Genotype and risk of major bleeding during warfarin treatment

Aim: To determine whether genetic variants associated with warfarin dose variability were associated with increased risk of major bleeding during warfarin therapy. **Materials & methods:** Using Vanderbilt's DNA biobank we compared the prevalence of *CYP2C9*, *VKORC1* and *CYP4F2* variants in 250 cases with major bleeding and 259 controls during warfarin therapy. **Results:** *CYP2C9**3 was the only allele that differed significantly among cases (14.2%) and controls (7.8%; $p = 0.022$). In the 214 (85.6%) cases with a major bleed 30 or more days after warfarin initiation, *CYP2C9**3 was the only variant associated with bleeding (adjusted odds ratio: 2.05; 95% CI: 1.04, 4.04). **Conclusion:** The *CYP2C9**3 allele may double the risk of major bleeding among patients taking warfarin for 30 or more days.

Original submitted 30 June 2014; Revision submitted 15 October 2014

Keywords: *CYP2C9* • *CYP4F2* • pharmacogenetics • risk of major bleeding • *VKORC1* • warfarin

Warfarin, the most commonly used oral anti-coagulant [1], reduces the risk of thrombosis in a variety of clinical situations [2]. However, the clinical use of warfarin is challenging. It has a narrow range of effective anticoagulation that is defined by the international normalized ratio (INR) with an INR <2 and >3 associated with increased risk of thrombosis and bleeding, respectively [3,4]. Moreover, the dose of warfarin required to produce an INR within the target range varies substantially among individuals [5]. Clinical and genetic factors contribute to this variability in warfarin dose requirement [6].

SNPs in genes that encode enzymes responsible for warfarin metabolism (*CYP2C9*), vitamin K cycling (*CYP4F2*) and the target of warfarin (*VKORC1*) contribute substantially to interindividual variability in warfarin dose requirement [7,8]. This genetic contribution to predicting warfarin dose is well-established [9] and is most important during the initial weeks of therapy [10–12], when an individual's stable warfarin dose is not yet known and is titrated empirically according to the INR response. Many pharmacogenetic studies,

and consequently guidelines for clinicians [13], have focused on the ability of genotype to improve the prediction of dose when initiating warfarin therapy. In contrast, the genetic contribution to bleeding associated with warfarin is poorly defined [14], although bleeding is the most common serious complication of therapy [15].

Studies of genotype and major bleeding in patients receiving warfarin have been relatively small [16–19] and it is unclear whether genotype is associated with bleeding after the dose-titration phase, often delineated as the first 30 days of therapy [20]. Genotype contributes little additional information to warfarin dose prediction after the dose-titration phase [12,21], and therefore the pharmacogenetic studies and guidelines have focused on the dose-titration phase [9]. However, most bleeding events occur after the dose-titration phase, and the contribution of genotype to this risk is poorly defined.

Knowledge of genotype was associated with a marked reduction in hospitalization for bleeding or thrombosis in the Medco-Mayo Warfarin Effectiveness Study [22]. However,

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much of the putative benefit of genotyping occurred after the warfarin dose-titration phase, an unexplained observation that raised the possibility that genotype affects bleeding risk after the initial 30 days of therapy. If true, knowledge of genotype could alter clinical care throughout warfarin therapy, not just during the dose-titration phase.

Accordingly, we hypothesized that variants in genes associated with altered warfarin sensitivity (*CYP2C9*, *VKORC1* and *CYP4F2*) are associated with increased risk of major bleeding, particularly after the warfarin dose-titration phase. To test this hypothesis, we conducted a retrospective case-control study in a large clinical practice-based DNA biobank.

Materials & methods

Study design

The study was performed using BioVU, the Vanderbilt University Medical Center's (VUMC) DNA Biobank [23]. A full description of BioVU, including its design, collection methods and ongoing IRB oversight, has been published [23]. Briefly, BioVU accrues DNA from blood samples obtained during routine clinical care from patients who have not opted out of participation. DNA is extracted from samples that would otherwise be discarded, de-identified and linked to a de-identified version of the electronic medical record (EMR) at VUMC.

We searched the de-identified EMR to identify a study population of potential cases and controls (Figure 1) that met the following criteria: age ≥ 18 years old, mention of warfarin/coumadin (and common misspellings) with an associated dose within 7 days of hospital admission and admitted to VUMC after 1 January 2006. The mentions of warfarin/Coumadin and its dose were extracted using a previously developed natural language processing system [24].

Definition of cases & controls

We defined a case as an individual with a qualifying bleed who had taken more than one dose of warfarin within 5 days of admission to VUMC. A qualifying bleed had to be classified as a major bleed using the Fihn criteria [25]; major bleeds included bleeds that were serious, life-threatening or fatal and triggered hospitalization (Table 1). Bleeds also had to be definite (direct visualization by a healthcare professional, imaging or investigation showing direct bleeding) or probable (history and clinical findings compatible with bleeding) according to criteria we have previously validated (Supplementary Table 1; see online at: www.futuremedicine.com/doi/suppl/10.2217/pgs.14.153) [26]. We excluded potential cases with bleeding that occurred after major trauma or medical procedures

(within 48 h of the procedure), or after hospitalization or that resulted in elective hospital admission. In cases with more than one qualifying bleeding event, we used the first.

We defined a control as an individual who had taken more than one dose of warfarin within 5 days of admission to VUMC. Controls did not have a history of bleeding that triggered hospitalization recorded in the EMR while taking warfarin (Figure 1).

Identification of cases and controls

We used the presence or absence of previously validated International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9) codes indicative of bleeding [26] associated with hospital admission to identify 517 potential cases and 4281 potential controls (Figure 1). From the potential cases we identified 277 who met the case definition and then selected approximately the same number of controls ($n = 307$) who had no evidence of a qualifying bleed while on warfarin. The case and control groups were frequency-matched for age, sex, race and year of hospital admission recorded in the EMR. Two of three physicians (V Kawai; S Vear; A Oginni independently reviewed the EMR for each patient to confirm that cases and controls qualified for inclusion). The agreement between reviewers was 93%. Disagreements between reviewers were adjudicated by consensus among four investigators (V Kawai; S Vear; A Oginni and CM Stein) without knowledge of genotype.

Outcome & clinical information

The outcome of interest was major bleeding as defined by Fihn [25], which included serious bleeds (requiring medical evaluation or treatment) and those that were life-threatening or fatal (Table 1). We manually reviewed the records of cases and controls and extracted relevant clinical information including medication use within the 2 weeks before admission (Table 2 & Supplementary Table 2). We defined the duration of warfarin therapy as the number of days between warfarin initiation and hospital admission.

Genotyping

We genotyped four SNPs most strongly related to altered responses to warfarin: rs9923231 (*VKORC1*), rs1799853 (*CYP2C9**2), rs1057910 (*CYP2C9**3) and rs2108622 (*CYP4F2*). As part of previous research in BioVU, 217 cases and 236 controls had been genotyped for at least one SNP of interest using the Illumina VeraCode ADME Core Panel or the Omni1-Quad BeadChip (Illumina, CA, USA). Samples that lacked genotype information for the SNPs of interest were genotyped by the Vanderbilt University Cen-

Figure 1. Algorithm used to identify cases and controls for the final analysis.

†517 potential cases and 839 potential controls were reviewed to identify 277 cases and 307 controls frequency matched for age, sex, race and year of hospital admission who met the case and control definition.

‡Not genotyped because they had either opted-out or had inadequate DNA concentration for genotyping.

EMR: Electronic medical record; ICD-9: International Classification of Diseases, 9th Revision, Clinical Modification.

ter for Human Genetics Research using TaqMan®, Drug Metabolism Genotyping Assays (Applied Biosystems, CA, USA). Quality control analysis was performed with PLINK version 1.07. Call rates were >95% for all SNPs with >98% concordance between duplicates. Among 411 individuals in whom duplicate genotype information was available, seven had discordant genotypes for *VKORC1* rs9923231. For these we selected the genotype from the platform with the higher call rate. Genotyping was not performed in 26 cases and 47 controls because they had either opted-out of the DNA biobank [23] or had inadequate DNA concentrations. We excluded one case and one control in whom race was not known. Thus, 250 cases with a qualifying bleed and 259 controls were analyzed.

Statistical analysis

Demographic and clinical characteristics were described as frequencies and percentages for categorical variables or mean and SD for continuous variables

unless otherwise specified. Clinical characteristics and genotypes were compared in cases and controls using Student's t-test or Pearson χ^2 test, as appropriate. We used logistic regression analysis to calculate odds ratios with 95% CIs (OR, 95% CI) to evaluate the individual effect of *VKORC1*, *CYP2C9**2, *CYP2C9**3 and *CYP4F2* variants on the risk of bleeding using an additive genetic model. In a separate analysis we studied the combined effect of *CYP2C9**2 and *CYP2C9**3 using an additive model. We first performed a logistic regression model that included age, sex, race, body surface area and time on warfarin (simple model); and then a full model that additionally included genotypes for the other SNPs, potentiating or inhibiting drugs, antiplatelet therapy, nonsteroidal anti-inflammatory drugs, previous bleeding without warfarin and atrial fibrillation and venous thrombosis as indications for anticoagulation. The distribution of duration of warfarin therapy was skewed and was log transformed for analysis. Because genotype contributes little to the prediction of an individual's warfa-

Table 1. Fihn criteria for bleeding severity.	
Criteria	Definition
Major	Serious: a bleed that requires treatment or medical evaluation (includes blood transfusion ≤2 units) Life-threatening bleeding: causes irreversible end-organ damage; or requires surgical or angiographic intervention; or two of the following: loss of ≥3 units of blood, systolic hypotension (<90 mmHg), critical anemia (hematocrit ≤20%) Fatal bleeding: led directly to patient's death
Minor	A bleed that required no additional testing, referral or outpatient visit but remarkable enough to report to the provider
Data taken from [25].	

rin dose requirement after the first weeks of therapy [12,21], we assessed the role of genotype on the risk of bleeding after the first 30 days of warfarin therapy including cases and controls that were on warfarin for more than 30 days before admission to VUMC. In a sensitivity analysis we analyzed Caucasians alone. All p-values are two sided and no statistical correction for multiple testing was performed; p-values <0.05 were considered statistically significant.

Results

Population characteristics

There were no significant differences between cases and controls as regards age, sex, race and cumulative dose of warfarin in the week before admission (Table 2). The most common indications for warfarin therapy were atrial fibrillation in controls and venous thromboembolism in cases. Time on warfarin was shorter in cases compared with control subjects.

Characteristics of bleeding events & cause of hospital admission for controls

Cases had definite (n = 197) or probable (n = 53) bleeding, and all meeting the criteria for major bleeds by the Fihn criteria [25]. Bleeds were serious in 178 (71.2%) cases, life threatening in 67 (26.8%) and fatal in 5 (2.0%). The most common site of bleeding was gastrointestinal (38.8%), followed by miscellaneous sites (including hematomas, epistaxis, hemoptysis, hemopericardium, retroperitoneal bleeding and hemarthrosis; 26.8%), CNS (16.8%), genitourinary (11.2%) and more than one site of bleeding (6.4%). At the time of admission to hospital, 85 (34.0%) cases received vitamin K, and during hospitalization 122 (48.8%) cases required blood transfusion (with or without plasma) and 54 (21.6%) plasma only. The median (interquartile range) duration of warfarin exposure before bleeding was 541.5 (92, 1914) days. Bleeding occurred within 30 days of warfarin initiation in 14.4% (36/250) of cases, and within the 5 years in 44.4% (111/250). The INR was

measured within 2 days of bleeding in 249 cases; the highest INR was >3 in 136 (54.4%), >4 in 88 (35.2%) and >6 in 49 (19.6%).

The most common causes of hospital admission among controls were miscellaneous (cancer, orthopedic surgery, gastrointestinal and CNS disorders, etc; 49.5%), arrhythmias/cardiac procedures (27.4%), infections (20.8%) and ischemic/thrombotic events (5.8%).

Allele frequency

Genotype information was obtained for *VKORC1* (n = 505), *CYP2C9*2* (n = 505), *CYP2C9*3* (n = 503) and *CYP4F2* (n = 509; Table 3). Genotypes were in Hardy–Weinberg equilibrium for Caucasians and the frequency of *VKORC1*, *CYP2C9*2*, *CYP2C9*3* and *CYP4F4* variants among Caucasians and African–Americans was as expected from the literature (Supplementary Table 3).

Genotype & the risk of bleeding

The *CYP2C9*3* allele (*1/*3 or *2/*3) was more prevalent in cases (14.2%) than in controls (7.8%; p = 0.022). Carriers of a *CYP2C9*3* allele had a significantly increased risk of bleeding (simple model, OR: 1.94; 95% CI [1.08,3.49]); this risk was attenuated in the fully adjusted model (OR: 1.75; 95% CI [0.95,3.21]; Table 4 & Figure 2A). There were no significant differences among cases and controls for *VKORC1*, *CYP2C9*2* and *CYP4F2* genotypes (Tables 3 & 4).

Genotype & risk of bleeding after the dose-titration phase

Carriers of the *CYP2C9*3* allele had an increased risk of major bleeding beyond the first 30 days of therapy in the fully adjusted model (OR: 2.05; 95% CI [1.04,4.04]; Figure 2B). There was no significant association between *VKORC1*, *CYP2C9*2* and *CYP4F2* alleles and the risk of bleeding after the first 30 days of warfarin therapy (Figure 2B). Findings in Cauca-

sians did not differ materially from those in the whole group (see [Supplementary Table 4](#) & [Supplementary Figure 1A & 1B](#)).

Discussion

The major finding of the study is that carriers of *CYP2C9*3* receiving warfarin have increased risk of

Table 2. Demographic and clinical characteristics of cases and controls.

Characteristics	Controls (n = 259)	Cases (n = 250)	p-value
Age, years	63.5 (15.6)	63.0 (15.6)	0.742
Male, n (%)	138 (53.3)	138 (55.2)	0.664
Race			
Caucasian	213 (82.2)	211 (84.4)	0.808
African-American	39 (15.1)	33 (13.2)	
Others (include Hispanic and Asians)	7 (2.7)	6 (2.4)	
Clinical variables within 48 h of admission			
Body surface area, m ²	1.99 (0.30)	1.97 (0.28)	0.351
Lowest systolic blood pressure, mmHg	119 (19)	114 (22)	0.004
Lowest hemoglobin, mg/dl	11.9 (2.2)	9.6 (2.5)	<0.001
Highest international normalized ratio	2.5 (1.6)	4.2 (3.2)	<0.001
Warfarin indication[†]			
Atrial fibrillation	148 (57.1)	111 (44.4)	0.004
DVT, PE, stroke, hypercoagulable state	98 (37.8)	124 (49.6)	0.007
Joint replacement	1 (0.4)	1 (0.4)	0.999
Prosthetic heart valve	24 (9.3)	33 (13.2)	0.159
Other	6 (2.3)	4 (1.6)	0.560
Warfarin dose [‡] , mg/week	33.1 (17.9)	34.2 (16.4)	0.483
Time on warfarin, days (median, IQR)	1064 (239, 2394)	542 (92, 1914)	0.001
Warfarin exposure ≤30 days	27 (10.4)	36 (14.4)	0.173
Previous bleeding without warfarin	7 (2.7)	14 (5.6)	0.100
Comorbidities[§]			
Liver disease	4 (1.5)	4 (1.6)	0.999
Chronic kidney disease	49 (18.9)	55 (22)	0.389
Diabetes	78 (30.1)	74 (29.6)	0.899
Hypertension	195 (75.3)	192 (76.8)	0.690
Peptic ulcer disease	2 (0.8)	5 (2.0)	0.278
Active cancer (on treatment or diagnosed)	14 (5.4)	24 (9.6)	0.072
Medication use within 2 weeks before admission			
Amiodarone	27 (10.4)	11 (4.4)	0.010
Antiplatelet agents [¶]	103 (39.8)	111 (44.4)	0.290
Nonsteroidal anti-inflammatory drugs	15 (5.8)	20 (8.0)	0.325
Antibiotics	25 (9.7)	38 (15.2)	0.057
Number of warfarin inhibitors, median (IQR)	1 (0, 1)	1 (0, 1)	0.273
Number of warfarin potentiators, median (IQR)	0 (0, 0)	0 (0, 0)	0.945

Data are expressed as mean and standard deviation, or n (%), unless specified.
[†]23 control subjects and 18 cases had more than one indication for anticoagulation with warfarin.
[‡]Warfarin dose in the week before admission.
[§]Comorbidities within the last 6 months, except for cancer that was required to be currently active and/or under treatment.
[¶]Antiplatelet agents include: aspirin, clopidogrel and ticlopidine.
DVT: Deep venous thrombosis; IQR: Interquartile range; PE: Pulmonary embolus.

major bleeding and that this risk persists after the first 30 days of therapy.

Although the genetic variants that affect sensitivity to warfarin are well known to alter dose requirements, less is known about their contribution to risk of bleeding [16–19]. In keeping with our findings, a recent meta-analysis of several small studies totaling 156 cases suggested that carriers of the *CYP2C9**3 variant, but not carriers of *CYP2C9**2 or *VKORC1*, had a significantly increased risk of bleeding [20]. Our study of 250 cases of major bleeding found that *CYP2C9**3 (OR: 2.05, 95% CI [1.04,4.04]), but not *CYP2C9**2, *VKORC1* or *CYP4F2*, increased the risk of major bleeding and that this risk persisted after the first 30 days of warfarin therapy. In our study the most common indication for warfarin therapy was atrial fibrillation in controls and thromboembolic disease in cases. We did not match cases and controls for indication for warfarin therapy and the prevalence of particular indications for warfarin therapy among controls may have been affected by factors that were not random. For example, patients with atrial fibrillation tend to be on warfarin for long periods of time and are re-admitted to hospital relatively frequently for recurrences of atrial fibrillation or other cardiac illness. Thus, atrial fibrillation may have been more likely to be the indication for warfarin therapy in hospitalized controls. Considering that the indication for warfarin therapy could affect the risk of bleeding we included atrial fibrillation and venous thromboembolism in the fully adjusted model

We found that *CYP2C9**3 was associated with increased risk of major bleeding, albeit with wide confi-

dence intervals. The low prevalence of *CYP2C9**3 likely contributed the wide confidence intervals. The association between *CYP2C9**3 and increased risk of major bleeding lost significance in the fully adjusted model. This may be because the inclusion of several variables may have reduced statistical power, or alternatively, some of the risk factors for bleeding incorporated in the model may also have been affected by genotype. A recent study with approximately the same number of cases and controls as our study suggested that *CYP4F2*, but not *VKORC1* or *CYP2C9*, was associated with a reduced risk of major bleeding [27]. Our results also showed the same directional trend for the *CYP4F2* variant although the trend was not statistically significant.

Variants in *VKORC1* and *CYP2C9* increase the risk of over-anticoagulation during the first month of warfarin therapy, before an individual’s stable dose of warfarin has been determined [28,29]. Thus, pharmacogenetic interventions have focused on better prediction of an individual patient’s stable warfarin dose. Randomized controlled trials studying the contribution of pharmacogenetics to clinical outcomes in patients receiving warfarin focused on short-term outcomes such as time to stable warfarin dose and time within the therapeutic INR range and had mixed results [30,31]. However, these trials were not powered to address the more important endpoint of bleeding [32]; major bleeding occurred in a total of 14 participants (ten in the control arm) in the COAG trial [30] and in none of the participants in the EU-PACT trial [31]. Our findings suggest that a genetically determined risk of bleeding is present and persists

Table 3. Genotype distribution in cases and controls.				
Variants	Controls	Cases	p-values	All
<i>VKORC1</i> rs9923231	n = 259	n = 246		n = 505
G/G	107 (41.5)	113 (45.7)	Reference	220 (43.6)
G/A	124 (48.1)	101 (40.9)	0.172	225 (44.6)
A/A	27 (10.5)	33 (13.4)	0.617	60 (11.9)
<i>CYP2C9</i>	n = 257	n = 247		n = 503
*1/*1	177 (69.1)	161 (65.2)	Reference	338 (67.2)
*1/*2	52 (20.3)	46 (18.6)	0.904	98 (19.5)
*2/*2	7 (2.7)	5 (2.0)	0.685	12 (2.4)
*1/*3	17 (6.6)	33 (13.4)	0.017	50 (9.9)
*2/*3	3 (1.2)	2 (0.8)	0.735	5 (1.0)
<i>CYP4F2</i>	n = 259	n = 250		n = 509
C/C	123 (47.5)	135 (54.0)	Reference	258 (50.7)
C/T	110 (42.5)	94 (37.6)	0.183	204 (40.1)
T/T	26 (10.0)	21 (8.4)	0.336	47 (9.2)
Genotypes are shown as n (%). No patient was <i>CYP2C9</i> *3/*3.				

Table 4. Genotype and risk of major bleeding.

Genotype	Simple model, OR (95% CI)	Full model, OR (95% CI)
<i>VKORC1</i> rs9923231	0.98 (0.75,1.29)	0.96 (0.72,1.27)
<i>CYP2C9</i> *2 carrier	0.82 (0.57,1.20)	0.84 (0.57,1.24)
<i>CYP2C9</i> *3 carrier	1.94 (1.08,3.49)	1.75 (0.95,3.21)
<i>CYP4F2</i>	0.83 (0.63,1.10)	0.85 (0.64,1.14)
<i>CYP2C9</i> *2 +*3 [†]	1.07 (0.77,1.48)	1.02 (0.73,1.43)

Simple model: Adjusted for age, sex, race, body surface area, log[time on warfarin].
 Full model: Adjusted for the same covariates as in the simple model + *VKORC1*, *CYP2C9**2, *CYP2C9**3, *CYP4F2* genotype, number of warfarin inhibitors, number of warfarin potentiators, use of antiplatelet agents and nonsteroidal anti-inflammatory drugs, previous bleeding without warfarin and atrial fibrillation and venous thromboembolism as indication for warfarin.
[†]Additive model where 0 allele = 0, 1 allele = 1 and 2 allele = 2 (e.g., *1/*1 = 0, *1/*2 = 1, *1/*3 = 1, *2/*2 = 2, *2/*3 = 2, *3/*3 = 2).
 OR: Odds ratio.

beyond the first month of treatment and therefore indicate that genotyping may have potential utility beyond the initial phases of warfarin therapy. The increased risk of bleeding associated with genotype, in turn, suggests potential clinical strategies of preemptive genotyping to consider anticoagulants other than warfarin in *CYP2C9**3 carriers, or to monitor warfarin therapy more carefully in these individuals.

*CYP2C9**3 was associated with increased risk of bleeding but *CYP2C9**2 was not. The function of the enzyme encoded by the *CYP2C9**3 variant is substantially more impaired than *CYP2C9**2 [33]. Reflecting this difference in enzyme activity, the mean warfarin dose reduction required per *3 allele (34–38%) is approximately twice that per *2 allele (17–19%) [34,35]. Thus, during long-term anticoagulation any environmental perturbations that inhibit residual *CYP2C9* enzyme activity could have greater clinical effects in individuals carrying *CYP2C9**3 than *CYP2C9**2 and thus increase the risk of bleeding.

CYP2C9 variants affect S-warfarin clearance whereas *VKORC1* variants affect sensitivity to warfarin. Nevertheless, *VKORC1* and *CYP2C9**3 variants have approximately the same magnitude of effect on warfarin dose requirements [9]. Previously, we and others have found that *VKORC1* variants increase the risk of overcoagulation before a stable dose of warfarin is reached [11,12,20,29,36–38]. However, *VKORC1* variants were not associated with increased risk of bleeding in either our study or a meta-analysis of previous reports [20]. Since most bleeding events in patients on long-term warfarin therapy occur after the stable dose is achieved, the observation that *VKORC1* variants are not associated with bleeding risk suggests that fluctuations in S-warfarin concentration, rather than fluctuations in sensitivity to warfarin are important determinants of long-term bleeding risk. Supporting this hypothesis are several studies showing that *VKORC1* variants were associated with increased risk of overcoagulation before, but not after, warfarin stable dose is achieved [29,37,39].

The addition of *CYP2C9* and *VKORC1* genotype to clinical information improves prediction of warfarin dose requirement when initiating therapy [9,13]. However, an individual's warfarin dose is rapidly determined empirically through titration according to the INR. Indeed, after approximately 14 days of warfarin, genotype contributes little information beyond that already obtained clinically [12,21,40]. Consequently, attention has focused on the ability of genotype to guide initial warfarin dose selection during this dose titration phase [13]. Our finding that *CYP2C9**3 affects the risk of bleeding, even after 30 days of warfarin initiation, suggest that genotype information has the potential to alter clinical practice beyond the initiation phase.

A major advantage of our EMR-based approach is that it allowed us to study a large number of cases with long follow-up. BioVU, the Vanderbilt biobank that is linked to de-identified clinical information has several advantages for the performance of pharmacogenetic studies [41] that allows the rapid accrual of patients with rare outcomes that would otherwise be logistically difficult; the study of patients that are unlikely to be enrolled in clinical studies (e.g., elderly or extremely ill patients), the use of left-over samples from clinical care that otherwise would be discarded. In addition, compared with traditional case–control studies, the design decreases selection and recall bias since cases and controls are retrospectively selected from the same population source. However, this approach has some disadvantages. First, some bleeding events may not have been captured (e.g., severe bleeding resulting in death before hospital admission would not be included, and controls that had a bleeding event at another hospital could be misclassified); this limitation would act to decrease our ability to detect an effect of genotype. Second, we relied on information recorded in the EMR and could not assess factors such as adherence to therapy. Third, our population included both African-Americans and Caucasians. Findings in Caucasians did not differ materially from those in the entire cohort, but we studied too few

Figure 2. Major bleeding risk. Genotype and risk of major bleeding (A) during warfarin therapy and (B) after 30 days of warfarin therapy. Adjusted for age, sex, race, body surface area, log[time on warfarin], *VKORC1*, *CYP2C9*2*, *CYP2C9*3*, *CYP4F2* genotype, number of warfarin inhibitors, number of warfarin potentiators, use of antiplatelet agents and nonsteroidal anti-inflammatory drugs, previous bleeding without warfarin and atrial fibrillation and venous thromboembolism as indication for warfarin. *CYP2C9*2+*3* represents the combined genotype information for *CYP2C9*2* and *CYP2C9*3* in an additive model (0 allele = 0, 1 allele = 1 and 2 allele = 2; e.g., **1/*1* = 0, **1/*2* = 1, **1/*3* = 1, **2/*2* = 2; **2/*3* = 2). OR: Odds ratio.

African-Americans to assess the rare *CYP2C9* variants that occur in this population [19,42].

Conclusion

We conclude that the *CYP2C9**3 variant is associated with increased risk of bleeding in patients receiving warfarin and that this risk persists after the first 30 days of warfarin therapy. Pharmacogenetic approaches to optimizing warfarin therapy may benefit from a greater consideration of the effects of genetics on the risk of bleeding and extending their focus beyond the dose titration phase.

Future perspective

Our study found that the *CYP2C9**3 genetic variant is associated with increased risk of bleeding in patients receiving warfarin. If this finding is confirmed, additional work to define the genetic and clinical risk factors that affect INR stability may have the potential to reduce the risk of major bleeds during warfarin treatment.

Acknowledgements

The authors acknowledge O De la Cruz Cabrera (Case Western Reserve University) for his advice regarding statistical analyses.

Financial & competing interests disclosure

The dataset(s) used for the analyses described were obtained from Vanderbilt University Medical Center's resources, Bi-

oVU and the Synthetic Derivative, which are supported by institutional funding and by the Vanderbilt National Center for Advancing Translational Science grant 2UL1 TR000445-06 from NCATS/NIH. Existing genotypes in BioVU were funded by NIH grants RC2GM092618 from NIGMS/OD and U01HG004603 from NHGRI/NIGMS. Additional support includes grants from the NIH U19-HL065962, P01HL056693, HL097036, 5T32GM007569, GM109145; the National Center for Research Resources grant UL1 RR024975-01; and the Vanderbilt Physician Scientist Development Award. The funding sources had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; and preparation, review or approval of the manuscript. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

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.

Executive summary

Background

- Warfarin is an oral anticoagulant commonly used worldwide. The use of warfarin in clinical practice is problematic because of large variation in individual dose requirements and the risk of overcoagulation and bleeding.
- Information about common genetic variants in *CYP2C9*, *CYP4F2* and *VKORC1* improves prediction of individual warfarin dose requirements when therapy is initiated.
- The contribution of these genetic variants to risk of bleeding during warfarin treatment, particularly after the initial dose-titration phase when the effect of genotype on warfarin dose requirements has incorporated into therapy, is not clear.

Aim

- We tested the hypothesis that genetic variants associated with warfarin dose requirement are associated with the risk of major bleeding in a large case-control study that includes 250 cases with major bleeding while receiving warfarin and 259 controls without a major bleed.

Results

- *CYP2C9**3 allele was present in 35 (14.2%) cases and 20 (7.8%) controls ($p = 0.022$).
- No other *CYP2C9*, *CYP4F2* or *VKORC1* allele differed significantly among cases and controls.
- Major bleeding occurred more than 30 days after initiation of warfarin therapy in 214 (85.6%) cases.
- In a logistic regression model that adjusted for clinical variables and other drugs, the only variant associated with a major bleed after 30 days of warfarin initiation was *CYP2C9**3, which had an adjusted odds ratio 2.05 (95% CI: 1.04, 4.04).
- *VKORC1*, *CYP2C9**2 and *CYP4F2* variants were not associated with the risk of major bleeding.

Conclusion

- Our findings suggest that the *CYP2C9**3 variant was associated with increased risk of major bleeding associated with warfarin, and this risk was present even after the initial dose-titration phase.
- Genotype information may have the potential to alter clinical practice beyond the warfarin initiation phase.

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