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Pharmacogenetic Association Study of Warfarin Safety Endpoints in Puerto Ricans

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Abstract

Objective—This study was intended to determine the incidence rate of warfarin-related adverse events (e.g., bleeding) in Puerto Ricans and whether a genetic association between warfarin pharmacogenes and any of these adverse events was observed over the initiation period (i.e., the first 90 days of therapy).

Methods—We conducted an observational, retrospective cohort study of pharmacogenetic association in 122 warfarin-treated, male, Puerto Rican patients (69.9 ±9.6 years) from the Veterans Affair Caribbean Healthcare System (VACHS) who consented to participate. Genotyping was performed using the CYP2C9 and VKORC 1 assays by Luminex. Event-free survival curves were estimated using the Kaplan–Meier method and analyzed by log-rank test. Cox regression models were constructed and hazard ratios (HR) calculated.

Results—Carriers of functional *CYP2C9* and *VKORC1* polymorphisms demonstrated a higher incidence rate of multiple adverse events (i.e., 5.2 vs. 1.0 cases per 100 patient-months; RR = 4.8, $p = 0.12$) than did wild types. A significant association was observed between multiple adverse

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events and carrier status (HR = 2.5; 95% CI : 1.0–6.3, $p = 0.04$). However, no significant associations between genotypes and individual outcomes over the first 90 days of therapy were found.

Conclusion—The association of *CYP2C9* and *VKORC1* genotypes and risks for adverse events due to exposure to warfarin was examined for the first time in Puerto Ricans. Despite a lack of association with individual events in this study population, our findings revealed a potential utility of genotyping for the prevention of multiple adverse events during warfarin therapy.

Keywords

Warfarin; *Genotypes*; *Hispanics*; *Safety endpoints*; *Pharmacogenetics*

Warfarin is a widely used anticoagulant with a narrow therapeutic window. Despite careful monitoring, bleeding and thrombotic complications are common, resulting in substantial morbidity, mortality, and cost. It is the second leading cause of drug-related emergency room visits for outpatients (1–2). Early reports suggested that bleeding rates range as high as 16.5–25% over the first 6 months of warfarin therapy in standard care settings (2–4). In patients starting oral anticoagulant therapy, the determination of the appropriate therapeutic dose of warfarin may require several weeks or months of trial and error, placing the patient at risk of bleeding or thromboembolisms and requiring excessive clinical resources (2).

CYP2C9 encodes the hepatic CYP450 2C9 isoenzyme, which is the major elimination pathway of S-warfarin in humans. *VKORC1*, on the other hand, encodes the C1 subunit of the vitamin K epoxide reductase enzyme, which is responsible for recycling vitamin K from its oxidized to its reduced form and is the target of warfarin activity (5–6). Common variants in the *CYP2C9* gene may decrease the rate at which warfarin is metabolized, while polymorphisms in the *VKORC1* gene could alter warfarin sensitivity and dose requirements. Together, these polymorphisms account for approximately one-third of the warfarin dose variability in various populations (2,5–12). These genetic markers in addition to other determinants (e.g., age, co-medications) explain up to 60 to 67% of the variability in warfarin sensitivity in non-Hispanic populations (5–6).

A gap exists in the literature with respect to genotyping persons of Hispanic ancestry in relation to their warfarin sensitivity. We found a significant association between the *CYP2C9* and *VKORC1* genotypes and warfarin dose requirements in Puerto Ricans (13). However, an association with the risks for adverse events due to exposure to warfarin among Puerto Ricans has yet to be determined. Such an examination would be of special interest, given the uniqueness of Puerto Ricans, whose genetic backgrounds encompass a mosaic of genomic haplotypes from different ancestries (mostly European, West African, and Amerindian), with the resulting high level of admixture having produced a rich repertoire of combinatorial *CYP2C9* and *VKORC1* genotypes (14–16). This study was intended to determine whether an association between genotypes and warfarin-related adverse events could be observed in a cohort of Puerto Ricans.

Methods

Study cohort

One hundred thirty-five warfarin-treated outpatients from the VACHS anticoagulation clinic in San Juan, Puerto Rico, were recruited from January 2008 through July 2010 and provided a written informed consent prior to their participation, as approved by the Institutional Review Boards (IRB) at both VACHS (#00558) and UPR-MSC (A4070109). The eligible candidates were Puerto Ricans (≥21 and <90 years-old). The subjects who met the inclusion criteria—being initially free of any adverse events—were selected from the study population based on their warfarin initiation date (after January 2001). Patients with low compliance (<80% medication possession ratio) were excluded.

Study design

This was an open-label, single-center, observational, population-based, retrospective cohort study (NCT01318057) conducted to test the pharmacogenetic associations between combinatorial warfarin genotypes and the rates of adverse events in Puerto Ricans during the induction phase (first 90 days). The participants provided a small blood sample (5ml) for cross-sectional genotyping.

Blood samples were processed for genomic DNA extraction using a QIAamp DNA whole-blood mini-kit (QIAGEN Inc., Valencia, CA, USA). DNA quantification was performed by the fluorescent staining of double-stranded DNA (PicoGreen® dsDNA Quantitation Kit, Molecular Probes, Eugene, OR, USA). Fluorescent intensity was measured using a fluorescent micro-titer plate reader (BMG Labtech, FLUOStar Optima, Ortenberg, Germany). Isolated DNA specimens were tested using the Tag-It™ Mutation detection assay for warfarin (LUMINEX xMAP® Technology). A full description of this assay and a complete list of variants ascertained can be found elsewhere (17–18). Departure from Hardy-Weinberg equilibrium (HWE) was estimated under the null hypothesis of the predictable segregation ratio of specific matching genotypes ($p>0.05$) by use of χ^2 goodness-of-fit test with 1 degree of freedom. Clinical non-genetic and demographic information from the participants was retrospectively extracted from the corresponding computerized patient record system (CPRS).

Outcomes

The primary outcome was the time to first major bleeding (MB) episode (7,19–25). Severe (major) bleeding in this study was defined according to the WHO criteria: lethal, life-threatening, permanently disabling, or leading to hospital admission or a prolongation of the hospital stay. Secondary outcomes were 1) time to stabilization; 2) time to first “non-severe” bleeding (NSB) event (e.g., petechiae, bruises, ecchymosis, or any other minimal or minor bleeding, as defined by the Thrombolysis in Myocardial Infarction [TIMI] Study Group classification); 3) time to first overanticoagulation level (INR>4); and 4) the composite of MB and either an INR greater than 4 or an NSB event's (regardless of cause) occurring in the same patient within the follow-up period, which was denoted as “multiple adverse events” (MAE) for the purpose of this study. Time to stabilization was defined as the lapse of time required to reach the first 3 consecutive INRs within the therapeutic range without

any further dose adjustment once this target was achieved (7,19). Therapeutic INR was defined as 2.0 to 3.0 for all patients, except for those undergoing valve replacement surgeries (2.5–3.5). An INR greater than 4 indicates the presence of overanticoagulation (22–25) and has been associated with an increased risk for serious bleeding. These outcomes have been earlier included in other clinical trials (e.g., the EUPACT [NCT01119300], CoumaGen-II [NCT00927862], and GIFT [NCT01006733] trials) aimed at assessing the effect of genotypes on the anticoagulation response.

Statistics

A sample size of 120 patients was estimated to have a power of 80% to detect a response hazard of 1.5 to 2 at a 2-sided significance level of 0.05. A hazard ratio (HR) of 2 was chosen on the basis of previously published hazard risk for primary outcomes given a particular carrier status (20, 26). Individuals, initially free of any adverse events but at risk due to initiation of warfarin therapy, were separated into 2 groups: variant carriers and wild-type (WT) carriers. Descriptive statistics were employed using mean, SD, median and range for continuous variables, and frequencies (%) for categorical variables. A comparison of baseline clinical parameters between carrier and non-carrier subjects was performed using an unpaired t-test (the Mann-Whitney *U* test if normal distribution assumption was not met) for continuous variables and using, as well, Pearson's chi-squared test (Fisher's exact test when applicable) for categorical variables. Incidence rates per person-month and relative risk (RR) ratio plus 95% confidence interval (CI) of the incidence in variant carriers to the incidence in WT were estimated for primary and secondary outcomes. The analysis was repeated for the composite of MAE. For each patient and each outcome, time at risk (i.e., event-free) was calculated as the time between the start of warfarin therapy (date of the index prescription) and either the occurrence of an outcome event or the termination of data collection (i.e., 90 days after initiation of therapy), whichever came first.

Association analyses

Because of sample-size limitations, all association analyses were based on combinatorial *CYP2C9* and *VKORC1* polymorphisms, without sub-stratifying by genotype. Survival analyses were employed to evaluate the association between covariates at baseline and the risk of developing an adverse event (primary and secondary outcomes) at 3 months after the initiation of warfarin therapy. Event-free survival curves between carriers and wild-types were constructed using the Kaplan-Meir method and analyzed by log-rank test. Cox regression models were constructed and hazard ratio (HR) calculated to determine whether genotypes explain the variability in the occurrences of these adverse events.

Subjects were eligible if their warfarin therapy was initiated during the period from January 2001 to April 30, 2010. A follow-up period began at the initiation of warfarin therapy and ended after the development of the first adverse event (i.e., should that event has occurred within 90 days post therapy). The follow-up period was extended to July 31, 2010, for those individuals entering in April 2010. The censoring of data was employed in the absence of an adverse event 90 days after the initiation of individual warfarin therapy. A fitted Multivariate Cox model was adjusted by the following covariates: the use of amiodarone, azoles, or statins; comorbidities; smoker status; weight (lbs.); and age (years). The numbers

needed to harm (NNH) were computed for each measured outcome. The significance level of all statistical analyses was set at $p<0.05$. Statistical analyses were performed using the Stata version 11 statistical package (Stata Corp LP, College Station, TX, USA; 2009).

Results

One hundred thirty-five eligible patients who were receiving warfarin oral anticoagulation therapy at the VACHS-associated anticoagulation clinic in San Juan, PR, were approached to participate in the study. Three declined to participate, resulting in 132 being enrolled after having completed full written consents. A total of 10 patients were excluded from further statistical analyses due either to their having poor genotyping call rates (2 individuals) or there being a lack of complete clinical data from the CPRS system (8 individuals). Table 1 summarizes the basic demographic and clinical non-genetic characteristics of our study cohort (N = 122).

Participants were all males. The distribution by decade was concentrated around the 60- to 70-year-old age group as follows: 30 to 39 years old, 1 patient; 40 to 49 years old, 1 patient; 50 to 59 years old, 18 patients; 60 to 69 years old, 46 patients; 70 to 79 years old, 43 patients; and 80 to 90 years old, 13 patients. The 2 groups were balanced with respect to co-medications (including *CYP2C9* inhibitors), in their primary indications for warfarin therapy and comorbidities, an action that minimized any effect of these potential confounding variables. The ethno-geographic distribution of these groups was also balanced (data not shown). Although it was a single-center study, participants in each group came from all the geographic regions of Puerto Rico. Since we enrolled patients with a similar socioeconomic status, health insurance coverage, and baseline characteristics (including dietary intake, smoking status, and body size), the chances of confounding were minimized. Unfortunately, we were not able to match both groups in terms of event rates for any cause during the time period prior to the date of index prescription because of the lack of such information in the medical records.

Loss-of-function alleles *CYP2C9**2, *3, *5, and *6 as well as the low-dose/high-sensitivity *VKORC1*-1639G>A polymorphism were found in the study cohort at frequencies slightly higher than those previously reported for Caucasians (7–9, 27–28). There were 33 (27%) participants with the wild-type genotype and 89 (73%) with a variant genotype, which last were sub-divided into 43.4% single, 24.6% double, 4.1% triple, and 0.8% quadruple carriers. Among all the patients, 29 (24%) had at least 1 polymorphism in *CYP2C9*, and 77 (63%) were carriers of the *VKORC1*-1639G>A variant. No deviations from Hardy–Weinberg equilibrium were detected.

Figures 1 and 2 depict the Kaplan-Meier plots for the effect of genotypes on the time to first occurrence of the corresponding primary and secondary outcomes measured in this study, with the log-rank *p*-values and NNH presented. No significant association was found between carrier status and the time to stabilization of warfarin therapy (Table 2). In addition, genotypes had no significant effect on the time to first MB episode, on NSB events, or on the risk of overanticoagulation (INR>4), according to the unadjusted and adjusted Cox proportional hazards regression model and log-rank tests. However, a marginal but

significant association ($p = 0.04$) was observed between MAE and the carrier status. A statistical analysis of Schoenfeld residuals and a visual inspection of log-minuslog plots revealed no significant variation from the proportional hazards assumption in Figure 2. Concordant with this finding, HR estimates (Table 2) for multiple events were significantly different from unity after adjustment for covariates by Cox regression analysis (HR = 2.5, 95% CI: 1.0–6.3).

Table 3 depicts the unadjusted incidence rates of adverse events per person-time as well as their corresponding RRs. The overall incidence of MB was 4.7 per 100 patient-months (n = 16), whereas the incidence of NSB events was 8.3 per 100 patient-months (n = 27). For patients with at least 1 variant allele, the rate of MB (n = 13) was 5.2 cases per 100 patient-months. For patients with the wild-type genotype, the rate of MB (n = 3) was 3.2 cases per 100 patient-months. The rate of NSB events was 9.9 cases per 100 patient-months (n = 23) and 4.3 cases per 100 patient-months (n = 4) for patients with the variant and for those with the wild-type genotypes, respectively.

Discussion

Because findings suggest that outcomes during the first 90 days would be most sensitive to the induction dosing regimen (29), we compared the time to first occurrence of any of these outcomes during the first 3 months of therapy between carriers and WT groups. These findings are in good agreement with some early reports (29). According to them, the monthly risk of MB events decreased over time, from 3% during the first month of outpatient therapy to 0.3% per month after the first year of therapy. Incorrect dosing, especially during the induction phase, confers a 10-times greater risk of severe bleeding (30).

Since the statistics in Table 3 are deemed a good measure of association strength, values indicate how much more likely these events are to develop in variant carriers than in wild-types. Accordingly, patients with variant genotypes showed a trend toward relatively higher MB and NSB rates than did those with WT genotypes. For the variant versus the wild-type genotype, the relative risk ratios were 1.6 (95% CI: 0.5–5.3) for serious bleeding and 2.1 (95% CI: 0.8–5.7) for NSB events, respectively. Accordingly, the risk of MB episodes is almost one and a half as common among variant carriers as it is among wild-types (i.e., a 60% increase in risk), whereas NSB events among carriers are approximately 2 times more likely to occur in wild-types. However, none of these 2 RR values were statistically significant under the experimental conditions of the clinical protocol.

The optimal prescribing of warfarin requires the correct, individualized assessment of warfarin-related bleeding risk, which a randomized controlled trial may underestimate. Notably, observational studies have reported a widely varying range of bleeding risks, differing ~40-fold amongst themselves (31–35). Comparatively, the overall incidence of serious bleeding in the prospective multicenter Warfarin Genetics (WARG) study cohort was 2.6 per 100 patient-years (95% CI: 1.7–3.5), but the risk of such bleeding was increased in patients with potentially interacting medication and in those who were male (31). Estimates for incidence rates in previous literature reports are in the range of 1.1 to 13.4

cases per 100 patient-years (4, 29, 31, 36–37), which obviously depends on selected criteria for event call; follow-up period; complete capture of incidents; patient selection; physician's judgment; outpatient heparinization; induction regimen; prospective versus retrospective, randomized controlled (internal validity) versus naturalistic observational design (external validity); healthcare environment (anticoagulation clinic versus usual care settings); etc. In addition, interethnic differences in warfarin disposition and genetic backgrounds underline the need for further studies in populations other than Caucasians, including medically underrepresented populations (e.g., Hispanics).

We also found that the carriers of functional *CYP2C9* and *VKORC1* polymorphisms demonstrated a higher but not statistically significant incidence rate of MAE (RR = 4.8; 95% CI: 0.7–35.4; $p = 0.12$) as well as an INR greater than 4 (RR = 1.2; 95% CI: 0.6–2.6; $p = 0.6$) than did wild-types (Table 3). Notably, the *VKORC1*-1639G>A variant had a stronger influence on adverse events than did the *CYP2C9* variants (data not shown). Many studies conducted over the last decade have demonstrated a significant contribution of polymorphisms on both pharmacogenes to lowering warfarin dose requirements, given the increasing risk of overdosing, bleeding, and having outof-range INR measures (7). However, the *VKORC1*-1639G>A polymorphism seems to play an important role as a critical modulator of early warfarin response in patients, particularly during the initiation of therapy (20,26). In addition, a higher minor allele frequency in *VKORC1* versus *CYP2C9* variants combined was found in this study cohort, making this gene more important on a population level.

No significant associations were found in the study cohort with regard to the individual endpoints. This lack of an association between combinatorial genotypes and risk of MB in our study cohort may be attributed in part to a closer monitoring of each patient's adherence, diet, lifestyle, and co-medications at the VACHS, which minimizes the occurrence of adverse events in this cohort compared with that of others (e.g., home warfarin users). On the other hand, outpatients often show a lower bleeding rate per year than do hospitalized patients who are newly starting on warfarin (35).

The findings from the 2010 Medco-Mayo Warfarin Effectiveness Study support the role of genotype-guided warfarin therapy by suggesting that, compared with controls, genotyping for patients whose therapy was initiated significantly reduced the hospitalization rate for bleeding or thromboembolic events by approximately 30% (2). Likewise, even though genotyping does not seem to improve the percent of time in therapeutic range during the initiation of therapy, and available evidence does not support the assertion that there are significant improvements in health outcomes in Medicare beneficiaries (38–39), the randomized and clinical effectiveness trial CoumaGen-II (NCT00927862) concluded that pharmacogenetic guidance was highly superior to standard dosing in achieving and maintaining therapeutic INRs and in reducing death, serious bleeding, and thromboembolic events (40). Although warfarin genotyping appears to be helpful whenever it is received during the first 2 months, the general consensus is that the sooner the test result is provided, the better the outcomes (2).

An NNH of 21.6 means that ~1 in 22 is at increased risk of MAE due to variant alleles, if exposed to standard doses of warfarin. That is also the number that needs to be genotyped to find 1 patient with an increased risk of MAE. In addition, the time points at which patients in each group experienced the very first adverse event to warfarin were 9 and 27 days, respectively (data not shown). This lag-time of 18 days in favor of wild-types denotes the size of the effect.

The limitations of the present study include the following: 1) gender (the participants were all men) and age (the participants were mostly elderly); 2) its retrospective design, which can increase chances for missing or overlooking some adverse events. We could neither estimate the absolute risk reduction after controlling by underlying risk factors (e.g., stroke based on CHADS2 scores) nor test for significant interactions given a sample size that precludes any further stratification by subgroups of risk. Although the present study was not powered enough to separately evaluate the effects of individual polymorphisms on responses, analyses were conducted to compare wild-types versus carriers. Accordingly, this study was powered to assess the effect of combinatorial *VKORC1* and *CYP2C9* genotypes on adverse events during the induction of oral anticoagulation therapy with warfarin in Puerto Rican Hispanic male patients.

It is noteworthy that aging was not significantly different between carriers and wild-types (i.e., 72.5 [±8.5] vs. 68.9 [±9.8] years; $p = 0.06$). Accordingly, we would not expect there to be any impact of aging on the differences observed between both groups. Being male has been previously found to be significantly related to bleeding (31). However, reports have been conflicting with regard to sex differences in bleeding risk (36–37, 40–42).

We concluded that genotyping might reduce the risk of MAE in warfarin-treated Puerto Ricans. Lower rates of a composite of warfarin-induced adverse events may offset the genotyping cost and thus provide leverage for the future adoption of genetic testing as a component of optimal warfarin therapy. Further prospective clinical studies are warranted to evaluate the effect these findings may have on current risk-benefit analysis involved in genotype-guided warfarin prescription in Hispanics.

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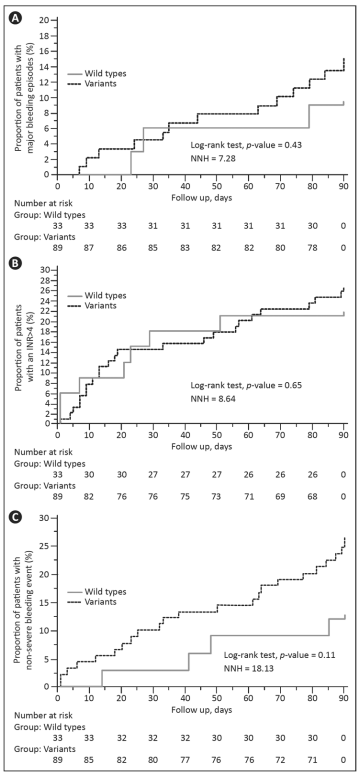

Figure 1.

The effect of genotypes on time to primary/secondary events. Kaplan–Meier plots (A–C) represent the lack of association for the attainment of the first major bleeding episode (plot A), first above-range INR (INR>4, plot B), and first “non-severe” bleeding event (plot C) among participants after the initiation of standard warfarin therapy (age-adjusted fixed-dose scheme). The statistic in each panel represents the log-rank p-value for testing the equality of survival function. NNH (Number Needed to Harm).

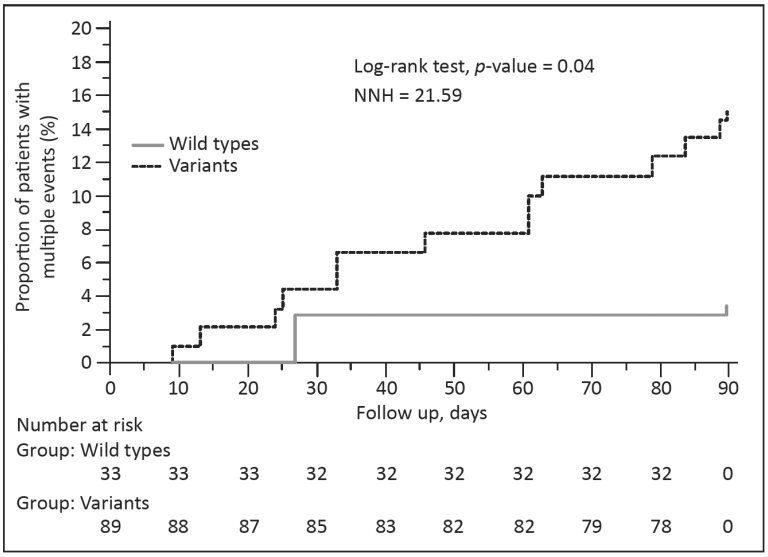

Figure 2.

The effect of carrier status on time to multiple adverse events. The Kaplan–Meier plot represents the marginal association of genotypes with multiple adverse events among participants after the initiation of standard warfarin therapy (age-adjusted fixed-dose scheme). The statistic represents the log-rank p-value for testing the equality of survival function. NNH (Number Needed to Harm).

Table 1

Descriptive characteristics of the study population (N - 122). The p-values represent significant differences between carriers and wild-types after comparison using unpaired *t*-test (continuous variables) or chi-squared/ Fisher's exact test (categorical variables). A 5% significance level was used.

Variable		All Patients N = 122	Wild-Type n = 33 (27%)	Carriers** n = 89 (73%)	*p*-value
Age (years)	Mean (sd)	69.9 (9.6)	72.5 (8.5)	68.9 (9.8)	0.06
	Median	71.0	72.0	70.0	
	Range (min–max)	31.0–90.0	56.0–89.0	31.0–90.0	
Weight (kg)	Mean (sd)	83.9 (14.7)	83.6 (15.2)	84.0 (14.6)	0.91
	Median	83.2	83.9	82.1	
	Range (min–max)	50.8–135.2	61.2–135.2	50.8–128.4	
Major bleeding episodes (%)*		16 (13.1)	3 (9.0)	13 (14.6)	0.16
Overanticoagulation Risk (%) (i.e., INR>4)		30 (24.6)	7 (21.2)	23 (25.8)	0.53
Non–severe bleeding events (%)		27 (22.1)	4 (12.1)	23 (25.8)	0.14
Time to	Mean (sd)	226 (159)	244 (168)	203 (144)	0.34
stabilization	Median	122	124	121	
(days)	Range (min–max)	59–412	67–412	59–385	
Smoker (%)		10 (8.2)	2 (6.1)	8 (9.0)	0.73
Chronic comorbidities (%)		85 (69.7)	23 (69.7)	62 (69.7)	0.99
Acute comorbidities (%)		24 (19.7)	8 (24.2)	16 (18.0)	0.44
More than 1 indication (%) (i.e., AF, DVT, PE)		62 (50.8)	15 (45.5)	47 (52.8)	0.54
Amiodarone Users (%)		16 (13.1)	5 (15.2)	11 (12.4)	0.76
Azole Users (%)		33 (27.1)	10 (30.3)	23 (25.8)	0.65
Statin Users (%)		87 (71.3)	24 (72.7)	63 (70.8)	0.99

*Values are the number of subjects (with percentages in parenthesis) having a given outcome, unless otherwise indicated. The count of subjects "without the outcome" is implied and, therefore, is not shown. INR: International Normalized Ratio. AF: atrial fibrillation; DVT: deep vein thrombosis; PE: pulmonary embolism.

**Carriers: all individuals with at least 1 polymorphism on either *CYP2C9* or *VKORC1*.

Table 2

Unadjusted and adjusted hazard-ratio (HR) estimates with 95% confidence intervals (CI) for different adverse events (primary and secondary outcomes) in warfarin-treated patients with variant genotypes. Cox proportional-hazard regression models were adjusted for age (years), weight (lbs.), smoker status, indication, comorbidities, and co-medications (i.e., amiodarone, azoles, statins).

Outcomes	Unadjusted		Adjusted	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Major bleeding episodes	1.64 (0.55–4.92)	0.43	1.64 (0.47–5.77)	0.36
INR>4 (overanticoagulation)	1.21 (0.54–2.71)	0.65	1.21 (0.52–2.83)	0.24
Non–severe bleeding events	2.31 (1.00–5.32)	0.11	1.47 (0.78–2.78)	0.12
Time to stabilization	1.18 (0.69–2.03)	0.55	1.17 (0.83–1.65)	0.38
Multiple events	5.05 (1.57–16.23)	0.08	2.49 (1.04–6.25)	0.04*

*stands for “statistically significant,” p<0.05

Table 3

Unadjusted incidence rates and relative risk (RR) of warfarin-related adverse events per person-time of follow-up, stratified by carrier status.

Outcomes	Incidence rates per 100 patient-months (number of events)				
	Total (N = 122)	Wild-type (n = 33)	Carriers (n = 89)	RR (95% CI)	p-value
Follow-up, average person-months*	329.87	91.72	238.15		
Major bleeding episodes	4.7 (n = 16)	3.2 (n = 3)	5.2 (n = 13)	1.61 (0.49–5.28)	0.43
Non-severe bleeding events	8.3 (n = 27)	4.3 (n = 4)	9.9 (n = 23)	2.13 (0.80–5.70)	0.13
INR>4	9.8 (n = 30)	8.5 (n = 7)	10.3 (n = 23)	1.22 (0.58–2.57)	0.60
Multiple events	4.1 (n = 14)	1.0 (n = 1)	5.2 (n = 13)	4.82 (0.66–35.4)	0.12

*estimate of the average actual time at risk (in months) that all the participants contributed to our study