)	38.9	[9]				Brown (816)	37.2, 39.0	[19, 59]
			Angolan (73)	40.4	[9]				>80% African ancestry (68)	46.9	[9]
rs17886199	6915C > T	intron 2				258	3.7	[33]			
rs59502288	1989_1990insTGGATGA	5′UTR				329	42.0	[26]			
rs62057090	5374C > G	5′UTR				329	29.0	[26]			
rs61162043	6051G > A	5′UTR				570	47.0, 48.3	[26, 27]			
rs2359612	7566C > T	intron 2	South African (974)	24.0	[60]	804	15.9–23.3	[26, 29, 32, 40, 61]			
rs7200749	8773G > A	L120L	South African (113)	25.7	[22]	353	18.2–26.5	[32, 33, 61]			
rs72547529	6557C > T	V66M	South African (110)	0.4	[22]	509	0–1.6	[23, 40, 55]			

n, number of individuals.

Table 3

CYP4F2, APOE, GGCX, FVII, CALU and EPHX1 polymorphisms in populations of African descent

Polymorphisms			Populations								
rs#	Nucleotide change	Effect	African			African-American			Brazilian		
			Cohort (*n*)	Frequency (%)	Reference	*n*	Frequency (%)	Reference	Cohort (*n*)	Frequency (%)	Reference
CYP4F2											
rs2108622	1347C > T	V433M	Mozambican (206)	8.7	[58]	717	7.2–11.7	[24, 40, 58, 63]	Black (74)	14.9	[48]
			YRI (113)	5.8	[61]				Brown (112)	22.8	[48]
APOE											
rs429358 +	ε2					328	11.5, 12.6	[15, 24]			
rs7412	ε3					328	66.2, 69.1	[15, 24]			
	ε4					328	19.4, 21.2	[15, 24]			
GGCX											
rs10654848	(CAA)8, (CAA)9	intron 6				338	2.1, 0.1	[27]			
	(CAA)10, (CAA)11					338	33.1, 13.9	[27]			
	(CAA)12, (CAA)13					338	17.6, 11.8	[27]			
	(CAA)14, (CAA)15					338	13.6, 5.0	[27]			
	(CAA)16, (CAA)17					338	2.5, 0.1	[27]			
FVII											
rs510335	–401G > T	5′UTR				112	29.9	[59]	Black (69)	18.0	[50]
									Brown (108)	21.0	[50]
rs510317	–402G > A	5′UTR							Black (69)	21.0	[50]
									Brown (108)	19.0	[50]
CALU											
rs339097	10587T > C	intron 4	YRI (113)	18.6	[61]	435	10.4–16.3	[18, 40, 61]			
			Mozambican (75)	18.5	*						
EPHX1											
rs2292566	26857G > A	K119K	YRI (113)	17.7	[61]	216	6.8–15.1	[32, 40, 61]	Black (73)	12.3	†
rs2740170	32001C > T	intron 3	YRI (113)	11.5	[61]	71	11.4, 19.4	[32, 61]			
rs2740171	32732C > A	intron 3	YRI (113)	38.5	[61]	71	31.6, 31.8	[32, 61]			
rs1051741	1071C > T	N357N	YRI (113)	11.1	[61]	71	7.1, 9.1	[32, 61]			

n, number of individuals. *Damasceno & Suarez-Kurtz, unpublished observations. †Suarez-Kurtz, unpublished observations.

Sudanese patients [28], but this was not verified in African Americans [29–31]. Two other *VKORC1* polymorphisms, rs2359612 and rs61162043 were found to impact on warfarin dose in African American cohorts [24, 26, 27, 29, 32], whereas contradictory results were reported for rs17886199 [32, 33]. The rs61162043, located in the 5'UTR and not in LD with the other SNPs analyzed by Perera *et al*. [26] in an African American cohort, contributed 3.2% of inter-individual dose variation among patients.

VKORC1 variants associated with higher warfarin requirements include rs7200749 in South Africans [22] and rs7294 in an admixed Brazilian cohort [22], in African Americans [26] and in Sudanese [28]. These two SNPs were tightly linked in black South Africans, and explained 7.4% of warfarin dose variation in this group [22]. The association between rs7294 and warfarin dose was retained in multivariate modelling for Sudanese [28], but not African American patients [24, 26]. Two other variants, rs62057090 and rs59502288, were significantly associated with warfarin dose in African Americans in univariate, but not multivariate analyses [26]. A non-synonymous *VKORC1* variant, V66M, conferred extreme warfarin resistance in one African-Caribbean [34], African Americans [23] and Black Brazilians [35], leading to the suggestion that inclusion of this SNP in genotyping panels might be considered for Black patients [23]. Another *VKORC1* warfarin-resistant SNP, namely D36Y, prevalent in Ethiopians (MAF 15%; [36]) was not detected in Black South Africans [21] or African Americans [23].

Several reports assessed the extent of LD of *VKORC1* polymorphisms in populations of African descent. Among rs9923231, rs2884737, rs8050894 and rs7294 no pairwise LD with a $r^2 > 0.5$ was detected in Angolans and Mozambicans, whereas in Brown and Black Brazilians, rs9923231 and rs2884737 showed r^2 values of 0.71 and 0.60, respectively [9]. Five haplotypes inferred from these four SNPs accounted for more 99% of the diversity in Angolans, Mozambicans and Brazilians with >80% African ancestry. Limdi *et al*. [33] reported that among 17 *VKORC1* SNPs with minor allele frequency (MAF) >0.02 in African Americans, only two pairs (rs9923231 and rs9934438; rs17883591 and rs17886199) were in strong LD ($r^2 > 0.9$). The strong LD between rs9923231 and rs9934438 was verified in another African American cohort [37]. The pairwise LD between all other SNPs identified by Limdi *et al*. [33] was weaker, with r^2 values ranging from 0.2 to 0.6. These results include rs7294 and rs7200749 which are tightly linked in Black South Africans [22]. Twelve haplotypes comprising 11 SNPs with MAF > 0.08 accounted for 98.4% of the diversity in African Americans. Haplotypes inferred from common *VKORC1* SNPs (MAF > 0.1) were equally informative as either rs9923231 and rs9934438 in capturing the genetic influence of *VKORC1* in a cohort of Black individuals from four continents [17]. This, however, does not exclude the possibility that other SNPs, either *per se* or in haplotypes, might be more informative than rs9923231 and rs9934438 alone in distinct African-derived populations.

EPHX1

Microsomal epoxide hydrolase 1 (mEH), encoded by *EPHX1*, is another putative subunit of the vitamin K epoxide reductase complex (VKOR), which contains the vitamin K epoxide binding site. Two *EPHX1* polymorphisms (rs2292566 and rs4653436) have been investigated in relation to warfarin dose requirements in Europeans, with controversial results and borderline associations at best (e.g. [38, 39]). No association between rs2292566 and warfarin dose was detected in African Americans [40] or Black Brazilians (Suarez-Kurtz, unpublished observations). Schelleman *et al*. [32] genotyped rs2292566 and 13 other SNPs in a small cohort of 22 African Americans and found that none associated with warfarin maintenance dose. The authors reported that two polymorphisms located in intron 3 (rs2740170 and rs2740171) and one exonic, synonymous SNP (rs1051741) were able to explain more than 10% of the dose variability ($P > 0.05$), and suggested that these SNPs should be retested in a study with larger sample sizes.

APOE

The main circulating form of vitamin K, phylloquinone, is bound to chylomicrons. The hepatic clearance of chylomicrons, and consequently vitamin K, is partly dependent on apolipoprotein E (APOE). Two variants in exon 4 of the *APOE* gene (rs429358 and rs7412), lead to formation of three alleles, ε2, ε3 and ε4, which are associated with low, intermediate and high vitamin K uptake by the liver, respectively. The frequency distribution of these *APOE* alleles varies across populations, ε3 being the most common in African populations. Cavallari *et al*. [41] observed that the time delay to achieve stable warfarin dose in African Americans was longer in patients with the *APOE* ε3/ε3 genotype, compared with other genotypes, and Kimmel *et al*. [15] reported that carriers of the ε4 allele required higher warfarin doses, but this was not replicated by others [24]. The *APOE* polymorphism has been included in some warfarin dosing algorithms for African Americans [42] (Table 4).

GGCX

The protein encoded by *GGCX*, γ-glutamil carboxilase, is responsible for the post translational carboxylation of hypofunctional clotting factors II, VII, IX and X, with reduced vitamin K serving as a necessary cofactor. Three independent studies investigated the influence of *GGCX* polymorphisms in warfarin dose requirements in African-Americans [27, 32, 40]. The rs699664, a non-synonymous SNP in exon 8 was examined in all three studies and none verified the significant association between the minor allele rs699664 and higher warfarin dose requirements, previously reported for European and Asian patients [43,

Table 4

Warfarin pharmacogenomic algorithms for populations of African descent

Study [reference]	*VKORC1*	*CYP2C9* alleles	Age	Body weight, BSA or BMI	Smoking status	INR	VTE/PE	Other factors	Cohort (*n*)	r^2 or variation explained (%)
Momary *et al.* [30]		*2, *3, *5	•	BSA					African American (115)	33.0
Gage *et al.* [14]	3673G > A	*2, *3	•	BSA	•	INR	VTE	African American race Co-medications	African American Derivation cohort (153) Validation cohort (45)	 31.0 40.0
Kimmel *et al.* [15]	6484G > A	*2, *3	•	BMI				Employment status Co-medications *APOE* (ε4)	African American (111)	54.6
Limdi *et al.* [33]	3673G > A	*2, *3, *5, *6, *11	•	BMI	•			Gender Alcohol intake Vitamin K intake Co-morbidities Co-medications	African American (273)	38.9
Perini *et al.* [19]	3673G > A	*2, *3, *5, *11	•	Body weight			VTE	Co-morbidities Co-medications	Admixed Brazilians (390)	50.4
Schelleman *et al.* [49]	6484G > A		•	BMI				Co-medications *APOE* (ε2, ε3, ε4) *FVII* (–401G > T)	African American (112)	28.0
IWPC [16]	3673G > A	*2, *3	•	Body weight				Height Ethnicity	Black/African American (353)	27.0
Suarez-Kurtz *et al.* [64]	3673G > A	*2, *3, *5, *11	•	Body weight		INR/dose	VTE	Co-morbidities Co-medications	Admixed Brazilians (260)	60.0
Cavallari *et al.* [24]	3673G > A	*2, *3, *5, *6, *8, *11	•	BSA				Co-morbidities	African American (226)	36.4
Voora *et al.* [18]	3673G > A	*2, *3	•	BSA	•	INR	VTE, PE	*CALU* (10587T > C) Co-medications	African American (241)	–
Mitchell *et al.* [22]	8773G > A, 9041G > A	*8, *9, *11 and 8 other alleles	•					Co-medications	South African (113)	45.2
Perera *et al.* [26]	6051G > A, 6484G > A	*2, *3, *5, *8, *11, 6787A > T	•	Body weight			VTE, PE		African American (330)	40.0
Shrif *et al.* [28]	6853G > A, 9041G > A	*2, *5, *6, *11		Body weight		INR		*POL3S* Co-medications	Sudanese (203)	36.7
Ramirez *et al.* [40]	3673G > A	*2, *3, *6, *8	•	BSA	•		VTE	*CALU* (10587T > C) *CYP4F2* (1347C > T) Gender Ethnicity Co-medications Co-morbidities	African American (145)	41.0

BMI, body mass index; BSA, body surface area; PE, pulmonary embolism; VTE, venous thromboembolism.

44]. Of the other polymorphisms investigated in African Americans, only the rs10654848 microsatellite $(CAA)_n$ in intron 6 associated significantly with warfarin stable dose [27]. Carriers of either (CAA)16 or 17 repeat were over-represented among patients requiring warfarin doses higher than 7.5 mg day^{-1} *vs*. those who required lower doses. The *GGCX* rs10654848 genotype remained associated with warfarin dose in multivariable regression modelling, and was proposed as a predictor of higher than usual warfarin requirements in African Americans [27].

CALU

Calumenin is a chaperone protein found in the endoplasmic reticulum, where γ-glutamyl carboxylase and VKORC1 also localize. Calumenin exerts an inhibitory effect on the biosynthesis of the functional vitamin K-dependent clotting factors II, VII, IX and X [45]. A conserved intronic SNP (rs339097), rare in Europeans, but common in sub-Saharan Africans and African Americans (Table 3) was reported to associate with higher expression of *CALU* in human lymphoblastoid cells, but it is unclear whether this SNP directly affects calumenin expression or mRNA stability, or is in LD with causative SNP(s) within a large haplotype block [18]. Two studies have shown a significant association between the variant rs339097 allele and higher warfarin dose requirements in African American patients. In both studies, the association remained significant in multivariate regression modelling [18, 40].

CYP4F2

The enzyme encoded by the *CYP4F2* gene catalyzes the conversion of vitamin K to hydroxyl vitamin K, acting as a counterpart to VKORC1 in limiting accumulation of vitamin K in hepatocytes. A genome-wide association in Europeans revealed an association of warfarin dose requirement with rs2108622 in *CYP4F2,* but only after adjusting for *CYP2C9* and *VKORC1* [46]. The frequency of rs2108622 differs markedly among populations, being considerably lower in sub-Saharan Africans and African Americans (0–9%) compared with Europeans and White North Americans (17–33%) [47]. From a population perspective, it might be anticipated that rs2108622 will have a smaller influence, if any, on the warfarin dose requirements in Black individuals. The available data are consistent with this notion. No significant association between the *CYP4F2* rs2108622 C > T genotype and warfarin dose was observed in African Americans [24, 40], Brown or Black Brazilians [48]. However, when rs2108622 was analyzed in a multivariate model in the overall Brazilian cohort, it was able to explain 1.1% of warfarin dose variability, an effect which the study authors considered too small to justify including this SNP in warfarin dosing algorithms for Brazilians [48].

FVII

Two common functional polymorphisms in the promoter region of the *FVII* gene, namely –401G > T and –402G > A have been investigated in relation to warfarin dose requirement in African-derived populations. In African Americans, the –401G > T genotype showed no statistically significant association with warfarin maintenance dose, but was nevertheless included in a dosing algorithm [49]. Neither –401G > T nor –402G > A associated with warfarin stable dose in an admixed Brazilian cohort, comprising 50% of self-identified Brown and Black individuals [50]. However, when the data were analyzed using the extreme discordant phenotype methodology [51] a significant difference emerged at the 5^{th} percentile, such that the GG genotype was over-represented among patients at the low dose end of the warfarin dose distribution, compared with the high dose [50]. This result was not reproduced at higher cutoff points, suggesting that the impact of *FVII* –402G > A genotype on warfarin requirement is small, compared with *VKORC1* and *CYP2C9* polymorphisms.

Warfarin dosing algorithms

Several pharmacogenomic warfarin algorithms have been described and their performance, expressed by the correlation coefficient (r^2) between predicted and prescribed doses ranges from 0.2 to 0.6, is consistently inferior in sub-Saharan Africans and African Americans, compared with European populations. This is best documented in studies enroling multiethnic cohorts, whether recruited worldwide (e.g. [16, 17]) or exclusively from the United States (e.g. [14, 49]). Indeed, Schelleman *et al*. [49] suggested that their pharmacogenetic dosing algorithms 'performed only marginally better for African Americans when compared with giving 5 mg (warfarin) empirically'. This discrepant performance of warfarin algorithms in Caucasian *vs*. African Americans is not reversed by inclusion of a 'race' term in some algorithms [14, 16], which is not surprising, considering the complex interplay of genetic and non-genetic factors in modulating drug response, plus the fluctuation of racial definitions according to social context, geographic location, historical period and personal experience [52].

Table 4 compares the covariates and predictive performance of warfarin dosing algorithms tested in sub-Saharan Africans, African Americans and admixed Brazilians. All listed algorithms include polymorphisms in *VKORC1* and *CYP2C9,* age and a measure for body mass. Most algorithms incorporate co-medication with CYP2C9 inhibitors (e.g. amiodarone) and inducers (e.g. carbamazepine), and some include African or Black 'race', gender, co-morbidities (notably thromboembolic conditions), smoking status, INR measurements and polymorphisms in other pharmacogenes (*APOE, CALU, CYP4F2* and *FVII*). Whereas a small number of polymorphisms in *CYP2C9* (alleles **2* and **3*, rs1799853 and rs1057910, respectively), *VKORC1* (3673G > A, rs9923231) and arguably *CYP4F2* (rs2108622) may capture most of the pharmacogenomic

influence on warfarin dose variance in White populations, additional pharmacogenetic variants increase the predictive power of warfarin dosing algorithms in sub-Saharan peoples and African Americans. This discordance is determined mainly by the absence or rarity of *VKORC1* rs9923231, *CYP2C9 *2* and *3 in Black populations (Tables 1 and 2). Other variants, notably *CYP2C9 *8*, replace *CYP2C9 *2* and *3 as the major determinants of CYP2C9 metabolic phenotype in African-derived populations, and therefore must be incorporated in warfarin dosing algorithms for these peoples. Regarding *VKORC1*, no identified polymorphism(s) or inferred haplotype(s) account for as large a fraction of warfarin dose variance in Black populations as the tightly linked rs9923231 and rs9934438 do in European/Caucasian patients (see above). Although other pharmacogenetic variants have been found to associate with warfarin requirements in Blacks (e.g. *CALU* rs339097), their inclusion in dosing algorithms does not compensate for the reduced *VKORC1* contribution, and consequently most algorithms explain only 27–41% of warfarin dose variation in sub-Saharan Africans and African Americans. Exceptions include one algorithm for Black South Africans ($r^2 = 45\%$; [22]) and another for African Americans ($r^2 = 54.6\%$; [15]). The latter algorithm was unique in comprising employment status as a covariate, and its higher predictive performance was not reproduced in subsequent studies in other African American cohorts.

Differently from North Americans, the performance of a warfarin dosing algorithm derived from an admixed Brazilian cohort, did not differ between White and Black patients, with r^2 values of 51% and 52%, respectively [19]. Incorporation of an INR term to the algorithm increased its r^2 to 60% in the overall cohort, irrespective of self-reported 'race/colour'. The interplay of three factors may account for the distinct influence of 'race' or racial/ethnic labels on the performance of warfarin dosing algorithms in Brazilians *vs*. North Americans: (i) European gene flow is, on average, considerably larger in Black Brazilians (Introduction), (ii) self-reported race/colour correlates poorly with biogeographical genetic ancestry in Brazilians [3, 4], and (iii) distinct cultural semantic criteria impact the definition and self-adoption of racial/colour definitions across time and geographical space.

An alternative approach to the development of warfarin dosing algorithms, based on combining genome-wide association (GWA) studies with machine learning techniques, has been recently shown to improve the predictive performance in African Americans [53]. The peak performance ($r^2 = 66.4\%$) was obtained with a model that incorporated 200 SNPs identified by GWA in the study cohort. The adoption of this model in a natural clinical setting is uncertain.

A personalized strategy for initiation of warfarin therapy, allowing for improved safety and cost-effectiveness for the Black populations examined in this review must take into account their pharmacogenomic heterogeneity. Socio-economical, cultural and medical factors must also be considered, including access to and reliability of warfarin formulations, compliance to prescription, diet, concurrent diseases and co-medications, etc. Accounting for this diversity in algorithms that are 'friendly' enough to be adopted by warfarin prescribers worldwide requires gathering information from trials at different population levels, but demands also a critical appraisal of racial/ethnic labels that are commonly used in the clinical pharmacology literature but do not accurately reflect genetic ancestry and population diversity.

Competing Interests

There are no competing interests to declare.

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