Pharmacogenetics of warfarin dosing in patients of African and European ancestry

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Despite the introduction of direct acting oral anticoagulants, warfarin remains the most commonly prescribed oral anticoagulant. However, warfarin therapy is plagued by the large inter- and inpatient variability. The variability in dosing fueled research to identify clinical and genetic predictors and develop more accurate dosing algorithms. Observational studies have demonstrated the significant impact of single nucleotide polymorphisms in *CYP2C9* and *VKORC1* on warfarin dose in patients of European ancestry and African-Americans. This evidence supported the design and conduct of clinical trials to assess whether genotype-guided dosing results in improved anticoagulation control and outcomes. The trial results have shown discordance by race, with pharmacogenetic algorithms improving dose and anticoagulation control among European ancestry patients compared with African-American patients. Herein, we review the evidence from observational and interventional studies, highlight the need for inclusion of minority race groups and propose the need to develop race specific dosing algorithms.

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Warfarin is an oral anticoagulant that acts by inhibiting *VKORC1*, thus interfering with clotting factor activation [1,2]. The most common indications for warfarin include venous thromboembolism (VTE) and thromboembolic events related to atrial fibrillation (AF) or prosthetic heart valves [3–5]. Warfarin is still the most commonly prescribed anticoagulant for the prophylaxis and treatment of thromboembolism despite the gradual increase in the uptake of direct oral anticoagulants [6,7]. However, warfarin therapy is complicated by large inter-patient variability in dose requirements and large inpatient variability in anticoagulation control [8]. The International Normalized Ratio (INR) is used to monitor the level of anticoagulation, with values between 2 to 3 considered optimal for most indications other than prosthetic heart valves (INR: 2.5–3.5) [9,10]. Doses vary by as much as 20-fold among patients initiating warfarin, often resulting in suboptimal response and leading either to an increased risk of thrombosis (INR <2) or an increased risk of bleeding (INR >4) [11–17].

Given the high rate of adverse outcomes, underutilization remains a major issue, with almost a third to half of the patients in need of anticoagulation not receiving warfarin [7,18,19]. To improve dose prediction and anticoagulation control and reduce the number of adverse events, investigations have identified clinical (e.g., age, comedications) [11,20–23] and genetic factors that explain a significant proportion of dose variability [24–28]. Although several genes influence warfarin dose [29–33], SNPs in the *CYP2C9* (the principal enzyme that metabolizes warfarin) and *VKORC1* (the target protein inhibited by warfarin) genes were determined to account for substantial dose variability [34–39].

In 2007, the US FDA revised its product label for the first time to include information on the pharmacogenomics of warfarin, describing the bleeding risk as well as percent dose reductions observed in patients of European ancestry (EA) in relation to the *CYP2C9* and *VKORC1* SNPs [40]. In 2011, the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines [28] refined these dosing recommendations based on the number of genetic variants present in a patient for the *CYP2C9* and *VKORC1* SNPs [41]. These changes were enabled by evolving evidence from large observational studies that supported the development of algorithms using genetic and clinical factors for the

Table 1. Allele frequencies from the 1000 Genomes Project for genetic polymorphisms that affect warfarin dose.

Gene (role)	SNP-id	MAF (%) [†]			
		African	European	Latino	Asian
<i>CYP2C9</i> (principal enzyme involved in warfarin metabolism)	*2 (rs1799853)	0.8	12.4	9.9	0.1
	*3 (rs1057910)	0.2	7.3	3.7	3.4
	*5 (rs28371686)	1.7	0.0	0.1	0.0
	*6 (rs9332131)	0.8	0.0	0.0	0.0
	*8 (rs7900194)	5.3	0.2	0.1	0.0
	*11 (rs28371685)	2.4	0.2	0.1	0.0
	18786A>T (rs7089580)	20.6	22.2	11.8	1.5
<i>CYP2C</i>	G>A (rs12777823)	25.1	15.1	10.7	31.4
<i>VKORC1</i> (target protein inhibited by warfarin)	1639G>A (rs9923231)	5.4	38.8	41.1	88.5
	1173C>T (rs9934438)	5.4	38.8	41.2	88.5
	8191A>G (rs61162043)	45.8	60.5	57.2	11.5
	6830T>C (rs17886199)	5.5	0.1	0.1	0.0
	6853C>G (rs8050894)	25.6	40.0	44.2	88.5
	2255C>T (rs2359612)	17.9	38.8	42.5	88.5
	3730G>A (rs7294)	45.4	36.6	40.2	11.2
<i>CYP4F2</i> (enzyme involved in vitamin K1 metabolism)	*3 (V433M) (rs2108622)	8.2	29.0	23.8	21.4
<i>GGCX</i> (enzyme activates vitamin-K dependent proteins)	C>G (rs11676382)	0.2	9.4	2.6	0.0
	(CAA) 16/17 (rs10654848)	2.5	0.0	0.0	0.0
<i>CALU</i> (inhibits GGCX)	T>C (rs339097)	14.4	0.0	1.2	1.4
<i>NQO1</i> (enzyme involved in vitamin K-dependent clotting factor synthesis)	*2 (rs1800566)	17.5	21.1	33.3	41.9
<i>EPHX1</i> (enzyme hydrolyses epoxide intermediates, i.e., vitamin K epoxide)	337T>C (rs1051740)	14.1	30.4	32.0	48.2
	357G>A (rs1131873)	11.6	16.2	14.8	28.7

[†] Minor allele frequencies were obtained from the 1000 Genomes Project using super population codes for African, European, Admixed American and East Asian populations. MAF: Minor Allele Frequency.

prediction of therapeutic warfarin dose. Since early 2000's, close to 105 such algorithms have been published [27,42], with the Gage [38] and the International Warfarin Pharmacogenetics Consortium (IWPC) [43] being the most widely used algorithms. A vast majority of these studies have been conducted in patients of European or Asian ancestry, with only a handful of studies evaluating other race/ethnicities such as patients of African descent [21,25,26,44–52].

Although race specific differences in allele frequencies are widely recognized, their differential influence on warfarin dose requirements across race groups is beginning to be appreciated. This is reflected in the updated warfarin dosing guidelines by CPIC (2017) which now include recommendations according to race [53].

Herein, we synthesize the evidence on pharmacogenetics (PGx) of warfarin dosing in EA and African-American (AA) patients from observational and interventional studies. We provide additional discussion of studies which recruited sizable African ancestry patients and developed either race-specific or race-stratified dosing algorithms.

Warfarin related genes & SNPs

To frame this discussion, we first present the allele frequencies for SNPs (Table 1) known to influence warfarin dose by race groups. *CYP2C9* is the primary enzyme involved in the metabolism of warfarin and thus the polymorphic nature of the gene encoding this enzyme greatly affects the pharmacokinetics of warfarin, resulting in wide inter-patient variability [2]. The most widely studied *CYP2C9* variants, *2 and *3, have very low minor allele frequencies (MAF) among AAs compared with the populations of European descent. AA-specific variants include *5, *6, *8, and *11, which together were reported to explain as much of the variability in warfarin dose among AAs as the *2 and *3 variants do in the European populations [24,48,54]. Recently, a genome-wide association study conducted specifically among AAs identified a novel variant (rs12777823) that alone explained about 5% of the variability in warfarin dose. This SNP is more prevalent in AA (MAF-25%) compared with EA (MAF-15%) populations.

Figure 1. Flowchart of warfarin pharmacogenetic dosing algorithm studies with a focus on those conducted in the European ancestry and African-American populations.
AA: African-American; EA: European ancestry.

Including this SNP in the model led to a 21% relative improvement in dose over the standard IWPC dosing algorithm [45].

Warfarin exerts its action by inhibiting the *VKORC1* enzyme that is responsible for the activation of clotting factors. Although, several SNPs in the *VKORC1* gene have been studied, including two common haplotypes (A and B), the rs9923231 (*-1639G>A*) SNP was demonstrated to explain the greatest variability in warfarin dose, similar to the haplotype groups tested, with differences in percent variation across racial groups largely explained by the differing allele frequencies [21,44,55]. As a result, this one SNP was included in the PGx algorithms evaluated by most studies.

The SNPs discussed so far result in lower warfarin dose requirements but the variant associated with the *CYP4F2* gene, rs2108622 (*3), results in higher warfarin dose requirements, as the variant greatly reduces the enzyme concentration involved in the metabolism of vitamin K1 [30,47]. However, this SNP only explains ~1% of the dose variability among EA patients and is not associated with dose among AAs [56]. Other genes that are either involved in the activation (*GGCX* or *NQO1*) or inhibition (*CALU* or *EPHX1*) of clotting factor synthesis have been shown to influence warfarin dose in some studies [31,52,57,58]. However, sufficient evidence does not exist to warrant their inclusion in dosing algorithms at present [53]. As such, we focus our discussion on racial differences in warfarin dosing in the context of SNPs related to the *CYP2C9*, *CYP2C*, *VKORC1* and *CYP4F2* genes.

Overview of warfarin dosing algorithm studies

Figure 1 presents both observational and interventional studies that have been developing and evaluating warfarin dosing algorithms, focusing particularly on studies of European and African ancestry populations. We found a total of 105 articles that have either tested the performance of new or established dosing algorithms or implemented them in clinical trials to determine the efficacy or effectiveness of using them in routine clinical care. The majority of the studies are observational, comprising 84% ($n = 88$) of the total, with clinical trials making up the rest ($n = 17$, 16%). We excluded studies that did not involve European or African ancestry populations, which resulted in the removal of 49 (56%) observational studies and 6 (35%) interventional studies. The remaining observational ($n = 39$) and interventional ($n = 11$) studies are discussed below.

Pharmacogenetic algorithms from observational studies

Of the 39 observational studies included in this review (Figure 1), only six have been conducted exclusively among AA populations, the remaining include only EA or combined EA and AA populations in the analysis. Although 49% of the studies listed include diverse populations, AA patients constitute a small fraction of the total population in many of these studies. The proportion of AAs is ~10% in more than half of the studies included, with as low as 2% in the study by Gong *et al.* [59] and as high as 49% in the study by Limdi *et al.* [60]. Thus, the limited subgroup size resulted in adjustment for race rather than stratification by race in model development. Of the 19 studies including racially diverse populations, 13 studies combined the EA and AA data when evaluating the performance

of the dosing algorithms. Of these, seven accounted for race in their models, the remaining six studies excluding race from their final models. Studies in the race-combined group that included >20% AA population had limited sample sizes and therefore used the combined AA and EA data to develop dosing algorithms [59,61–67]. Therefore these studies, including the study by Gage *et al.* adjusted for race [38,39,68–70].

Table 2 provides the details of the warfarin dosing algorithms, including the performance of the genetic and clinical predictors separately when available, the performance of the combined (clinical + genetic) dosing algorithms, and whether those studies also evaluated the performance of the Gage or IWPC algorithms. We focus our discussion on studies that included a racially diverse population and either adjusted or stratified for race. We initially discuss the studies that adjusted for race in their model development. The earliest dosing algorithm by Gage *et al.* included only the *CYP2C9* variants and explained 39% of the variation in warfarin dose after adjusting for race [71]. The *CYP2C9* variants (including *2, *3 and *5) alone explained 10% of the variability in dose in this diverse cohort of patients that included 29% AAs. With the discovery of *VKORC1* in 2004, algorithms incorporated both *CYP2C9* *2, *3 and the *VKORC1* -1639G>A or -1173C>T variants. Limdi *et al.* evaluated the predictive ability of a single *VKORC1* (-1639G>A) SNP versus *VKORC1* haplotype and haplotype clusters in both AA and European-Americans [44]. The single *VKORC1* SNP explained 5% of the variability in warfarin dose among AA and 19% among European-Americans, the latter finding being consistent with the report from Gage *et al.* [38]. Limdi *et al.* then extended these findings to include Asian patients, matching the performance of the single *VKORC1* -1639G>A SNP with the haplotype in all three race groups (EA: 23%, AA: 4%, Asian: 29%) [21]. The equivalent performance of the *VKORC1* -1639G>A SNP enabled the inclusion of a single SNP in the algorithm, improving the feasibility of PGx testing in a clinical care setting. Another commonly used race-adjusted algorithm, the IWPC algorithm [39], was found to have similar predictive performance as the Gage algorithm [38] although it incorporated different predictors, including enzyme inducers and excluding smoking status and VTE which were part of the Gage model.

The Gage and IWPC algorithms include genetic and clinical predictors to guide dose initiation. Extending this work further, Lenzini *et al.*, Ramos *et al.* and Horne *et al.* incorporated INR values and day(s) of therapy (Horne) or prior dose (Lenzini) to further guide warfarin dose [65,68,69]. These models demonstrate significantly better performance as they incorporate intermediate responses such as changes in INR to modify dose. Comparing the R² values across all studies for the PGx dosing algorithms, the models by Horne *et al.* and Ramos *et al.* explained the greatest proportion of variation in warfarin dose, 72 and 67%, respectively [65,69]. The PGx algorithms by Horne *et al.* and Lenzini *et al.* were derived as dose-revision algorithms at days 6–11 and 4–5, respectively. The model by Ramos *et al.*, although not a dose-revision algorithm, includes dose-adjusted INR at day 3 which requires the use of other methods to determine the dose for the first two days. INR and prior dose/day of therapy were influential in improving the R² of these models. For example, the dose-adjusted INR at day 3 alone explained 19% of the variability in warfarin dose in the model by Ramos *et al.* [65]. However, Lenzini *et al.* demonstrated that inclusion of genetic information in their model reduced the R² for INR compared with the R² noted for INR in the clinical algorithm [68]. Thus, including SNPs together with dose and INR may improve our ability to predict dose-revisions. Yet, it is not clear whether genetic predictors would improve dose-revision if they are already accounted for in the dose-initiation algorithms [72]. Future studies are needed to test whether the use of PGx algorithms for both dose-initiation and dose-revision result in better percentage time in therapeutic range (PTTR) and clinical outcomes in patients.

Race-adjusted or race-combined models assume that the predictors have the same impact on dose across race groups. However, as noted in all the race-stratified analyses, the proportion of variability explained by the PGx algorithms in AA populations remains considerably lower compared with the EA populations. For instance, Gage *et al.* noted that the same PGx algorithm that explains 57% of the variability in EA only explained about 31% of the variability in AA patients [38]. The IWPC model reports a similar disparity (45% EA vs 26% AA) [43]. Limdi *et al.* recently evaluated the Gage algorithm and demonstrated that the PGx model explained 46, 51 and 29% of the variability, in the race-combined, EA and AA populations, with the genetic factors alone explaining 22, 34 and 7% of the variation, respectively [46].

Other studies evaluating the Gage or the IWPC algorithm by race have reported similar findings wherein the dose variability explained among AAs is considerably lower compared with the EA populations [70,73,74]. Thus, the three genetic variants most often included in the PGx algorithms are not good predictors of warfarin dose in the AA populations, with clinical algorithms even outperforming PGx algorithms in some studies. As a result, there was an impetus to study minority populations alone or stratified by race in diverse cohorts to discover novel variants or develop dosing algorithms that predict dose better among these populations.

Table 2. Pharmacogenetic dosing algorithms and their performance in observational studies.

Study (year)	n [†]	Treatment of race in the model	Clinical and genetic predictors	R ² explained (%)			Assessed prediction with the IWPC or Gage'08 algorithm	Ref.
				Genetic	Clinical	Total		
Kamali <i>et al.</i> (2004)	E: 119	Restricted to EA	Age, CYP2C9*2, *3	6	17	20	–	[24]
Hillman <i>et al.</i> (2004)	E: 453	Restricted to EA	Age, BSA, valve replacement, diabetes, CYP2C9*2, *3	–	–	34	–	[88]
Gage <i>et al.</i> (2004)	E: 261 A: 108	Adjusted for race	Age, gender, BSA, race, target INR, amiodarone, simvastatin, CYP2C9*2, *3	10 (includes CYP2C9*5)	–	39	–	[71]
Voora <i>et al.</i> (2005)	E: 40 A: 5	Adjusted for race	Age, gender, race, weight, height, simvastatin, amiodarone, CYP2C9*2, *3	–	–	42	–	[67]
Sconce <i>et al.</i> (2006)	E: 297	Restricted to EA	Age, height, CYP2C9*2, *3; VKORC1-1639A	–	–	55	–	[89]
Herman <i>et al.</i> (2006)	E: 165	Restricted to EA	Age, BSA, CYP2C9*2, *3; VKORC1-1639A	–	–	60	–	[90]
Carlquist <i>et al.</i> (2006)	E: 213	Restricted to EA	Age, gender, weight, CYP2C9*2, *3; VKORC1-1639A	33	12	45	–	[91]
Aquilante <i>et al.</i> (2006)	E: 319 A: 25	Unadjusted for race	Age, weight, smoking status, mean and goal INR, vitamin K intake, enzyme inhibitors and inducers, CYP2C9*2, *3, *5; VKORC1-1639A; Factor VII I/D, Factor X I/D	–	–	51	–	[66]
Caldwell <i>et al.</i> (2007)	E: 570	Restricted to EA	Age, gender, BSA, valve replacement, diabetes, CYP2C9*2, *3; VKORC1-6853C	–	–	56	–	[92]
Limdi <i>et al.</i> (2007)	E: 249 A: 239	Stratified by race	Age, gender, BMI, drug interactions, average alcohol intake, average vitamin K intake, # comorbid conditions, CYP2C9 *2, *3, *5, *6, *10 or *11	6 1	– –	19 28	– –	[60]
Momary <i>et al.</i> (2007)	A: 115	Restricted to AA	Age, BSA, CYP2C9*2, *3, *5	–	–	33	–	[93]
Zhu <i>et al.</i> (2007)	E: 65	Restricted to EA	Age, gender, weight, CYP2C9*2, *3; VKORC1-1639A	–	–	61	–	[94]
Gage <i>et al.</i> (2008)	E: 838 A: 153	Adjusted for race	Age, race, BSA, current smoking, target INR, VTE, amiodarone, CYP2C9*2, *3; VKORC1-1639A	–	17	57 31	53	[38]
Perini <i>et al.</i> (2008)	E: 196 A: 76	Unadjusted for race	Age, weight, valve replacement, VTE, amiodarone, simvastatin, CYP2C9*2, *3, *5, *11; VKORC1-1639A	–	–	51	–	[61]
Limdi <i>et al.</i> (2008)	E: 302 A: 273	Stratified by race	Age, gender, BMI, follow-up clinic, income, smoking status, education, health insurance, drug interactions, alcohol intake, vitamin K intake, comorbid conditions, CYP2C9 *2, *3, *5, *6, *11 and VKORC1-1173C>T	– –	– –	42 36	– –	[25]
Schelleman <i>et al.</i> (2008)	E: 147 A: 112	Stratified by race	E: Age, gender, BMI, # medications that potentiate warfarin, most # drinks on one occasion, h/o DVT, CYP2C9 *2, *3 and VKORC1 -1173C>T A: Age, BMI, # medications that potentiate warfarin, VKORC1 -1173C>T, Factor VII -401 G>T, APOE	– –	– –	43 28	42 34	[75]
Wu <i>et al.</i> (2008)	E: 33 A: 31	Adjusted for race	Age, race, gender, height, weight, smoking, amiodarone, sulfamethoxazole, CYP2C9*2, *3; VKORC1-1639A, 2255T	–	–	59	–	[62]
Michaud <i>et al.</i> (2008)	E: 131	Restricted to EA	Age, BSA, INR and Model 1: S:R-warfarin ratio at 14 h Model 2: dose-adjusted INR at day 4, CYP2C9*2, *3 VKORC1-1639A, 3730T	–	–	51	–	[95]

[†] Individuals of other race/ethnic groups are not listed in this table.

A: African descent; BMI: Body mass index; BSA: Body surface area; CHF: Congestive heart failure; CKD: Chronic kidney disease; DVT: deep vein thrombosis; E: European descent; INR: International normalized ratio; IWPC: International Warfarin Pharmacogenetics Consortium; PE: Pulmonary embolism; VTE: Venous thromboembolism.

Table 2. Pharmacogenetic dosing algorithms and their performance in observational studies (cont.).

Study (year)	n [†]	Treatment of race in the model	Clinical and genetic predictors	R ² explained (%)			Assessed prediction with the IWPC or Gage'08 algorithm	Ref.
				Genetic	Clinical	Total		
Wadelius <i>et al.</i> (2009)	E: 1496	Restricted to EA	Age, gender, drugs that increase INR, CYP2C9*2, *3; VKORC1-1639A	–	–	59	–	[96]
Klein <i>et al.</i> (2009) (IWPC)	E: 2233 A: 353	Adjusted for race	Age, race, height, weight, amiodarone, enzyme inducers, CYP2C9*2, *3; VKORC1-1639A	–	27	45 26	47	[43]
Cavallari <i>et al.</i> (2010)	A: 226	Restricted to AA	Age, BSA, stroke/TIA, CYP2C9 *2, *3, *5, *6, *8, or *11, VKORC1 -1639G>A	15	–	36	–	[48]
Wells <i>et al.</i> (2010)	E: 232	Restricted to EA	Age, BMI, height, exercise level, angiotensin II receptor antagonists, beta blockers, CYP2C9*2, *3; VKORC1-1639A; CYP4F2*3	–	–	58	–	[97]
Lenzini <i>et al.</i> (2010)	E: 818 A: 95	Adjusted for race in the PGx model	Age, BSA, diabetes, stroke, amiodarone, fluvastatin, target INR, log INR, dose 2–4 days before INR measurement, CYP2C9*2, *3; VKORC1-1639A	–	48	63	–	[68]
Zambon <i>et al.</i> (2011)	E: 274	Restricted to EA	Age, BSA, CYP2C9*2, *3; VKORC1-1639A; CYP4F2*3	–	–	65	63	[98]
Botton <i>et al.</i> (2011)	E: 279	Restricted to EA	Age, weight, amiodarone, carbamazepine, beta blockers, amlodipine, diuretics, CYP2C9 *2, *3; VKORC1 haplotype based on -1639A, -1173T, -3730A; CYP4F2*3, F2 -494T	–	–	63	IWPC: 46 Gage: 42	[99]
Gong <i>et al.</i> (2011)	E: 159 A: 4	Unadjusted for race	Age, weight, gender, amiodarone, CYP2C9*2, *3; VKORC1-1639A; CYP4F2*3	–	–	42	–	[59]
Perera <i>et al.</i> (2011)	A: 330	Restricted to AA	Age, weight, DVT/PE, CYP2C9*2, *3, -18786T; VKORC1-1173T, -8191A	–	22	40	26	[49]
Avery <i>et al.</i> (2011)	E: 671	Restricted to EA	Age, height, weight, amiodarone, CYP2C9*2, *3; VKORC1-1639A	–	–	42	–	[100]
Cini <i>et al.</i> (2012)	E: 55	Restricted to EA	Age, height, weight, gender, smoking, dietary intake, VTE, diabetes, CYP2C9*2, *3; VKORC1 -1639A, 3730A	–	–	44	IWPC: 47 Gage: 54	[101]
Ramos <i>et al.</i> (2012)	E: 146 A: 17	Unadjusted for race	Age, PE, dose-adjusted INR at day 3, amiodarone, CYP2C9*2, *3, *5; VKORC1-1639A	34	–	67	36	[65]
Horne <i>et al.</i> (2012)	E: 1115 A: 64	Unadjusted for race	Age, BSA, stroke, target INR, INR values, day of therapy, amiodarone, simvastatin, fluvastatin or enzyme inducers, CYP2C9*2, *3; VKORC1-1639A	–	65	72	49	[69]
Ramirez <i>et al.</i> (2012)	E: 1022 A: 145	Stratified by race	Age, gender, BSA, smoking, atrial fibrillation vs. VTE, amiodarone, CYP2C9*2, *3, *6, *8; VKORC1 -1639A; CALU rs339097; CYP4F2*3	– –	23 24	53 41	50 29	[70]
Shuen <i>et al.</i> (2012)	E: 264 A: 52	Unadjusted for race	Age, gender, target INR, CYP2C9*2, *3; VKORC1-1639A, VKORC1 Asp36Tyr	–	–	54	–	[63]
Bress <i>et al.</i> (2012)	A: 260	Restricted to AA	Age, BSA, hypertension, CYP2C9*2, *3, *5, *6, *8, *11; VKORC1 -1639A	–	23	37	–	[58]
Hernandez <i>et al.</i> (2014)	A: 349	Restricted to AA	Age, weight, VTE, CYP2C9*2, *3, *5, *8, *11, 18786T, VKORC1 -1639A, -8191A; rs12777823	–	–	27	IWPC Clinical: 16, Total: 15	[76]
Santos <i>et al.</i> (2015)	E: 574 A: 67	Adjusted for race	Age, gender, race, weight, height, amiodarone, enzyme inducers, CYP2C9*2, *3; VKORC1-1639A	–	–	40	–	[64]

[†] Individuals of other race/ethnic groups are not listed in this table.

A: African descent; BMI: Body mass index; BSA: Body surface area; CHF: Congestive heart failure; CKD: Chronic kidney disease; DVT: deep vein thrombosis; E: European descent; INR: International normalized ratio; IWPC: International Warfarin Pharmacogenetics Consortium; PE: Pulmonary embolism; VTE: Venous thromboembolism.

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Study (year)	n [†]	Treatment of race in the model	Clinical and genetic predictors	R ² explained (%)			Assessed prediction with the IWPC or Gage'08 algorithm	Ref.
				Genetic	Clinical	Total		
Limdi <i>et al.</i> (2015)	E: 762	Stratified by race	Age, BSA, amiodarone, CKD, <i>CYP2C9</i> *2, *3, *5, *6, *11; <i>VKORC1</i> -1639A, <i>CYP4F2</i> *3, rs12777823	35	16	54	51	[46]
	A: 595			10	23	34	29	
Alzubiedi <i>et al.</i> (2016)	A: 163	Restricted to AA	Age, height, weight, CHF, amiodarone, aspirin, paracetamol, enzyme inducers, <i>CYP2C9</i> *2, *3, *5; <i>VKORC1</i> -1639A, <i>CYP4F2</i> *3, rs12777823	–	–	38	26	[75]
Wiley <i>et al.</i> (2017)	E: 1928	Four models related to race - unadjusted, adjusted, % ancestry and stratified	Age, BSA, smoking, amiodarone, enzyme inducers, <i>CYP2C9</i> *2, *3, *5, *6, *8, *11, <i>VKORC1</i> -1639A; rs12777823 Other <i>CYP2C9</i> and <i>VKORC1</i> SNPs – either alone, combined or as haplotypes	–	20	56	45	[73]
	A: 253			–	22	40	20	

[†]Individuals of other race/ethnic groups are not listed in this table.

A: African descent; BMI: Body mass index; BSA: Body surface area; CHF: Congestive heart failure; CKD: Chronic kidney disease; DVT: deep vein thrombosis; E: European descent; INR: International normalized ratio; IWPC: International Warfarin Pharmacogenetics Consortium; PE: Pulmonary embolism; VTE: Venous thromboembolism.

We found six studies that evaluated race-stratified dosing algorithms, three of which were conducted by Limdi *et al.* [25,46,60], one by Schelleman *et al.* [74], one by Ramirez *et al.* [70] and the last by Wiley *et al.* [73]. The study by Schelleman *et al.* is the only one that presents two completely separate dosing algorithms for EA and AA patients, with different predictors included in the model for each. The genetic predictors included in the two models were especially different except for the *VKORC1* -1173C>T variant. Only the EA algorithm included the *CYP2C9**2, *3 variants whereas the AA algorithm included *Factor VII* -401 G>T, and *APOE* instead. Their dosing model in EAs performed similarly to the Gage model (43 vs 42%), however, their AA model was only able to explain 28% of dose variability compared with 34% explained by the Gage model. This low R² was attributed to likely unmeasured or unknown genetic or environmental factors, including the AA-specific *CYP2C9* variants. The remaining race-stratified studies evaluate the same dosing algorithms with the same set of predictors, but modeled separately in each race group. Thus, the coefficients and the performance parameters obtained from these studies are different for the EA and AA populations. However, only the study by Limdi *et al.* presents the beta coefficient values for the two groups separately, showing how the same predictors have a differential effect between the two race groups [46]. For example, the presence of the *VKORC1* -1639G>A variant required a significant dose reduction in both EA and AA groups. However, the dose reduction needed was significantly higher among EA than AA patients (29 vs 20%, *p* = 0.003). A race-combined algorithm would not achieve sufficient dose reduction among EA patients but at the same time result in a higher dose reduction among AA patients, thus failing to improve dose prediction in both race groups.

The earlier studies including AA patients modeled SNP genotypes as variant versus wild-type, most likely due to the low prevalence of these variants and/or the limited sample size of this subgroup. As a result, independent influences of each genotype (e.g., *CYP2C9* *1/*2, *1/*3, etc.) were not assessed until 2012 when Ramirez *et al.* showed that *CYP2C9**2 did not influence warfarin dose in AA patients using Vanderbilt's BioVU data repository [70]. Perera *et al.* also demonstrated the same finding in their genome-wide association study among AAs, reporting no association of the *CYP2C9**2 variant with dose [45]. On the other hand, *CYP2C9**2 is significantly associated with dose decrease among EA patients. This difference in the SNP effect could be multifactorial but still highlights the need for race-stratified algorithms that would include predictors significantly influencing warfarin dose. For instance, the inclusion of the rs12777823 SNP in our dosing algorithm predicted a 12% dose decrease in the AA patients [46]. The inclusion of AA-specific SNPs considerably improve the dose variability explained by the algorithm among AAs, as noted by the R² values observed in some studies – for example, 41% – Ramirez *et al.* [70], 40% – Perera *et al.* [49], 38% – Alzubiedi *et al.* [75] – among others. In addition, Cavallari *et al.* noted that the removal of the *CYP2C9**5, *8, and *11 SNPs from their PGx model decreased the variability explained from 36 to 30%, showing that these AA-specific variants undoubtedly improve dose prediction among AAs [48]. Overall, we see an improvement in dose prediction with the inclusion of AA-specific SNPs, but these improvements still

do not match or supersede the improvements noted in the EA populations. Inclusion of more SNPs in the model does not appear to improve the model performance substantially, as observed from the study by Wiley *et al.* [73]. Discovery of new genetic variants and/or clinical/environmental factors may further explain the variability, but would require larger studies with sample sizes that match EA populations. Another factor that may contribute to the variability is genetic ancestry which is a more accurate measure for self-reported race.

Very few studies have examined the effect of genetic ancestry on warfarin dose. Bress *et al.* and Hernandez *et al.* accounted for west African ancestry (WAA) but reported no significant association with model performance or improvement in warfarin dose prediction after inclusion in their PGx models [58,76]. However, Hernandez *et al.* reported an inverse association of WAA with warfarin dose that was predicted using the IWPC PGx algorithm among patients requiring substantially higher doses (≥ 60 mg/week). They suggested that the lack of AA-specific genetic variants in the IWPC model possibly diminished its warfarin dose predictability. On the other hand, Wiley *et al.* demonstrated that their haplotype model including percent African ancestry explained 42% of the variation in warfarin dose, the highest across all dosing algorithms among AAs in their study [73]. This is in contrast to the earlier findings where African ancestry did not remain significant after the inclusion of AA-specific SNPs in the PGx models. The adjustment for genetic ancestry, however, has been used in reports of race-combined models, improving upon the use of self-reported race. Accounting for genetic ancestry, both local and global, in race-specific models has the potential to further improve dose prediction and SNP parameter estimates by removing any underlying confounding due to admixture in these populations [77].

Sufficient evidence exists that indicates the need for AA-specific algorithms or the inclusion of AA-specific SNPs in the current warfarin dose prediction models. However, before we consider clinical implementation and utility of any of these algorithms, a more uniform or standardized dosing algorithm needs to be developed that maximizes dose prediction and minimizes adverse outcomes in AA populations.

Pharmacogenetic algorithms from interventional studies

Of the 11 interventional studies included in this review (Figure 1), not a single one has been conducted exclusively among AA patients whereas two trials were performed exclusively among EA populations. Except for the Genotype-guided Warfarin Therapy Trial (WARFPGX) [78] and the Clarification of Oral Anticoagulation through Genetics (COAG) trial [79] which included $\sim 27\%$ AA patients in both studies, the seven remaining trials included at least 90% EAs with the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) [80] and the trial by Burmester *et al.* [81] including $\sim 98\%$ EA patients in their trials. Since most of these trials only report the percent EA populations, it is hard to discern the race/ethnicities of the remaining trial population.

Table 3 provides the details of the warfarin dosing algorithms used in the interventional trials, the performance of the PGx algorithms and the clinical and genetic components separately if available. Finally, we have also included PTTR achieved by genotype-guided therapy compared with clinical or standard therapy in these trials. PTTR is an established measure of anticoagulation control which has been used as a surrogate marker of hemorrhage in almost all the clinical trials so far and thus provides a standard measure of comparison across all trials. Only the COAG trial provides a thorough comparison between the EA and AA populations, including but not limited to the model performances as well as PTTR [79]. Despite having a higher proportion of AAs, the WARFPGX trial was not powered to detect differences in their outcomes, let alone race-stratified ones [78]. The only other trial to compare PTTR between the two ancestry populations is the Genetics Informatics Trial (GIFT) [82]. The remaining studies present their findings for the race-combined analyses since they included a largely EA population.

The trial by Hillman *et al.* is one of the first trials that was conducted to determine the feasibility of using a genotype-guided dosing algorithm in clinical practice [83]. It only included the *CYP2C9* SNPs and reported warfarin dose, response and INR-related outcomes in addition to its feasibility measures. However, they found no significant differences in the two groups related to these outcomes, including PTTR. The remaining ten trials included both the *CYP2C9* (*2 and *3) and the *VKORC1* -1639A SNPs, with the GIFT and the trial by Burmester *et al.* including the *CYP4F2**3 variant as well.

The genotype-guided dosing algorithms were compared with standard therapy in seven of 11 trials whereas the GIFT [82], COAG [79], WARFPGX [78] and Burmester [81] trials used clinically derived dosing algorithms for the control arm. The PTTR reported by most studies was not significantly different between the genotype-guided and the clinically-guided/standard therapy groups, except in the case of CoumaGen II [84], Personalized Medicine Interface Tool (PerMIT) [85] and the EU-PACT [80] trials. In the GIFT trial, PTTR was significantly different between the intervention and control arms, but only among the non-AA group (55 vs 51%, $p = 0.003$) [82]. Despite

Table 3. Pharmacogenetic dosing algorithms and their performance in interventional studies.

Study (year)	n [‡]	Treatment of race in the model	Clinical and genetic predictors [†]	R ² explained (%)			PTTR (%) (genotype guided vs clinical/standard)	Ref.
				Genetic	Clinical	Total		
Hillman <i>et al.</i> (2005)	E: 38	Restricted to EA	Age, BSA, indication of therapy, diabetes, <i>CYP2C9</i> *2, *3	–	–	34	42, 42	[83]
Anderson <i>et al.</i> (2007) (CoumaGen)	E: 189	Restricted to EA	Age, gender, weight, amiodarone, <i>CYP2C9</i> *2, *3; <i>VKORC1</i> -1639A	32	15	47	70, 69	[86]
McMillin <i>et al.</i> (2010)	E: 206	Restricted to EA	Age, height, <i>CYP2C9</i> *2, *3; <i>VKORC1</i> -1639A	18	–	–	43, 45	[102]
Burmester <i>et al.</i> (2011)	E: 225	Restricted to EA	Age, gender, BSA, valve replacement, <i>CYP2C9</i> *2, *3; <i>VKORC1</i> -1639A, <i>CYP4F2</i> *3 Age*Valve replacement as well in the clinical arm	–	35	65	29, 29	[81]
Anderson <i>et al.</i> (2012) (CoumaGen II)	E: 2744	Restricted to EA	Age, height, weight, race, amiodarone, enzyme inducers, <i>CYP2C9</i> *2, *3; <i>VKORC1</i> -1639A	37	13	49	71, 59	[84]
Borgman <i>et al.</i> (2012) (PerMIT)	E: 24	Restricted to EA	Age, gender, bodyweight, <i>CYP2C9</i> *2, *3; <i>VKORC1</i> -1639A	–	–	–	63, 55	[85]
Jonas <i>et al.</i> (2013) (WARFPGX)	E: 79 A: 30	Adjusted for race	Age, race, BSA, smoking status, indication for warfarin, target INR, amiodarone, <i>CYP2C9</i> *2, *3; <i>VKORC1</i> -1639A	–	–	–	45, 49	[78]
Pirmohamed <i>et al.</i> (2013) (EU-PACT)	E: 447 A: 5	Unadjusted for race	Age, height, weight, amiodarone (BSA, log INR, target INR, dose given 2–4 days before INR measurement), <i>CYP2C9</i> *2, *3; <i>VKORC1</i> -1639A	–	–	–	67, 60	[80]
Kimmel <i>et al.</i> (2013) (COAG)	E: 675 A: 275	Adjusted for race	Age, race, smoking status, BSA, amiodarone, target INR, DVT/PE (diabetes, stroke, fluvastatin, log INR, dose given 2–4 days before INR measurement), <i>CYP2C9</i> *2, *3; <i>VKORC1</i> -1639A	–	17 (51)	52 (75)	49, 46	[79]
				–	33 (50)	21 (40)	35, 44	
Pengo <i>et al.</i> (2015)	E: 180	Restricted to EA	Age, BSA, <i>CYP2C9</i> *2, *3; <i>VKORC1</i> -1639A; <i>CYP4F2</i> *3	–	–	50	52, 53	[87]
Gage <i>et al.</i> (2017) (GIFT)	E: 1454 A: 102	Adjusted for race	Age, race, BSA, current smoking, baseline and target INR, indication for warfarin, amiodarone, azoles and other antibiotics, <i>CYP2C9</i> *2, *3; <i>VKORC1</i> -1639A; <i>CYP4F2</i> *3	–	–	–	55, 51	[82]
				–	–	–	51, 51	

[†] Parentheses include variables that were part of the dose revision algorithm, in addition to the variables listed in that cell that were part of the dose initiation algorithm.

[‡] Individuals of other race/ethnic groups are not listed in this table.

A: African descent; BSA: Body surface area; COAG: Clarification of Oral Anticoagulation through Genetics; DVT/PE: Deep vein thrombosis or pulmonary embolism; E: European descent; INR: International Normalized Ratio; PTTR: Percentage time in therapeutic range; COAG: Clarification of Optimal Anticoagulation through Genetics; EU-PACT: European Pharmacogenetics of Anticoagulant Therapy; GIFT: Genetics Informatics Trial of warfarin; PerMIT: Personalized Medicine Interface Tool for genotype-based warfarin dosing; WARFPGX: Genotype-guided warfarin therapy trial.

finding no difference in PTTR, Burmester *et al.*, reported an improved predictive ability of the PGx algorithm compared with the clinically-guided dosing algorithm (65% vs. 35%) [81]. The CoumaGen trials (I and II) also reported the performance of their algorithms, with the PGx models (47 and 49%) performing better than the genetics only (32 and 37%) as well as the clinical models (15 and 13%) [84,86]. Similarly, the trial by Pengo *et al.* among Italian Caucasians also demonstrated that their PGx algorithm accounted for ~50% of the variability in warfarin dose [87].

The EU-PACT [80] and COAG [79] trials published their findings in 2013 but reported very disparate results. Both employed dose-initiation as well as dose-revision algorithms for their genotype-guided dosing groups. However, these two trials differed in some fundamental ways. The EU-PACT trial included almost all EA patients (98.6%) compared with ~73% in the COAG trial. COAG used a clinically guided dosing algorithm for the control arm whereas EU-PACT employed a standard dosing method. Furthermore, the PGx dosing algorithms in themselves

were different, varying in the clinical predictors that were included during the dose-initiation and dose-revision algorithms. Finally, the EU-PACT trial used a modified IWPC algorithm to incorporate loading doses during days 1–3. Loading doses reduce the time taken to reach therapeutic INR, which may have contributed to a higher PTTR in the genotype-guided group. As such, the EU-PACT trial was able to report a significant difference in PTTR between the PGx and standard dose therapy groups (67 vs 60%; $p < 0.001$) while the COAG trial reported no benefit in the overall population (45 vs 45%; $p = 0.91$). However, significant differences were observed in the subgroup analysis by race wherein the PTTR was significantly higher among AAs (44 vs 35%; $p = 0.01$) but in the clinically-guided dosing arm. A possible explanation for this discrepancy is the use of the race-adjusted algorithm [38] which often resulted in the dispensation of doses that were significantly different (≥ 1 mg/day higher or lower) among the AA group (versus the non-AA group), both during dose-initiation (62 vs 42%; $p = 0.002$) and dose-revisions (50 vs 34%; $p = 0.01$). The results demonstrate that the PGx algorithm predicted dose more accurately among EAs compared with AAs during both dose-initiation (52 vs 21%; $p = 0.001$) and dose-revision (75 vs 40%; $p < 0.001$) stages. On the other hand, the clinical algorithm was better at dose prediction only among AAs and only during dose-initiation (33 vs 17%; $p = 0.08$).

The success of the EU-PACT trial can be attributed to the use of the IWPC algorithm that was developed and implemented among a largely EA population and which also included SNPs that are known to substantially influence warfarin dose in this race group. On the other hand, the COAG trial was designed to allow the examination of differences by race, but still used a PGx algorithm that was derived and validated in a mostly EA population. It did not include any AA-specific SNPs, and could not include the new *CYP2C* rs12777823 AA-specific variant, the results for which were not published until after the COAG trial was completed. As mentioned before, the use of the race-adjusted algorithm [38] assigned the same effect sizes for a predictor in both EA and AA. And, as the algorithm was derived mainly from EA, the parameter estimates were most likely weighted towards the EA population, resulting in less than optimal doses in the AA population. Thus, these findings highlight the need for adequate representation of minority populations, assessing race-specific SNPs and implementing race-specific algorithms to evaluate their efficacy and safety.

Conclusion & future perspective

The field of warfarin PGx has made tremendous strides in the last two decades. The discovery of genetic variants that explain a substantial proportion of the variability in warfarin dose among EA populations paved the way for the development of PGx algorithms that have improved dose prediction significantly in this race group. These dosing algorithms have undergone several iterations, each involving some variation in the clinical or genetic predictors included in the models. The *CYP2C9**2, *3 and *VKORC1* variants have been the most consistent genetic predictors across all models, with the AA-specific ones also including other *CYP2C9* variants and more recently the rs12777823 SNP. The improvements in dose variability explained have been substantial, however, ~40% still remains unexplained. Dose revision algorithms including INR, warfarin dose as well as genetic variants add to the variability explained but warrant further investigation to determine if the results are consistent across all populations and clinical settings. Interventional trials have established the role of genotype-guided dosing algorithms in improving anticoagulation control and are now moving towards the evaluation of safety outcomes. These trials show that implementation of the PGx algorithms in clinical care is feasible and would be beneficial at least in populations of European descent.

The focus should now shift to under-represented populations to enable similar discoveries and developments so that dose prediction in these populations can at least be brought to par with what has been achieved in the EA populations. This requires adequate representation of minority populations, especially in clinical trials, and the inclusion of race-specific SNPs if not the use of race-specific algorithms to better predict dose in these race-groups. However, as with the Gage or IWPC models that are considered established algorithms for the EA populations, there is a need to develop and validate an algorithm that can be considered a gold standard among the AA population, which best explains the variability in warfarin dose and can be used in routine practice across all care settings.

Studies in patients from Asian, Hispanic and Middle-Eastern populations are growing, however, we have not included these studies in the current review. As we show herein, the impact of genetic factors on warfarin dose varies among African vs. European populations, but it is also known to differ among other groups (e.g., Hispanic). The evaluation of these other race groups would be best served through a separate focused review.

Executive summary

Warfarin pharmacogenetic dosing algorithms

- The discovery of the *CYP2C9* and *VKORC1* polymorphisms resulted in the development and evaluation of dosing algorithms to better predict warfarin dose.
- Most of the observational as well as interventional studies on warfarin dosing algorithms have been conducted among populations of European descent.
- Observational studies among other race/ethnic groups such as individuals of African descent led to the discovery of novel and race-specific genetic variants that improved dose prediction in these racial groups.

Pharmacogenetic algorithms from observational studies

- A major proportion of the evidence for pharmacogenetic (PGx) algorithms comes from observational studies. Of these, 39 included European ancestry (EA) or African-Americans (AA) populations but only half of them included both groups.
- Among the racially diverse studies, only six performed a race-stratified analyses to evaluate the performance of these algorithms in EA and AA separately.
- The differences in prediction identified in race-stratified analyses highlight the need for race-specific dosing algorithms.

Pharmacogenetic algorithms from interventional studies

- Genotype-guided dosing has been evaluated in 17 studies, of which 11 were conducted in either EA or both EA and AA populations.
- Two of the interventional studies also evaluated outcomes by race but only the Clarification of Oral Anticoagulation through Genetics trial presented the performance of the models in both race groups.
- PGx algorithms improved dose prediction and percentage time in therapeutic range among patients of European descent but not among AA.

Future directions

- Dosing algorithms including race-specific genetic variants need to be developed and validated in minority populations.
- Similar to the Gage and International Warfarin Pharmacogenetics Consortium algorithms, a dosing algorithm needs to be developed for the AA populations, one that explains the greatest variability in warfarin dose and can serve as a gold-standard of therapy in this population.

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