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Predicting warfarin dosage in European–Americans and African–Americans using DNA samples linked to an electronic health record

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The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Abstract

Aim—Warfarin pharmacogenomic algorithms reduce dosing error, but perform poorly in non-European-Americans. Electronic health record (EHR) systems linked to biobanks may allow for pharmacogenomic analysis, but they have not yet been used for this purpose.

Patients & methods—We used BioVU, the Vanderbilt EHR-linked DNA repository, to identify European-Americans ($n = 1022$) and African-Americans ($n = 145$) on stable warfarin therapy and evaluated the effect of 15 pharmacogenetic variants on stable warfarin dose.

Results—Associations between variants in *VKORC1*, *CYP2C9* and *CYP4F2* with weekly dose were observed in European-Americans as well as additional variants in *CYP2C9* and *CALU* in African-Americans. Compared with traditional 5 mg/day dosing, implementing the US FDA recommendations or the International Warfarin Pharmacogenomics Consortium (IWPC) algorithm reduced error in weekly dose in European-Americans (13.5–12.4 and 9.5 mg/week, respectively) but less so in African-Americans (15.2–15.0 and 13.8 mg/week, respectively). By further incorporating associated variants specific for European-Americans and African-Americans in an expanded algorithm, dose-prediction error reduced to 9.1 mg/week (95% CI: 8.4–9.6) in European-Americans and 12.4 mg/week (95% CI: 10.0–13.2) in African-Americans. The expanded algorithm explained 41 and 53% of dose variation in African-Americans and European-Americans, respectively, compared with 29 and 50%, respectively, for the IWPC algorithm. Implementing these predictions via dispensable pill regimens similarly reduced dosing error.

Conclusion—These results validate EHR-linked DNA biorepositories as real-world resources for pharmacogenomic validation and discovery.

Keywords

anticoagulants; bioinformatics; electronic health record; genes; pharmacogenomics; warfarin

Warfarin is a widely used anticoagulant with a narrow therapeutic index [1]. Dose varies up to tenfold between individuals, and is only partially predicted by clinical factors including age, weight, diet and concurrent medications [2,3]. Genetic variants in the pharmacokinetic

gene *CYP2C9* [4] and pharmacodynamic gene *VKORC1* [5], the target of warfarin, explain over 35% of the total dose variation in European-descent populations [6]. Integrating this information into dosing algorithms has been shown to reduce dosing error in large studies [2,7].

Apart from these variants, several other common genetic variants in other genes have also been implicated as influencing this trait. For example, a variant in *CALU*, a cofactor in the VKOR complex, is associated with stable warfarin dose, but its role in predictive modeling is not well established [8–10]. Additional genes in the vitamin-K cycle containing variants associated with stable warfarin dose include the CYP enzyme *CYP4F2* [11], *EPHX1* [12] and *GGCX* [13], but studies are conflicting on individual variant associations and their role in predictive modeling [14–16].

Population-specific frequencies and genetic variants also contribute to the dosing distribution: African-Americans have lower frequencies of the *VKORC1* and *CYP2C9* predictive alleles, and, consequently, stable dose is less accurately predicted [17,18]. In addition, variants in *CYP2C9* (e.g., *CYP2C9**5, *6, *8 and *11) associated with warfarin dosing are polymorphic in African-Americans, but rarely so in European-Americans. Only a few studies have examined candidate genes and their relationship with warfarin dose in African-Americans, and no genome-wide association study has yet been published for this population [18–21].

Rates of major bleeding in the initiation phase of therapy are between 16 and 25% [22]. While small prospective randomized trials have shown conflicting results using pharmacogenomic-guided therapy [23,24], a comparative effectiveness study using historical controls showed genotyping can reduce major adverse bleeds by up to 48% [25]. Despite this evidence and the inclusion of genotyped-based recommended dose ranges in the US FDA warfarin label in February 2010, prospective genotyping is not yet widespread [101].

Electronic health records (EHRs) may offer a mechanism to bring pharmacogenomic information to the point of care through decision-support systems. We have shown that the associations between genetic variation and common diseases can be replicated in the Vanderbilt University (TN, USA) DNA Biobank, BioVU, which as of August 2011 contains over 127,000 DNA samples linked to de identified EHR data [26]. However, the extension of this approach to pharmacogenomics has not been evaluated.

Here we test: whether the published associations between steady-state warfarin dose and variants in warfarin pharmacogenes could be replicated in BioVU; how implementing published pharmacogenomic algorithms affects dosing error; and if an improved algorithm for African-Americans can be generated using variants associated with stable dose in this population. This work lays the foundation for implementing prospective genotyping in patients initiating warfarin by a decision-supported electronic model as a next step to evaluating the efficacy of such an approach to reducing drug-associated morbidity and mortality.

Patients & methods

■ Study population

Cases were identified in BioVU, the Vanderbilt DNA Biobank, which accrues DNA samples extracted from blood remaining from routine clinical testing after it has been retained for 3 days and is scheduled to be discarded [27]. BioVU is linked by research-unique identifiers to the synthetic derivative, a copy of the Vanderbilt EHR with all identifying patient

information such as names and addresses removed, clinic and provider information anonymized and dates shifted uniformly across a record within 1 year [27]. BioVU and individual studies using the resource are classified as nonhuman subjects research in accordance with the provisions of Title 45 Code of Federal Regulations Part 46, and have ongoing oversight from the Institutional Review Board and internal and external ethical boards [28]. The synthetic derivative contains data from the early 1990s, and since 2005 includes nearly all inpatient and outpatient billing codes, laboratory values, reports and clinical documentation; almost all are in electronic formats available for searching. The synthetic derivative currently contains data on over 1.7 million individuals and is refreshed regularly to add new clinical information from the EHR as it is accrued.

In this study, individuals were identified with International Normalized Ratio (INR) values between 2.0 and 3.0 for at least 3 weeks with no out-of-range INRs and two clinical encounters at least 3 weeks apart. Clinician-determined target therapeutic INR range was required to be between 1.9 and 3.2. Cases were followed either by primary-care providers or anticoagulation -specialty clinics (i.e., a 'Coumadin® clinic'). Primary care notes were manually reviewed for dose information. Records from anticoagulation clinics contain semistructured dose information amenable to natural language processing [29]. We developed a computer program to extract warfarin dose per day, then summed to the weekly dose [29]. Physician review verified the weekly dose extracted by the regular expression program in a subset of cases with positive predictive value of 97.4%, and all individuals outside the fifth and 95th percentiles of the dose ranges were manually verified. If more than one-weekly dose occurred, the median value was used.

Cases were limited to race/ethnicity recorded as 'non-Hispanic' European-American (designated 'white' in the EHR) or African-American (designated 'black' in the EHR). We have previously shown a high degree of concordance between EHR-ancestry designation and genetic ancestry determined from ancestry-informative markers [30]. Additional covariates extracted from the EHR included age, height, weight and gender. Concomitant medications (e.g., amiodarone and enzyme inducers including carbamazepine, phenytoin and rifampin) and indication for warfarin therapy were extracted using natural language processing and verified by manual review [29]. Current smoking status (yes or no) was identified by manual review.

■ Genotyping

Fifteen SNPs in six genes (*CYP2C9*, *VKORC1*, *EPHX1*, *GGCX*, *CYP4F2* and *CALU*) were selected from literature-reported associations in 2010. All SNPs were genotyped in the entire dataset regardless of race/ethnicity with the exception of *CYP2C9**8, which was subsequently added and only genotyped in African-Americans due to low frequency in European-Americans [31]. The location and rs numbers for variants genotyped are given in **Table 1**. Genotyping for eight of the 15 SNPs was conducted by the Vanderbilt DNA Resources Core using the Sequenom (Sequenom, Inc., CA, USA) platform, which is based on a single-base primer extension reaction coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. PCR primers and multiplexed-homogeneous MassEXTEND primers were designed by SPECTRODESIGNER software (Sequenom, Inc.). The remaining seven SNPs (rs1799853, rs1057910, rs2108622, rs229566, rs699664, rs11676382 and rs339097) were genotyped by the Vanderbilt DNA Resources Core using TaqMan® (Applied Biosystems, Inc., CA, USA). DNA samples were genotyped using TaqMan SNP Genotyping Assays according to the manufacturers' recommended protocols with probes and primers designed and synthesized by Applied Biosystems, Inc. Fluorescence data was captured using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Inc.). Genotype calls were determined by investigators blinded to dose

taken by individuals. Quality-control procedures included examination of marker and sample genotyping efficiency, duplicate and HapMap concordance checks, allele frequency calculations, and tests of Hardy–Weinberg equilibrium (HWE). The PLINK and Plato software packages were used to generate quality-control and linkage-disequilibrium metrics [32,33]. SNPs out of Hardy–Weinberg equilibrium at $p < 0.05$, with call rate $< 95\%$, or with minor allele frequency (MAF) $< 1\%$ were excluded. Samples with SNP call rates $< 50\%$ across all 15 SNPs or failing both Sequenom and TaqMan assays were excluded.

■ Analysis

Linear regression with a log-transformed weekly warfarin dose response was used for tests of association with clinical covariates and single-locus tests of association for SNPs with MAFs greater than 1%. For the multivariate trait indication, each indication was compared against all others. Additive genetic models were assumed and adjustments were made for covariates: age (continuous), gender, race, current smoking status and BMI (continuous). BMI was missing in 37 individuals who, due to the low percentage of missing data, were excluded from the adjusted analyses. Tests of association were conducted in European–Americans and African–Americans, separately, and on the entire dataset with race included as an adjustment variable.

Predictions for steady-state warfarin doses were obtained using the algorithms developed by the International Warfarin Pharmacogenomics Consortium (IWPC)[7] and, using the ‘FDA table’, recommended dose found in the warfarin package insert which are based exclusively on *VKORC1* and *CYP2C9* (*1, *2, *3) genotypes (0.5–2 mg/day, 3–4 mg/day, 5–7 mg/day) [101]. For the FDA table recommendation, the midpoint of the recommended range was used for predictions (1.25 mg/day, 3.5 mg/day, 6 mg/day). We also used regression modeling to develop algorithms to predict the warfarin dose based on clinical and genetic factors. As a practical approach to variable selection, clinical variables present in less than 10% of cases were not considered for analysis, due to the high likelihood of inadequate power. The one exception to this rule was amiodarone usage, which was only present in 8% of individuals, but was still included in final algorithms due to its known strong association with warfarin dose.

To validate model summaries and to correct for potential overfitting, we used a nonparametric bootstrap procedure with 1000 replicates. Mean absolute error (MAE) was used as the measure of prediction accuracy. We calculated the coefficient of determination, R^2 , for each algorithm and the sensitivity and positive predictive value for low, medium and high steady-state warfarin doses. For each bootstrap replicate, we fitted a regression model to the individuals sampled with replacement (i.e., the training sample). We then applied the regression model to the entire sample (i.e., the testing or validation sample) and calculated the summary measures for example, MAE, R^2 , sensitivity and specificity. Reported validated estimates and confidence intervals are based on the mean and the 2.5th and 97.5th percentiles of the bootstrap-based summaries that were calculated in the validation sets.

To examine the practical utility of implementing these algorithms, predicted weekly doses were rounded to the nearest discrete weekly dose using one of three regimens: any single pill size (1, 2, 2.5, 3, 4, 5, 6, 7.5 or 10 mg) in any combination of fractions (0.5, 1, 1.5 or 2 tablets) that could vary on different days (e.g., using 5-mg pills, take one pill three-times weekly and 1.5 pills four-times weekly); the subset of those regimens with the same dose all 7 days of the week (e.g., take 1.5 5-mg tablets every day); and the predicted weekly dose divided by seven and rounded to the nearest full pill strength, forcing a complete tablet dose each day. All three options allow a single-strength pill to be prescribed to minimize confusion with multiple bottles. The calculation of the rounded dose for each algorithm and

dosing regimen was incorporated into all the bootstrap analyses and the dose calculations from the dosing regimens were adapted into a webpage available at [102].

The R programming language was used for regression analyses, diagnostic-test calculations, and to implement and evaluate the algorithms (R Foundation for Statistical Computing, Vienna, Austria).

Results

Natural language processing and manual review identified the median weekly dose of 1175 individuals on warfarin with stable therapeutic INR values in BioVU, of which 718 individuals were seen in anticoagulation specialty clinics. Eight samples failed genotyping at >50% of loci and were therefore excluded. Of the remaining 1167 individuals, 145 (12.4%) were African-American and 522 (44.7%) were female. The median age was 66. The median dose was 35 mg/week. Additional summary statistics are shown in **Table 2**.

Genotyping quality control and MAFs of the 15 SNPs are shown in **Supplementary Table 1** (see www.futuremedicine.com/doi/suppl/10.2217/pgs.11.164). Two rare *VKORC1* SNPs associated with warfarin resistance, rs28940305 and rs72547529, were monomorphic in this dataset and were not analyzed further. We compared the MAFs in European-Americans and African-Americans for the 13 remaining SNPs in this dataset. As has already been noted, frequencies of alleles currently used in predictive algorithms including *CYP2C9*2*, *CYP2C9*3* and *VKORC1* rs9923231 were higher in European-Americans compared with African-Americans [17]. MAFs of variants more common in African-Americans including *CYP2C9*6*, *CYP2C9*8*, *CYP2C9*11* and *CALU* rs339097 were similar to population reports (**Supplementary Table 1**). *CYP2C9*6*, *CYP2C9*11* and *CALU* rs339097 were rare in European-Americans (two, six, and one European-American individuals were heterozygous for these variants, respectively).

Unadjusted tests of association of warfarin dose with clinical covariates based on simple linear regression analyses are shown in **Table 3**. Female gender, older age, atrial fibrillation indication and amiodarone use were strongly associated with lower stable warfarin dose ($p < 2.2 \times 10^{-5}$). African-American race, current smoking, larger body habitus and blood clot indication were associated with higher stable warfarin dose ($p < 3.4 \times 10^{-4}$). No associations at the 0.05 significance threshold were observed with use of enzyme inducers or other indications.

Genetic associations with weekly warfarin dose, adjusted for age, gender, BMI and smoking status are shown in **Table 1** for European-Americans, African-Americans and combined adjusted for race. As expected, in European-Americans, six of the nine SNPs tested for association with stable warfarin dose were highly statistically significant at $p < 10^{-5}$. The strongest associations were observed between steady-state dosage and *CYP2C9*2* (rs1799853; $p = 1.4 \times 10^{-11}$), *CYP2C9*3* (rs1057910; $p = 8.7 \times 10^{-25}$), *CYP4F2* (rs2108622; $p = 3.1 \times 10^{-6}$), and *VKORC1* (rs9923231, rs9934438 and rs2359612; $p < 5.3 \times 10^{-58}$). Among African-Americans, seven out of 13 SNPs were associated with steady-state warfarin dose at $p < 0.05$, four of which remained statistically significant even after using a conservative Bonferroni multiple comparisons adjustment (i.e., using $p < 0.0038 = 0.05/13$ to define statistical significance). Both *CYP2C9*3* (rs1057910; $p = 0.013$) and *VKORC1* (rs9923231 and rs9934438; $p = 0.001$) replicated associations previously reported in European-Americans. *VKORC1* rs2359612, which is in strong linkage disequilibrium with rs9923231 and rs9934438 ($R^2 > 0.99$) in European-Americans but not African-Americans ($R^2 = 0.37$), did not replicate in African-Americans. Several SNPs were associated with warfarin dose in African-Americans (e.g., *CYP2C9*6* rs9332131, $p = 0.001$; *CYP2C9*8*

rs7900194, $p = 0.002$; *CALU*rs339097, $p = 0.02$), but were not associated with warfarin dose in European–Americans due to low MAFs. Irrespective of whether results were statistically significant, the direction of SNP–warfarin dose associations in African–Americans was consistent with that of European–Americans. Unadjusted single-SNP tests of associations are provided in **Supplementary Table 2**, and the distribution of stable weekly warfarin dose for each genotype is shown in **Supplementary Table 3**.

Table 4 shows the performance of the fixed 35 mg/week (5 mg/day) dosing strategy, the dosing table published by the FDA on the warfarin package insert [101], the IWPC algorithm [7], and three novel regression-based algorithms. For comparison with previous reports, performance was quantified with the MAE, that is, the average absolute value of the difference between the predicted and the actual stable dose, as done in the IWPC. The three regression-based algorithms are given by: clinical: clinical covariates only (i.e., age, smoking status, amiodarone use, race, gender, venous thromboembolism indication, atrial fibrillation indication and body surface area); limited genetic: clinical covariates and the IWPC variants (i.e., *CYP2C9**2, *CYP2C9**3 and *VKORC1* rs9923231); and expanded genetic: clinical covariates, IWPC variants, plus *CALU*rs339097, *CYP4F2*rs2108622, *CYP2C9**6 and *CYP2C9**8. The equation form of the expanded genetic algorithm is provided in **Supplementary Table 4**.

The use of the FDA table to guide therapy based on *CYP2C9**2, *CYP2C9**3 and *VKORC1* rs9923231 genetic information improved the MAE in European–Americans by 1.1 mg/week and African–Americans by 0.2 mg/week compared with a fixed dose of 35 mg/week. Addition of this genetic information and of clinical covariates using the published IWPC algorithm led to improvement of the MAE in European–Americans from 13.5 mg/week to 9.5 mg/week (clinical plus genetic), but more modestly in African–Americans from 15.2 mg/week to 13.8 mg/week. The expanded genetic algorithm incorporating additional genotypes performed similarly to the IWPC algorithm in European–Americans, but added additional benefit in predicting stable warfarin dose in African–Americans, reducing the MAE to 12.4 mg/week, (95% CI: 10.0–13.2). **Supplementary Table 5** presents the performance of the new algorithms on the dataset with and without bootstrap estimates. There was no evidence of model overfitting based on the bootstrap procedure except in the expanded genetic algorithm in the African–American set, where it was modest (MAEs in the training and validation sets were 11.9 and 12.4, respectively).

Rounding continuous weekly doses to the nearest possible discrete weekly dosing regimen had little effect on performance, as shown with the expanded genetic algorithm in **Table 4**. Performance of all discrete dosing regimens are provided in **Supplementary Table 6**.

The R^2 values for the regression-based algorithms are shown in **Figure 1**. Clinical information alone explained 23% of variation in stable dose in European–Americans and 24% in African–Americans. Addition of the variants used in the IWPC algorithm, (*CYP2C9**2, *CYP2C9**3 and *VKORC1* rs9923231), improved R^2 to 50% in European–Americans and 30% in African–Americans, which was similar to results of the IWPC model (50 and 29%, respectively). The expanded genetic algorithm, including *CYP2C9**6, *CYP2C9**8, *CYP4F2*rs2108622, and *CALU*rs339097 improved explanation of variability in European–Americans to 53% and African–Americans to 41%. Reported values were adjusted for overfitting with the bootstrapping algorithm. **Supplementary Table 5** provides nonbootstrapped MAE and R^2 values.

The performance of the algorithms for discriminating between and predicting low (<21 mg/week), medium (21–49 mg/week) and high (>49 mg/week) steady-state warfarin dose are reported in **Figure 2 & Supplementary Table 7**. The IWPC algorithm predicted low-dose

warfarin users (<21 mg/week) with a positive predictive value of 72% in European-Americans but in African-Americans, only three of 11 individuals (27%) predicted to require a low dose were correct. The expanded genetic algorithm with the addition of *CYP2C9**6, *CYP2C9**8, *CYP4F2* and *CALU* variants, predicted that four African-Americans would require the low warfarin dose which was correct in three cases (75%). For African-Americans requiring high-doses (> 49 mg/week), the positive predictive value for both algorithms was similar, however, the sensitivity was improved with the expanded genetic algorithm (n = 42; 67 vs 20%).

Discussion

This study validates pharmacogenomic influences on steady-state warfarin dose in a diverse EHR-linked DNA biobank. Previously-associated variants in *VKORC1*, *CYP2C9*, *CYP4F2* and *GGCX* were significantly associated with warfarin dose in European-Americans, and variants in *CYP2C9* (*6 and *8) and *CALU* significantly impacted warfarin dose in African-Americans. In this study, we demonstrate reduced dosing error using published algorithms for European-Americans, although we found that existing algorithms did not dramatically improve warfarin dosing for African-Americans. However, a new expanded pharmacogenomic dosing algorithm incorporating additional variants improved prediction of warfarin dose, explaining 53% of the variability in European-Americans and 41% of the variability in African-Americans. This study, along with a recent study with clopidogrel, demonstrates the effectiveness of EHR-linked DNA biobanks for pharmacogenetic studies [34].

While most comparative studies have shown that clinical and genetic information reduce dosing error [35–39], some have found no benefit from additional genetic information [40], and the use of predictive algorithms remains widely unimplemented despite the inclusion of genetic information in the FDA label for warfarin [101]. Use of the FDA table provided in the package insert (which only uses genetic information for select *VKORC1* and *CYP2C9* variants) performed poorly in this study, in agreement with findings of other recent table comparisons [41]. Indeed, frequent INR monitoring by experienced clinicians also effectively guides warfarin-dose adjustment [42]. The demonstration of these findings using an EHR reflecting a diverse, real-world population improves generalizability of results and argues further for the possible utility of such sophisticated algorithms in clinical practice. Barriers to implementation of pharmacogenetic algorithms include algorithm complexity, genotyping cost and means to quickly order and return genotype data for use at the point of care. A future vision includes an EHR-based decision support tool leveraging genotypic data already embedded within the patient's chart, such that the use of a pharmacogenetic algorithm becomes as simple to the clinician as current clinical advisors such as those responding to impaired renal function. This environment is the goal of a recently launched effort at Vanderbilt, the Pharmacogenomic Resource for Enhanced Decisions in Care and Treatment (PREDICT), which prospectively genotypes patients at known pharmacogenetic variants and has implemented clinical decision support for clopidogrel therapy [34,43]. As more pharmacogenomic relationships are characterized and genotyping and sequencing costs decline, genetic data is more likely to be already available and cost effective [44].

Current published warfarin-prediction algorithms predict a continuous weekly dose; the conversion of these continuous dosage recommendations to discrete warfarin pill regimens for patients has been unclear. However, these data demonstrate that rounding the predicted dose on a daily or weekly basis does not diminish the improvement observed using genetic information compared to the traditional, nongenetic dosing. To enable others to implement such continuous outcomes in discrete dosing, we have provided our dose regimens online at [102].

The variability explained by an algorithm is a useful statistic to compare the fitness of an algorithm. By that measure, the greatest opportunity to improve safety lies in the ability of an algorithm to detect users with low-warfarin requirements who are at higher risk from of bleeding during the initiation phase of therapy with traditional 5 mg/day dosing. In addition, a clinically useful algorithm must especially avoid suggesting high initial doses in warfarin-sensitive individuals. An ideal test would have high sensitivity and positive predictive values at low doses, and high specificity at high doses to avoid overdosing and causing harm. We demonstrate improved positive-predictive value using the novel expanded genetic algorithm compared with the IWPC algorithm in warfarin-sensitive African-Americans, while maintaining high-positive predictive value at high doses among all groups. This enhanced prediction in warfarin-sensitive individuals is likely due to improved detection of warfarin sensitivity alleles more common in African-Americans compared with European-Americans, such as *CYP2C9*6* and *CYP2C9*8*. However, there is still substantial unexplained dose variability, highlighting the need for ongoing discovery efforts such as a genome-wide association study of warfarin dose in African-Americans and better characterization of influential pathologic and environmental factors, such as diet.

New direct thrombin inhibitors such as dabigatran may reduce the usage of warfarin over time. However, it is unlikely that warfarin will disappear from clinical use given dabigatran's gastrointestinal side effects, cost issues, inability to urgently reverse anticoagulation and lack of available longitudinal efficacy data in very high-risk patients (such as valve replacements) at the current time [45]. Additionally, as pharmacogenomic algorithms are more widely implemented, it may be desirable to conduct clinical trials comparing new anticoagulants with pharmacogenomically guided warfarin therapy.

Limitations of this study include restriction to European-Americans and African-Americans, as insufficient numbers of other races are present in our biobank to permit large-scale study at this time. While EHRs can enable the rapid accumulation of data, it does reflect local practice patterns, which may not generalize to other institutions. Additionally, this population was limited to users with a target INR range of 1.9–3.2, which may affect generalizability for patients requiring higher target INRs. The algorithms developed here have not been tested on an external dataset. Although bootstrapping adjusts for overfitting within our population, unmeasured site-specific differences may influence performance in other populations and remains an area needing testing. Nonetheless, the algorithm improvement through incorporation of additional genetic variants suggests that improvement in warfarin dosing for African-Americans, and potentially other races, may be achieved with more inclusive genetic models. This study considered only a limited set of known variants influencing warfarin variability, not including some in African-Americans, discovered after genotyping [46]; future genome-wide association studies on non-European-Americans may further elucidate the genetic architecture behind warfarin dose variability. While suggestions are included on the FDA label regarding initial-dose guidance based on genotype [101], the current CHEST guidelines on warfarin therapy do not support using genetic information in dosing prediction [47]. Small randomized controlled trials to date have shown conflicting impact of genotyping on various end points [10,23,48–51]. Results of large, multicenter trials are expected soon [52,53].

Incorporation of additional variants improves warfarin dose prediction in African-Americans while maintaining high performance in European-Americans. EHR-linked DNA bio-repositories provide a real-world resource for pharmacogenomic validation, discovery and clinical algorithm development. Continued research including the prospective study of clinical outcomes, implementation of computerized decision support and refinement of genetic dosing in non-European-descent populations is needed to enhance genetic-guided drug dosing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Executive summary

Background

- Warfarin pharmacogenomic algorithms reduce dosing error, but perform poorly in non-European-Americans and remain widely unimplemented.
- Electronic health record (EHR) systems linked to biobanks may allow for pharmacogenomic analysis, but have not yet been used for this purpose.

Patients & methods

- We used BioVU, the Vanderbilt University EHR-linked DNA repository, to identify Americans of European and African ancestries on stable warfarin therapy.

Results

- European-Americans and African-Americans share some variants in association with stable warfarin dose; however, others are ancestry-specific.
- Implementing published algorithms using clinical and genetic information derived from studies in European ancestry populations reduced error in European-Americans more so than in African-Americans.
- Incorporating additional variants found more often in African-Americans in a novel predictive model improved performance in African-Americans while maintaining high performance in European-Americans.
- Implementing these predictions via dispensable pill regimens reduced dosing error to a similar degree.

Conclusion

- These results validate EHR-linked DNA biorepositories as real-world resources for pharmacogenomic discovery and implementation.

Figure 1. Percentage of variation explained by models
IWPC: International Warfarin Pharmacogenetics Consortium.

Figure 2. Sensitivity and positive predictive value of IWPC and new models

Error bars represent 95% CIs.

AA: African-Americans; EA: European-Americans; IWPC: International Warfarin Pharmacogenetics Consortium.

Table 1

Single SNP tests of association for stable weekly warfarin dose[†].

SNP	Chr.	Position	Gene	Minor allele	All (n = 1167)			European-American (n = 1022)			African-American (n = 145)		
					Median ratio (95% CI)	p-value		Median ratio (95% CI)	p-value		Median ratio (95% CI)	p-value	
rs9923231	16	29189811	VKORC1	T	0.87 (0.86–0.88)	1.51 × 10 ⁻⁶⁰		0.87 (0.86–0.88)	5.31 × 10 ⁻⁵⁸		0.89 (0.83–0.95)	0.001	
rs9934438	16	29192625	VKORC1	A	0.87 (0.86–0.88)	4.48 × 10 ⁻⁶¹		0.87 (0.86–0.88)	1.58 × 10 ⁻⁵⁸		0.89 (0.83–0.95)	0.001	
rs2359612	16	29193707	VKORC1	A	0.88 (0.87–0.89)	3.40 × 10 ⁻⁵⁵		0.87 (0.86–0.88)	1.99 × 10 ⁻⁵⁸		0.97 (0.92–1.09)	0.207	
rs1799853	10	96702047	CYP2C9*2	T	0.92 (0.89–0.94)	8.27 × 10 ⁻¹²		0.91 (0.89–0.94)	1.38 × 10 ⁻¹¹		0.95 (0.83–1.10)	0.509	
rs1057910	10	96741053	CYP2C9*3	C	0.83 (0.80–0.86)	2.70 × 10 ⁻²⁶		0.83 (0.80–0.86)	8.70 × 10 ⁻²⁵		0.81 (0.69–0.96)	0.013	
rs9332131	10	90449157	CYP2C9*6	–	–	–		–	–		0.77 (0.66–0.90)	0.001	
rs7900194	10	90442184	CYP2C9*8 [‡]	A	–	–		–	–		0.87 (0.80–0.95)	0.002	
rs28371685	10	90481096	CYP2C9*11	T	–	–		–	–		0.94 (0.81–1.09)	0.423	
rs2108622	19	15888374	CYP4F2	T	1.04 (1.03–1.06)	1.31 × 10 ⁻⁶		1.05 (1.03–1.06)	3.05 × 10 ⁻⁶		1.04 (0.97–1.12)	0.296	
rs339097	7	123194979	CALU	C	–	–		–	–		1.09 (1.01–1.17)	0.020	
rs699664	2	85608724	GGCX	T	1.02 (1.00–1.03)	0.041		1.02 (1.00–1.04)	0.039		1.00 (0.96–1.05)	0.847	
rs11676382	2	85605821	GGCX	G	0.99 (0.96–1.01)	0.305		0.99 (0.96–1.02)	0.368		0.96 (0.84–1.11)	0.597	
rs2292566	1	199210714	EPHX1	A	1.00 (0.97–1.02)	0.850		0.99 (0.96–1.01)	0.407		1.05 (0.99–1.12)	0.093	

The median ratio is the exponentiated parameter estimate from the multiple linear regression model of the log-transformed warfarin dose. For example, with all clinical covariate values being equal (e.g., age, gender and so on), one minor allele at rs9923231 is associated with a 13% reduction in the median weekly warfarin dose compared with two major alleles.

Chr.: Chromosome.

[†] Adjusted for age, gender, BMI, smoking status and race.

[‡] Genotyped in African-Americans only.

Table 2

Study subject characteristics.

Variable	Median (5%, 95%) or %
Dose (mg/week)	35 (14, 70)
Age (years)	66 (35, 87)
% Female	44.7%
% African-American	12.4%
Height (cm)	173 (155, 188)
Weight (kg)	86 (56, 130)
BMI (kg/m ²)	28 (21, 43)
Body surface area (m ²)	2.1 (1.6, 2.6)
Current smokers	11.2%
Amiodarone	8.0%
Enzyme inducer	2.7%
Indication[†]	
Venous thromboembolism	26.7%
Atrial fibrillation	51.9%
Stroke	5.7%
Orthopedic	0.3%
Other (e.g., cardiomyopathies, coagulopathies and pulmonary hypertension)	17.3%

Median values or proportions are given for each variable for 1167 study individuals. Values in parentheses represent the 5th and 95th percentiles.

[†]Twenty three individuals had multiple indications.

Table 3

Clinical covariates associated with steady-state warfarin dose.

	Median ratio (95% CI)	R ²	p-value
Age (per 10-year change)	0.945 (0.938–0.953)	0.154	3.02×10^{-44}
Height (per 10-cm change)	1.042 (1.030–1.054)	0.042	3.34×10^{-12}
Weight (per 5-kg change)	1.015 (1.012–1.017)	0.105	7.12×10^{-30}
BMI (per 5-kg/m ² change)	1.036 (1.028–1.044)	0.061	4.92×10^{-17}
Body surface area (per 0.2 m ² change)	1.048 (1.040–1.056)	0.112	6.23×10^{-31}
Female gender	0.946 (0.922–0.971)	0.015	2.23×10^{-5}
African–American race	1.088 (1.051–1.125)	0.018	4.13×10^{-6}
Current smokers	1.072 (1.033–1.111)	0.011	3.40×10^{-4}
Amiodarone use	0.856 (0.811–0.902)	0.032	5.73×10^{-10}
Enzyme-inducer use	1.016 (0.939–1.093)	0.000	0.684
Indication			
Venous thromboembolism	1.076 (1.048–1.103)	0.024	9.68×10^{-8}
Atrial fibrillation	0.912 (0.888–0.936)	0.041	2.24×10^{-12}
Stroke	1.003 (0.950–1.056)	0.000	0.912
Orthopedic	1.142 (0.897–1.387)	0.001	0.255

Effect sizes (represented as median ratios with 95% CI and R²) and p-values are given for each test of association. The median ratio is the exponentiated parameter estimate from the linear regression model of the log-transformed warfarin dose. To aid in interpreting the results in the table, for example, a 10-year increase in age is associated with a 5.5% decrease in the median weekly warfarin dose.

Table 4

Performance of predictive dosing algorithms.

Algorithm	Mean absolute error (mg/week)		
	EA and AA	EA	AA
Fixed 35 mg/week dose	13.7	13.5	15.2
US FDA table mid-value of range	12.7	12.4	15.0
IWPC (three variants)	10.0	9.5	13.8
New models			
Clinical [†]	12.1 (11.3–12.6)	11.8 (11.1–12.4)	13.4 (11.3–14.9)
Limited genetic [‡]	9.8 (9.1–10.1)	9.3 (8.7–9.8)	12.9 (10.8–14.5)
Expanded genetic [§]	9.6 (8.9–9.8)	9.1 (8.4–9.6)	12.4 (10.0–13.2)
Dispensable regimen [¶]	9.6 (8.8–9.9)	9.1 (8.6–9.6)	12.4 (9.8–13.4)

For the novel models, the mean absolute error represents the validated bootstrap estimate and 95% CI in parentheses.

AA: African–American; EA: European–American; IWPC: International Warfarin Pharmacogenetics Consortium.

[†]Includes age, gender, body surface area, race/ethnicity, smoking, amiodarone usage and indication (venous thromboembolism vs atrial fibrillation).

[‡]Includes clinical and IWPC genetic variants (e.g., CYP2C9*2, CYP2C9*3 and VKORC1 rs9923231) as modeled in IWPC [37].

[§]Includes clinical and VKORC1 rs9923231, CYP2C9 variants (*2, *3, *6, *8), CYP4F2 rs2108622, and CALU rs339097.

[¶]Includes expanded genetic model variables rounded to nearest dispensable regimen using any single pill strength in 0.5, 1, 1.5 or 2 pill increments.