



Genetic variants associated with warfarin dose in African-American individuals: a genome-wide association study

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Summary

Lancet 2013; 382: 790–96

Published Online

June 5, 2013

[http://dx.doi.org/10.1016/S0140-6736\(13\)60681-9](http://dx.doi.org/10.1016/S0140-6736(13)60681-9)

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Background *VKORC1* and *CYP2C9* are important contributors to warfarin dose variability, but explain less variability for individuals of African descent than for those of European or Asian descent. We aimed to identify additional variants contributing to warfarin dose requirements in African Americans.

Methods We did a genome-wide association study of discovery and replication cohorts. Samples from African-American adults (aged ≥ 18 years) who were taking a stable maintenance dose of warfarin were obtained at International Warfarin Pharmacogenetics Consortium (IWPC) sites and the University of Alabama at Birmingham (Birmingham, AL, USA). Patients enrolled at IWPC sites but who were not used for discovery made up the independent replication cohort. All participants were genotyped. We did a stepwise conditional analysis, conditioning first for *VKORC1* –1639G→A, followed by the composite genotype of *CYP2C9**2 and *CYP2C9**3. We prespecified a genome-wide significance threshold of $p < 5 \times 10^{-8}$ in the discovery cohort and $p < 0.0038$ in the replication cohort.

Findings The discovery cohort contained 533 participants and the replication cohort 432 participants. After the prespecified conditioning in the discovery cohort, we identified an association between a novel single nucleotide polymorphism in the *CYP2C* cluster on chromosome 10 (rs12777823) and warfarin dose requirement that reached genome-wide significance ($p = 1.51 \times 10^{-8}$). This association was confirmed in the replication cohort ($p = 5.04 \times 10^{-5}$); analysis of the two cohorts together produced a p value of 4.5×10^{-12} . Individuals heterozygous for the rs12777823 A allele need a dose reduction of 6.92 mg/week and those homozygous 9.34 mg/week. Regression analysis showed that the inclusion of rs12777823 significantly improves warfarin dose variability explained by the IWPC dosing algorithm (21% relative improvement).

Interpretation A novel *CYP2C* single nucleotide polymorphism exerts a clinically relevant effect on warfarin dose in African Americans, independent of *CYP2C9**2 and *CYP2C9**3. Incorporation of this variant into pharmacogenetic dosing algorithms could improve warfarin dose prediction in this population.

Funding National Institutes of Health, American Heart Association, Howard Hughes Medical Institute, Wisconsin Network for Health Research, and the Wellcome Trust.

Introduction

Warfarin is the most widely prescribed oral anticoagulant in the USA, with more than 35 million prescriptions in 2011.¹ Complicated by a narrow therapeutic index, warfarin contributes to 33% of hospital admissions related to adverse drug events in individuals aged at least 65 years from the USA.² Rapid and predictable therapeutic anticoagulation is difficult because dose requirements vary substantially between patients, but is crucial for safe and effective treatment.^{3,4}

Dose variability is affected by single nucleotide polymorphisms (SNPs) in genes encoding cytochrome P450 2C9 (*CYP2C9*), which metabolises the S-enantiomer of warfarin (more active than the R-enantiomer), and vitamin K epoxide reductase complex 1 (*VKORC1*), which is the target enzyme for the drug. Both candidate gene studies and genome-wide association studies (GWAS)

have consistently shown that *VKORC1* and *CYP2C9* genotypes explain up to 30% of the total variability in warfarin dose requirements in people of European or Asian origin.^{5–12} The International Warfarin Pharmacogenetics Consortium (IWPC) has shown that the *VKORC1* –1639G→A SNP (rs9923231), *CYP2C9**2 (rs1799853), and *CYP2C9**3 (rs1057910) can be used with clinical variables to predict warfarin dose.^{13,14}

Unfortunately, these commonly studied genetic variants explain substantially less variability in individuals of African descent than in those of European or Asian origin.^{5,13,14} African Americans have been absent in previous warfarin GWAS. Importantly, the genomes of people of African ancestry have decreased linkage disequilibrium and increased diversity.¹⁵ Therefore, we aimed to identify novel variants contributing to warfarin dose requirements in African Americans.

Methods

Study population

We did a GWAS of two study samples: one from IWPC sites across the USA and one from the University of Alabama at Birmingham (UAB; Birmingham, AL, USA). Together, these samples made up the discovery cohort. Patients enrolled in IWPC sites, but who were not used for discovery (enrolled at local site after samples from that site were sent for genotyping or their local site did not join IWPC until after IWPC samples were sent for genotyping), made up an independent replication cohort. All study participants (discovery and replication cohorts) were adults (aged ≥ 18 years) of self-reported African ancestry who were taking a stable maintenance dose of warfarin, and for whom clinical and demographic data had been obtained as previously reported.^{5,13} Definitions of stable dose varied between sites (appendix), but most sites insisted that patients had to have been on a stable dose for at least three consecutive clinic visits, which is indicative of the maintenance phase of warfarin treatment after initial stable dosing has been achieved. Patients provided a DNA sample (whole-blood, saliva, or mouthwash sample). All participants gave written informed consent to participate, and all protocols were approved by local institutional review boards.

Procedures

For the IWPC samples, data curation and quality control procedures were as described previously.^{13,14} Data for several variables were obtained for participants from IWPC sites in the discovery cohort (appendix). These individuals were genotyped with the Illumina 610 Quad BeadChip (Illumina, San Diego, CA, USA) at the RIKEN Center for Genomic Medicine (Yokohama, Japan).

Information about demographic and clinical variables was obtained for the UAB study sample as previously described.⁵ Participants from UAB were genotyped at the University of Washington's Department of Genome Sciences (Seattle, WA, USA) with the Illumina Human1M-Duo 3.0 (Illumina, San Diego, CA, USA). SNPs previously associated with warfarin dose were genotyped independently (ie, via single SNP genotyping and at local institutions) in both datasets.

In the replication cohort, samples were tested for a genome-wide significant signal ($p < 5 \times 10^{-8}$). To ensure important signals approaching genome-wide significance were not missed, we identified an additional 12 SNPs with $10^{-5} > p > 5 \times 10^{-8}$ as being of interest that were also tested in the replication cohort. IWPC replication samples were genotyped at Vanderbilt University's DNA Resources Core (Nashville, TN, USA) with the Sequenom (San Diego, CA, USA) platform, and UAB replication samples at the University of Washington (Seattle, WA, USA) by Sanger sequencing.

To provide insight into potential functional mechanisms of an identified SNP, we assessed the pharmacokinetics of warfarin in an independent cohort of

60 African Americans on stable warfarin doses, as previously described.¹⁶ Briefly, venous blood was obtained 12–16 h after a warfarin dose. Warfarin enantiomer concentrations were established by a chiral high-performance liquid chromatography-based method, and the oral clearance of both enantiomers, corrected for body surface area, was calculated.¹⁷ Warfarin is almost exclusively cleared by liver metabolism, thus oral clearance approximates the hepatic metabolism of warfarin.

The phenotypic data and a restricted genotypic dataset (eg, *VKORC1*, *CYP2C9*, *CYP4F2*, and rs12777823) are accessible for download at PharmGKB. The IWPC GWAS data are made available under the IWPC Data Usage Agreement and can be accessed at PharmGKB.

Statistical analysis

Conventional GWAS quality control procedures were undertaken, such as checks for sex mismatches, identity by descent testing, deviation from Hardy–Weinberg Equilibrium, and SNP and sample exclusions on the basis of call rates (appendix).

Imputation of additional SNPs was done with hidden Markov models via MACH (version 1.0),¹⁸ on the basis of HapMap Phase 2 Release 22 data (appendix). The quality metric (r^2) was calculated for all imputed SNPs; no SNPs reported here had a value of less than 0.8.

Global estimates of ancestry (appendix) were established by the first and second principal components, with the first principal component used as a global estimate of African ancestry. All covariates obtained in each dataset (including drugs; appendix) and the first ten principal components obtained through EIGENSTRAT (version 4.2)¹⁹ were tested as single covariates in a PLINK (version 1.07) additive model analysis for association with stable warfarin dose. Mean height and weight by sex were used for participants missing these variables in the IWPC cohort.

For all analyses, the primary phenotype (weekly stable warfarin dose) was log transformed to give a normal distribution. We excluded individuals missing data for non-genetic covariates associated with warfarin dose. For the discovery cohort, GWAS analysis was done in PLINK with a linear regression additive genetic model, by use of covariates significantly associated with stable warfarin dose. We combined GWAS results for the IWPC and UAB study samples with fixed effects meta-analysis. We used the same quality-control procedures and analysis approaches in the replication cohort.

Previous warfarin GWAS in individuals of European and Asian ancestry emphasised the importance of conditioning analyses for the well recognised *VKORC1* and *CYP2C9* SNPs.^{10,12,20} Therefore, a-priori analysis plans also included a stepwise conditional analysis, conditioning first for *VKORC1* –1639G → A, followed by the composite genotype (presence or absence of a variant allele) of *CYP2C9**2 and *CYP2C9**3 (appendix).

We prespecified a genome-wide significance threshold of $p < 5 \times 10^{-8}$ for the discovery cohort. For the replication

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See Online for appendix

To download phenotypic data from PharmGKB see <http://www.pharmgkb.org/downloads.jsp>

| | Discovery cohort | | Replication cohort (n=432) |
|--|--|---|----------------------------|
| | International Warfarin Pharmacogenetics Consortium (n=327) | University of Alabama at Birmingham (n=206) | |
| Stable warfarin dose (mg/wk) | 45.6 (18.6) | 42.2 (18.4) | 45.7 (23.8) |
| International normalised ratio | 2.46 (0.44) | 2.41 (0.40) | 2.48 (0.31) |
| Age (years) | 57.1 (14.8) | 59.1 (15.3) | 58.6 (16.0) |
| Height (cm) | 169.4 (10.1) | 170.9 (10.5) | 170.4 (27.2) |
| Weight (kg) | 94.4 (10.1) | 89.6 (22.9) | 93.5 (27.8) |
| Sex | | | |
| Male | 121 (37%) | 92 (45%) | 168 (39%) |
| Female | 206 (63%) | 114 (55%) | 264 (61%) |
| Primary warfarin indication* | | | |
| Pulmonary embolism or deep vein thrombosis | 153 (47%) | 97 (47%) | 186 (59%) |
| Atrial fibrillation or flutter | 81 (25%) | 68 (33%) | 67 (21%) |
| Cardiomyopathy or left ventricle dilation | 5 (2%) | 0 | 0 |
| Heart valve replacement | 26 (8%) | 0 | 7 (2%) |
| Stroke | 28 (9%) | 21 (10%) | 29 (9%) |
| Other | 34 (10%) | 20 (10%) | 27 (9%) |
| Current smoker | 53 (16%) | 34 (17%) | NA |
| Amiodarone use | 16 (5%) | 7 (3%) | 21 (5%) |
| Aspirin use | 92 (28%) | 93 (45%) | 149 (34%) |
| Genotype† | | | |
| CYP2C9*2 | 12 (2%) | 6 (1%) | 19 (2%) |
| CYP2C9*3 | 5 (1%) | 6 (1%) | 13 (2%) |
| VKORC1-1639G→A | 59 (10%) | 38 (10%) | 77 (11%) |

Data are mean (SD) or n (%). NA=not available. *Primary indication was not available for 116 individuals in the replication cohort. †Reported as number of people carrying one or two variant alleles; percentage is minor allele frequency.

Table 1: Baseline characteristics

cohort, we defined the significance level on the basis of the number of samples tested (13 SNPs). Therefore, significant replication was defined as a Bonferroni-corrected *p* value of less than 0.05/13 or 0.0038.

Additionally, we looked at a previous GWAS of individuals of European and Asian ancestry^{9,10,12} to identify any association between the identified SNP and warfarin dose. We tested the SNP in a previously described Egyptian cohort.²¹

We modelled the residual dose (actual dose minus predicted IWPC dose) with linear regression, with genotypes of a replicated SNP as covariates in participants in the discovery cohort for whom complete data were available for calculation of warfarin dose with the IWPC algorithm. We tested the difference between the models (with and without rs12777823 as a covariate) by ANOVA. All statistical analyses were done with R (version 2.10.1).

Pharmacokinetic variables were compared between genotype groups by ANOVA, and between the GG genotype and both the AA and AG genotypes by the one-sided Student's unpaired *t* test, with the expected direction defined by the data from the GWAS.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. MAP, LHC, NAL, NJC, RBA, TEK, and JAJ had complete access to the data, and JAJ had final responsibility for the decision to submit for publication.

Results

For the IWPC study sample, eight institutions contributed genetic and clinical data for 345 African-American individuals, although 18 were subsequently excluded because of missing data or quality-control issues. The UAB study sample consisted of 206 African Americans; no participant was excluded from analysis because of quality control issues. Therefore, the discovery cohort contained 533 participants after quality control (table 1). The replication cohort consisted of 432 African Americans enrolled from six IWPC sites, including UAB (table 1; appendix).

Participants in both the IWPC and UAB cohorts clustered between the HapMap CEU (northern and western European ancestry) and YRI (African ancestry) samples, as was expected (appendix). Mean proportion of African ancestry in the discovery cohort was 85% (SD 9%) in participants from IWPC sites and 86% (8%) from UAB. The proportion of African ancestry was not associated with warfarin dose in participants from IWPC sites ($p=0.08$) or from UAB ($p=0.944$). 557286 SNPs passed quality-control checks in the IWPC samples (mean call rate 99.96%) and 950007 in the UAB samples (99.95%).

Age, weight, height, aspirin use, and amiodarone use were associated with warfarin dose in the discovery cohort ($p<0.05$ for all in both IWPC and UAB samples; appendix). A glomerular filtration rate of less than 30 mL/min in UAB samples was also associated with warfarin dose ($p<0.05$).

None of the SNPs reported here that were associated with stable warfarin dose significantly deviated from Hardy-Weinberg equilibrium. SNPs on chromosome 16 reached genome-wide significance, including *VKORC1*-1639G→A (rs9923231; $p=2.08\times 10^{-9}$; figure 1, appendix). Associations with SNPs in linkage disequilibrium with rs9923231 on chromosome 16 were also significant (appendix).

After the prespecified conditioning on the *VKORC1* locus, *CYP2C9**2, and *CYP2C9**3, the most significant SNP association with warfarin dose was rs12777823 on chromosome 10 ($p=1.51\times 10^{-8}$; figure 1, appendix). The minor allele frequency was about 25% in the discovery cohort (appendix). Other top signals after conditioning are shown in the appendix.

The SNP that achieved genome-wide significance in the discovery cohort after conditioning (rs12777823) was also significantly associated with stable warfarin dose in the replication cohort after conditioning ($p=5.04\times 10^{-5}$; figure 2, appendix). This *p* value is well below the threshold of 0.0038 that had been defined for significant replication. A combined analysis of the discovery and

replication cohorts for rs12777823 leads to a combined p value of 4.5×10^{-12} . None of the 12 SNPs of interest were associated with stable warfarin dose (appendix). rs12777823 was also not associated with warfarin dose in individuals of European and Japanese ancestry, or Egyptian individuals (data not shown).

The IWPC warfarin dosing equation is less predictive for African Americans than for other populations.¹³ We modelled the residual dose with linear regression in 504 participants from the discovery cohort. Addition of rs12777823 genotype to the IWPC algorithm improved the ability to predict actual therapeutic warfarin dose with the IWPC algorithm by an absolute 5% (relative 21%; table 2). Individuals heterozygous or homozygous for the rs12777823 A allele have reduced stable warfarin doses (table 2).

In the independent cohort of 60 African Americans, the hepatic metabolism of the S-enantiomer of warfarin (as estimated by oral clearance) differed significantly with rs12777823 genotype (table 3). Additionally, the ratio of the R-enantiomer and S-enantiomer differed significantly by rs12777823 genotype, consistent with genotypic differences in metabolism of the S-enantiomer, but not of the R-enantiomer (table 3).

*CYP2C9**2 and *CYP2C9**3 occurred at low frequency (table 1) and were not significantly associated with warfarin dose variability (*CYP2C9**2 nominal $p=0.834$; *CYP2C9**3 nominal $p=0.023$). Of the *CYP2C9* non-synonymous polymorphisms specific to individuals of African descent, only *CYP2C9**11 was imputed in the meta-analysis and had a significant association with warfarin dose ($p=0.0001$). The *CYP2C9**5, *CYP2C9**6, and *CYP2C9**8 alleles were not included on the GWAS arrays and could not be imputed. Analyses with additional *CYP2C9* alleles (eg, *CYP2C9**5, *CYP2C9**6, and *CYP2C9**8) had low power, because few participants were genotyped for these alleles independent of the GWAS. Notably, rs12777823 is in low linkage disequilibrium with *CYP2C9**2, *CYP2C9**3, and *CYP2C9**11 (all $r^2 < 0.05$), on the basis of the imputed genotypes for these alleles in the GWAS cohorts. Additionally, on the basis of participants independently genotyped for *CYP2C9**5, *CYP2C9**6, and *CYP2C9**8, rs12777823 is also in low linkage disequilibrium with these SNPs (all $r^2 < 0.10$), suggesting that the rs12777823 signal is independent of previously studied *CYP2C9* polymorphisms. The *CYP4F2* Val433Met variant (rs2108622) that has been previously associated with warfarin dose in those of European and Asian descent^{10,12} was not associated with warfarin dose in this study ($p=0.07$).

Discussion

Our results show that *VKORC1* genotype is the primary genetic determinant of variability in warfarin dose requirements in African Americans, which is consistent with previous GWAS data from European and Japanese populations.^{9,10,12} After adjustment for *VKORC1*

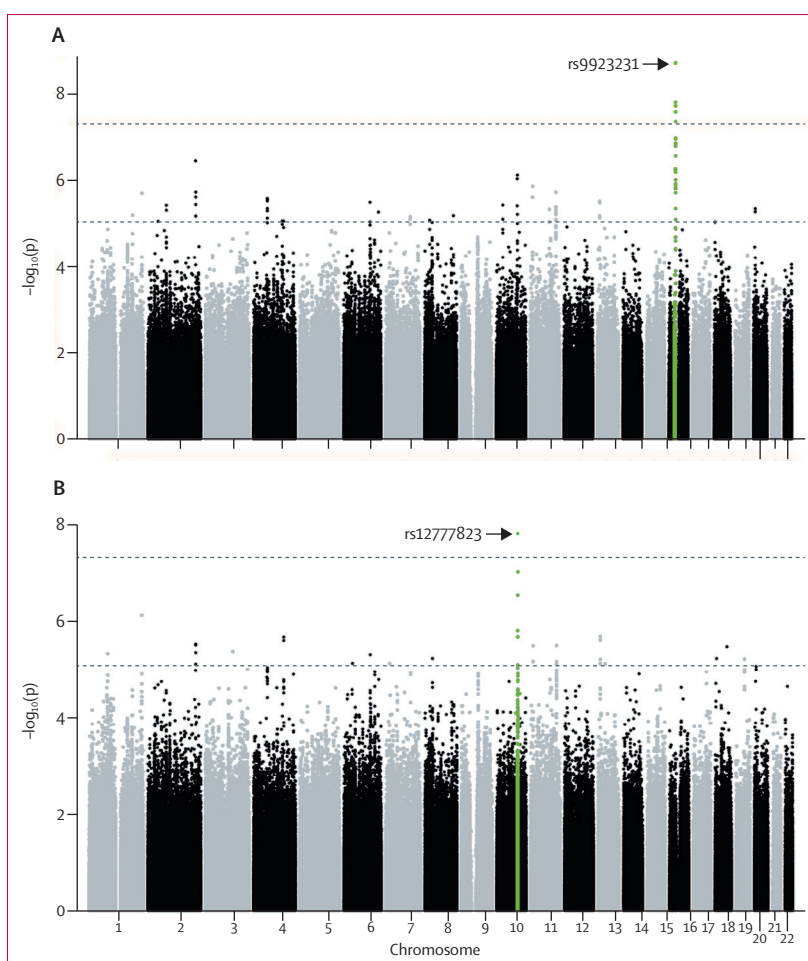


Figure 1: Manhattan plots showing associations between single nucleotide polymorphisms and stable warfarin dose in the discovery cohort

(A) Without and (B) with prespecified conditioning. The top line shows $p=5 \times 10^{-8}$. Green points indicate genome-wide significant regions.

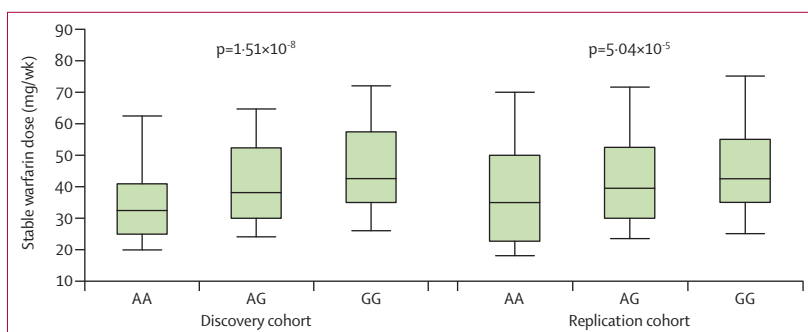


Figure 2: Stable warfarin dose by rs12777823 genotype

Lines within boxes represent medians; lower and upper boundaries represent IQRs; and whiskers represent tenth and 90th percentiles. p values calculated after conditioning on the *VKORC1* locus, *CYP2C9**2, *CYP2C9**3, and non-genetic covariates associated with warfarin dose.

–1639G→A, *CYP2C9**2, and *CYP2C9**3, we identified a novel association between rs12777823 and warfarin dose variability; we replicated this finding in an independent African American cohort.

| | Coefficient* | p value | Adjusted model R ² † |
|-------------------------------------|--------------|-----------------------|---------------------------------|
| IWPC dosing equation Predicted Dose | .. | <2×10 ⁻¹⁶ | 0.2193 |
| rs12777823 AG | -6.92 | 6.76×10 ⁻⁶ | .. |
| rs12777823 AA | -9.34 | 0.00050 | .. |
| IWPC dosing equation and rs12777823 | .. | 1.7×10 ⁻⁶ | 0.2666 |

IWPC=International Warfarin Pharmacogenetics Consortium. *Represents change in warfarin dose (mg/week). †Increase indicates increase in amount of variability explained.

Table 2: Effects of novel CYP2C variation on warfarin dose prediction with the IWPC dosing algorithm

| | AA (n=6) | AG (n=24) | GG (n=30) |
|---|-------------|-------------|-------------|
| Oral clearance of S-enantiomer (mL/min/m ²)* | 1.20 (0.69) | 1.38 (0.48) | 1.80 (0.83) |
| Plasma concentration ratio of R-enantiomer to S-enantiomer† | 1.29 (0.50) | 1.42 (0.43) | 1.76 (0.68) |

Data are mean (SD). Clearance values are normalised for body surface area. *p=0.042 for association between the three genotype groups (one-way ANOVA); p=0.049 for comparison between AA and GG genotypes, and p=0.016 for comparison between AG and GG genotypes (one-sided Student's unpaired t test). †p=0.049 for association between the three genotype groups (one-way ANOVA); p=0.039 for comparison between AA and GG genotypes, and p=0.018 for comparison between AG and GG genotypes (one-sided Student's unpaired t test).

Table 3: Pharmacokinetic parameters of warfarin R-enantiomer and S-enantiomer by rs12777823 genotype

Panel: Research in context

Systematic review

We searched PubMed for reports of studies and meta-analyses published in any language before Dec 1, 2012. We used the search terms “warfarin” and “genome wide association study”. We identified three genome-wide association studies of warfarin dose requirement^{9,10,12} and one of acenocoumarol.²⁰ All previous studies were done in either European or Asian populations. They all showed that that *VKORC1* -1639G→A (rs9923231), *CYP2C9**2 (rs1799853), *CYP2C9**3 (rs1057910), and *CYP4F2* (rs2108622) are the main genetic determinants of drug dose. The study of acenocoumarol²⁰ identified a single nucleotide polymorphism (SNP) upstream of *CYP2C18*, although it did not reach genome-wide significance after conditioning for *CYP2C9**2 and *CYP2C9**3. To identify previous studies testing genetic variation associated with warfarin dose requirements in African Americans, we did another search of PubMed with the same date criteria and no language restrictions, but used the search terms “warfarin” and “African American”. We identified five studies,^{22–26} all of which used a candidate-gene approach.

Interpretation

As far as we are aware, ours is the first genome-wide association study to investigate warfarin dose requirements in African Americans. Our results identified a SNP upstream of *CYP2C18*, rs12777823, that is significantly associated with warfarin dose requirement in African Americans and is independent of previous associations with *VKORC1* and *CYP2C9*. Patients carrying this SNP have a significantly lower stable dose of warfarin than do those without this variant, and the addition of this SNP improved the International Warfarin Pharmacogenetics Consortium algorithm by 21%. We have also shown that this SNP significantly alters warfarin clearance. To our knowledge, no other studies of warfarin dose requirement have identified this SNP.

In the context of other genotypes and clinical factors in the IWPC model, the rs12777823 genotype was associated with a reduction in dose of almost 7 mg/week in heterozygotes and of 9 mg/week in homozygotes—an effect similar to that of the *CYP2C9**2 allele in the IWPC dosing algorithm.¹³ Thus, the effect is consistent with a polymorphism that is well accepted to be clinically relevant for individuals. The effect is also important at a population level, because of both the effect size and the frequency of the polymorphism. Specifically, rs12777823 explains 5% of the variability in warfarin dose in African Americans, which is consistent with the variability explained by *CYP2C9**2 and *CYP2C9**3 in individuals of European ancestry,⁸ and greatly exceeds the variability explained by *CYP2C9**2 and *CYP2C9**3 in African Americans (1–2%).^{5,22}

Our findings have important implications for prediction of warfarin maintenance dose in individuals of African ancestry, for whom current warfarin pharmacogenetic algorithms are less predictive.^{13,14} On the basis of our data, the IWPC algorithm could be used to estimate warfarin dose, and then the rs12777823 variant in African Americans could be accounted for with dose reductions (panel). Further improvement in dose estimation might eventually be possible for African Americans when additional SNPs specific to individuals with African ancestry are identified.

rs12777823 is located within the *CYP2C* gene cluster, upstream from *CYP2C18* on chromosome 10q23, and includes the *CYP2C9*, *CYP2C8*, *CYP2C18*, and *CYP2C19* genes. In a GWAS of clopidogrel antiplatelet effect in Amish individuals,²⁷ rs12777823 was the strongest signal, although it was in strong linkage disequilibrium ($r^2=0.87$) with *CYP2C19**2 (rs4244285), which produces a non-functional truncated *CYP2C19* protein and is believed to be the causative variant. We reported low linkage disequilibrium ($r^2=0.5$) between rs12777823 and *CYP2C19**2, and the association between warfarin dose requirements and *CYP2C19**2 was not significant at the genome-wide level and was absent when conditioned on rs12777823. Therefore, the effect of rs12777823 in African Americans is probably independent of *CYP2C19**2.

The functional basis for the reported association between warfarin dose and rs12777823 is unknown, but the pharmacokinetic data indicate an effect on metabolism of the S-enantiomer. The S-enantiomer is metabolised primarily by *CYP2C9*, and the stereoselective nature of the genetic association suggests rs12777823 affects *CYP2C9* activity. Importantly, the low linkage disequilibrium between this SNP and other known functional SNPs in *CYP2C9* suggest that it has an independent and novel genetic effect on *CYP2C9* metabolism, which could have clinically important implications for many other drugs that are *CYP2C9* substrates.

rs12777823 is common in African Americans: in our study, the minor allele frequency was 25% in both the discovery and replication cohorts. Although common in European (minor allele frequency 14%) and Japanese (32%) populations, rs12777823 did not have a significant

association with warfarin dose in previous GWAS,^{9,10,12} or when we interrogated the European and Japanese GWAS datasets for this SNP. Similarly, this SNP did not have an association with warfarin dose in about 200 individuals from Egypt. Collectively, these data suggest that the association in African Americans might not be due to rs12777823 itself, but a causal SNP in linkage disequilibrium with rs12777823 in African Americans but not in other populations.

Although the *CYP2C9*2* and *CYP2C9*3* alleles contribute to warfarin dose variability in populations of European descent,^{9,10} we recorded no genome-wide significant association in the GWAS of African Americans. The low frequency of these alleles in this population might have contributed to this finding.

GWAS has limitations because not all variants are interrogated—eg, a novel *VKORC1* variant (rs61162043) identified through resequencing and associated with increased warfarin dose requirements in African Americans²² is not captured on the GWAS genotyping platforms, nor is it in HapMap.²⁸ *CYP2C9*5*, *CYP2C9*6*, *CYP2C9*8*, and *CYP2C9*11* have been previously associated with warfarin dose variability,^{5,22,29} but were not included on the GWAS genotyping platforms; only *CYP2C9*11* was available through imputation. Because few sites independently genotyped for these variants, we could not reliably estimate their contribution to warfarin dose requirements in our study. However, we could rule out the *CYP2C9*2*, *CYP2C9*3*, and *CYP2C9*11* alleles as causes of the recorded association with the rs12777823 variant on the basis of conditional analyses and the computed pairwise linkage disequilibrium between these SNPs. Additionally, linkage disequilibrium between rs12777823 and *CYP2C9*5*, *CYP2C9*6*, and *CYP2C9*8* was low in participants for whom these data were available.

Although use of new oral anticoagulants is expected to increase with time, warfarin will probably be widely used for the foreseeable future. A study reported in 2012¹ suggested that dabigatran use has risen consistently since its approval; 8·1% of oral anticoagulant prescriptions in the USA in late 2011 were for this drug. In Sweden, the proportion of prescriptions for oral anticoagulants that were for new drugs rose from 3·0% in 2011, to 4·7% in 2012.³⁰ Thus, although new oral anticoagulant use is increasing, warfarin remains the predominant oral anticoagulant. Additionally, in December, 2012, the US Food and Drug Administration issued a safety notice that outlined that dabigatran is contraindicated in patients with mechanical heart valves, meaning new oral anticoagulants will not be used for all the indications for which warfarin is presently used.

Importantly, because of cost issues, warfarin will probably remain the mainstay oral anticoagulant for the uninsured or underinsured in the USA and individuals in developing countries—particularly in Africa—for many years to come. Therefore, identification of a way

to achieve therapeutic anticoagulation efficiently with warfarin is a priority. Pharmacogenetic warfarin dosing algorithms, such as the IWPC algorithm, could reduce the time needed to reach stable therapeutic dosing;^{8,13,31} genotype-guided warfarin dosing better predicts warfarin maintenance dose than does standard dosing, and reduces out-of-range international normalised ratio values.³¹ Secondary analyses in a previous study³¹ also suggested the potential for improved outcomes via a pharmacogenetic approach. Randomised controlled trials are assessing the clinical utility of genotype-guided warfarin dosing.^{32,33} If the trials show benefits for pharmacogenetic approaches to warfarin, then future consensus guidelines will probably support warfarin pharmacogenetics in the clinical setting.

In conclusion, we have shown that *VKORC1* is the major gene affecting warfarin dose variability in African Americans, with the *CYP2C* locus exerting influence independent of well known *CYP2C9*2*, *CYP2C9*3*, and *CYP2C19*2* polymorphisms. Our findings suggest that warfarin dose variability is affected by variants other than the well established *VKORC1* and *CYP2C9* ones in African Americans; these new variants could improve dose prediction in these individuals.

Contributors

MAP, LHC, NAL, PD, MJW, DMR, RBA, TEK, and JAJ did the literature search. MAP, LHC, NAL, DCC, RJD, SIK, SAL, MJR, SAS, AHBW, JKB, PD, MJW, TM, DMR, NJC, RBA, TEK, YN, and JAJ designed the study. MAP, LHC, NAL, DCC, NT, HT, YB, BMB, JLH, TYL, EAN, MO, MHS, SRP, HS, MT, KEW, MJR, SAS, MJW, TM, and MK gathered data and generated laboratory data. MAP, LHC, NAL, ERG, AK, and AP prepared figures. MAP, LHC, NAL, ERG, AK, RD, AP, DCC, JW, NL, NT, SB, HT, MJR, MK, and NJC analysed data. MAP, LHC, NAL, ERG, AK, RD, AP, DCC, JL, NL, NT, SB, HT, SAS, AHBW, JKB, PD, MJW, TM, DMR, NJC, RBA, TEK, YN, and JAJ interpreted data. MAP, LHC, NAL, RBA, TEK, and JAJ drafted the report. All authors contributed to revision of the report.

Conflicts of interest

NAL, RJD, JLH, JAJ are principal investigators; TYL, SAS, and JKB are members of the genotyping committee; and JAJ is a member of the executive committee for the National Institutes of Health Clarification of Optimal Anticoagulation through Genetics trial. KEW is an unpaid consultant for Roche Diagnostics and a paid consultant for the Gentris Corporation. JKB is a site principal investigator for the Iverson Warfarin Trial and holds a patent pending for CYP4F2 use for warfarin dosing. RBA is founder, equity holder, and consultant for Personalis. The other authors declare that they have no conflicts of interest.

Acknowledgments

MAP (K23 HL089808), NAL (K23 NS45598; RO1 HL092173; UL1 TR000165), NL (R01GM081488), MT and JKB (UL1 TR000427), SAS (KL2 TR000069), DMR (U19 HL 065962; RC2 GM092618), NJC (U19 GM61393; U19 HL065962), RBA and TEK (R24 GM061374), and JAJ (U01 GM074492) were supported by National Institutes of Health grants. LHC was supported by an American Heart Association Grant-In-Aid (10GRNT3750024). RD was supported by the Howard Hughes Medical Institute. MT and JKB were also supported by the Wisconsin Network for Health Research. PD was supported by the Wellcome Trust (grant 098051).

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