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Long-term outcomes of elderly patients with CYP2C9 and VKORC1 variants treated with vitamin K antagonists

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Running head: Clinical outcomes in CYP2C9 and VKORC1 variants

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Essentials

- The long-term effects of *VKORC1* and *CYP2C9* variants on clinical outcomes remains unclear.
- We followed 774 patients ≥ 65 years with venous thromboembolism for a median duration of 30 months.
- Patients with *CYP2C9* variants are at increased risk of death and non-major bleeding.
- Patients with genetic variants have a slightly lower anticoagulation quality only.

Abstract

Background

The long-term effect of polymorphisms of the vitamin K-epoxide reductase (*VKORC1*) and the cytochrome P450 enzyme gene (*CYP2C9*) on clinical outcomes remains unclear.

Objectives

We examined the association between *CYP2C9/VKORC1* variants and long-term clinical outcomes in a prospective cohort study of elderly patients treated with vitamin K antagonists for venous thromboembolism (VTE).

Methods

We followed 774 consecutive patients aged ≥ 65 years with acute VTE from nine Swiss hospitals for a median duration of 30 months. The median duration of initial anticoagulant treatment was 9.4 months. The primary outcome was the time to any

clinical event, i.e. the composite endpoint of overall mortality, major- and non-major bleeding, and recurrent VTE.

Results

Overall, 604 (78%) patients had a *CYP2C9* or *VKORC1* variant. Three hundred thirty-four patients (43.2%) had any clinical event, 119 (15.4%) died, 100 (12.9%) had major and 167 (21.6%) non-major bleeding, and 100 (12.9%) recurrent VTE.

After adjustment, *CYP2C9* (but not *VKORC1*) variants were associated with any clinical event (hazard ratio [HR] 1.34; 95% confidence interval [CI] 1.08-1.66), death (HR 1.74; 95% CI 1.19-2.52), and clinically relevant non-major bleeding (sub-hazard ratio [SHR] 1.39; 95% CI 1.02-1.89), but not with major bleeding (SHR 1.03; 95% CI: 0.69-1.55) or recurrent VTE (SHR 0.95; 95% CI 0.62-1.44). Patients with genetic variants had a slightly lower anticoagulation quality.

Conclusions

CYP2C9 was associated with long-term overall mortality and non-major bleeding.

While genetic variants were associated with a slightly lower anticoagulation quality, there was no relationship between genetic variants and major bleeding or VTE recurrence.

Keywords

Venous Thromboembolism; anticoagulants; phenprocoumon; cytochrome P-450 CYP2C9; mortality

Introduction

Personalized medicine based on genetic factors is seen as a promising strategy to improve patient care and save healthcare costs, in particular with regard to anticoagulation treatment.[1-7] Despite the increasing use of direct oral

anticoagulants, vitamin K antagonists (VKAs) will continue to play a major role and improving the safety of anticoagulant treatment with VKAs remains an important goal.[8, 9] Main safety issues related to VKAs are bleeding as well as thromboembolic events, which both depend on the quality of anticoagulation.[10, 11] Achieving a stable anticoagulation control is difficult because of a large variability of VKA response across individuals.[12] A major part of this variability results from polymorphisms of the vitamin K-epoxide reductase (*VKORC1*) and the cytochrome P450 enzyme gene (*CYP2C9*), which both influence VKA metabolism.[13-22] Patients with a *CYP2C9* genetic variant metabolize VKA to a lesser degree, resulting in higher levels of VKA metabolites and in decreased VKA dose requirements [6, 23]. *VKORC1* encodes for Vitamin K-epoxide reductase subunit 1, which converts vitamin K into its active form [6]. Patients with the *VKORC1* G1639A variant are more sensitive to VKA and dose requirements are lower as well [6].

To date, the clinical impact of measuring genetic polymorphisms in patients receiving VKAs still remains unclear.[4, 9, 24-27] Genetics-based dosing algorithms focusing on the initiation of anticoagulation did not demonstrate a clear benefit in terms of anticoagulation quality, as shown in several recent randomized-controlled trials.[24-27] In addition, a recent systematic review did not detect relevant differences in bleeding events between patients with and without *VKORC1* variants, and only a slightly higher bleeding risk in patients with *CYP2C9**3 variants.[22] However, these studies were limited by relatively small sample sizes and/or short follow-up periods, and methodologically more rigorous studies were requested.[22, 27] Moreover, previous studies focused on the initiation of anticoagulation only and the long-term effects of genetic polymorphisms are unknown. To fill this gap of knowledge, we examined the association between *CYP2C9* and *VKORC1* variants

and long-term clinical outcomes in a prospective multicenter cohort study of elderly patients treated with VKA for venous thromboembolism (VTE).[28, 29]

Methods

Cohort sample

The study was conducted between September 2009 and December 2013 as part of the SWISS venous Thromboembolism COhort (SWITCO65+), a prospective multicenter cohort study that assessed long-term medical outcomes and quality of life in elderly patients with acute VTE from all five university hospitals and four high-volume non-university hospitals in Switzerland.[28, 29] Consecutive patients aged 65 years or older with an acute, objectively confirmed VTE were identified in the inpatient and outpatient services of all participating study sites. Patients were excluded in case of inability to provide consent (e.g., severe dementia), thrombosis at different site than DVT or PE, impossible follow-up (e.g. terminal disease), or insufficient German or French language skills. For the sake of this analysis, we considered patients treated with VKAs only. A detailed description of the study methods was published elsewhere.[28, 29] The study was approved by the ethics committees at each participating site.

Baseline data collection

Trained study nurses prospectively collected baseline demographics (age, sex), VTE-related information (localization of the index VTE [PE, proximal/distal DVT], type of VTE [provoked, unprovoked, cancer-related], history of prior VTE, and family history of VTE), vital signs (heart rate, systolic blood pressure), body mass index, co-morbid conditions (recent immobilization, history of major bleeding, acute

rheumatic disease, inflammatory bowel disease, cerebrovascular disease, chronic renal and pulmonary disease), laboratory findings (hemoglobin, creatinine), concomitant antiplatelet and non-steroidal anti-inflammatory therapy, polypharmacy, and VTE-related treatments (type and duration of initial parenteral anticoagulation and duration of VKA therapy) from all enrolled patients using standardized data collection forms.

Genetic analyses

A specifically designed haemostasis biobank was implemented before inclusion of patients.[29] At the day of enrollment, one EDTA-whole blood sample (2.7ml, Sarstedt, Nümbrecht, Germany) was collected. After plasma removal by centrifugation, blood cells were stored at -80°C until DNA extraction (Qiacube automated extraction plus Qiagen DNA blood mini kit, Qiagen, Hombrechtikon, Switzerland). Allelic identifications of *VKORC1* and *CYP2C9* were made by melting curve analysis of fluorescent Q-PCR amplification products. “LightMix for the detection of human *VKORC1* G1639A” and “LightMix for the detection of human *CYP2C9**2 and *CYP2C9**3” kits (TIBMOLBIOL, Berlin, Germany), containing primers and probes, were used. PCR reactions were performed as advised by the manufacturer on a LightCycler 1.8 (Roche Diagnostics, Rotkreuz, Switzerland). Patients and managing physicians were blinded to the results of these analyses.

Study outcomes

The primary outcome was the time to any clinical event, i.e. a composite outcome of death, major bleeding, clinically relevant non-major bleeding, or recurrent VTE. Major bleeding was defined as fatal bleeding, symptomatic bleeding in a critical

organ (intracranial, intraspinal, intraocular, retroperitoneal, intraarticular, pericardial, or intramuscular with compartment syndrome), bleeding with a reduction in hemoglobin ≥ 20 g/l, or bleeding leading to a transfusion ≥ 2 units of packed red blood cells. [30] Clinically relevant non-major bleeding was defined as bleeding that did not meet the definition of major bleeding but required medical attention (i.e., a physician contact). Recurrent VTE was defined as objectively confirmed, new or recurrent proximal or distal DVT or PE, as previously described.[28, 29]

The secondary outcomes were the individual outcomes of overall mortality, major bleeding, clinically relevant non-major bleeding, and recurrent VTE. Additional outcomes were the percentage of time within the therapeutic INR range (TTR; 2.0-3.0),[31] the percentage of time above the therapeutic INR range (>3.0), the time to a therapeutic INR (defined as three consecutive INR measurements in the range between 2.0 and 3.0), and the time to first INR above 4.5.

During follow-up, patients were contacted semi-annually, alternating between face-to-face evaluations and telephone calls. Detailed information regarding the date, type and circumstance of outcomes were collected from the patient, and/or the patient's family members, primary care physician and medical chart. We also recorded all INR values measured by the family physician during the follow-up period.

All outcomes were reviewed and adjudicated by an independent committee of three blinded clinical experts. Based on the full consensus of this committee, deaths were classified as definitely due to PE (e.g., confirmed by autopsy or following severe PE), possibly due to PE (e.g., sudden death without obvious cause), due to major bleeding and due to other causes. Bleeding-related death was defined as

death following intracranial hemorrhage or hemodynamic deterioration due to major bleeding.[32]

Statistical analyses

We present Kaplan-Meier curves for any clinical event and mortality, as well as cumulative incidence curves adjusted for the competing risk of death for major bleeding, clinically relevant non-major bleeding, and recurrent VTE [33]. We examined the association between *CYP2C9* and *VKORC1* variants and any clinical outcome event and death using Cox regression using robust standard errors, adjusting for previously published predictors of the respective outcome as well as periods of anticoagulation as a time-varying covariate.[34-36]

We examined associations between *CYP2C9* and *VKORC1* variants and the time to a first VTE recurrence, major bleeding, and clinically relevant non-major bleeding using competing risk regression according to Fine and Gray,[37] accounting for non-PE- or non-bleeding-related death as a competing event. We adjusted the models for previously published predictors of bleeding and VTE recurrence.[34, 38-54] As we aimed to predict overall adverse events, we considered the full observation period from diagnosis until the end of follow-up, adjusting for periods of anticoagulation as a time-varying covariate.

We calculated the percentage of time spent within one of three specified INR ranges (<2.0, 2.0-3.0, >3.0) using the Rosendaal method [31] and compared groups using linear regression. We examined the association between *CYP2C9* and *VKORC1* variants and the time to the first therapeutic INR and the time to the first INR >4.5 using Cox-regression with robust standard errors. Only the initial VKA treatment was considered. Time was censored at stop of initial treatment, death, or

withdrawal/loss of follow-up. Analyses were adjusted for known influencing factors of anticoagulation quality, including age, sex, provoked VTE, active cancer, diabetes, heart failure, chronic pulmonary disease, chronic liver disease, renal failure (GFR <30 ml/min.), body mass index, hypertension, and smoking status.[55-57] All analyses were done using Stata 13 (Stata Corporation, College Station, Texas).

Results

Patient characteristics

Of 1863 patients screened, 1003 were included in the study cohort and 744 were analyzed for the purpose of this study (Figure 1). Analyzed patients did not differ from non-analyzed patients in terms of age (median age 75 vs. 74 years) and sex (women 46% vs. 50%).

Overall, 78% of patients (n=604) had a *CYP2C9* or *VKORC1* variant. *CYP2C9**2 polymorphisms were found in 26% of patients (n=200), *CYP2C9**3 in 12% (n=95), *VKORC1* in 66% (n=510), and both *CYP29C* and *VKORC1* variants in 24% (n=187). Patient baseline characteristics did not differ in patients with and without a genetic variant (Table 1). Although patients with a genetic variant had a shorter initial parenteral anticoagulation duration than patients without variant (9 vs. 12 days), the duration of the initial anticoagulation with vitamin K antagonists was comparable (9.7 vs. 8.4 months) (Table 1).

Clinical outcomes

The median observation time was 30 months (interquartile range 24 to 41 months). The primary outcome, the composite endpoint of death, major or non-major clinically relevant bleeding, or VTE recurrence, occurred in 43% of patients (n=334).

Overall, 15% of patients died (n=119), 13% (n=100) had major bleeding, 22% (n=167) clinically relevant non-major bleeding, and 13% (n=100) recurrent VTE.

While patients with a *CYP2C9* variant had a higher cumulative 3-year incidence of any clinical outcome event than those without these variants (52.3% vs. 41.1%; Figure 2), the cumulative incidence did not differ much in patients with or without *VKORC1* variants (47.1% vs. 41.8%). Patients with a *CYP2C9* variant had a higher cumulative 3-year incidence of death and clinically relevant non-major bleeding but not major bleeding or recurrent VTE (Figure 3).

After adjustment, *CYP2C9* variants were associated with any clinical outcome event (hazard ratio [HR] 1.34; 95% confidence interval [CI] 1.08, 1.66), death (HR 1.74; 95% CI 1.19, 2.52), and clinically relevant non-major bleeding (sub-hazard ratio [SHR] 1.39; 95% CI 1.02, 1.89), but not with major bleeding (SHR 1.03; 95% CI 0.69, 1.55) or recurrent VTE (SHR 0.95; 95% CI 0.62, 1.44) (Table 2). Patients with both *CYP2C9* and *VKORC1* variants had a higher risk of any clinical event (HR 1.40; 95% CI 1.11, 1.77) and clinically relevant non-major bleeding (SHR 1.78; 95% CI 1.30, 2.44).

Quality of anticoagulation

Associations between *CYP2C9* and *VKORC1* variants and measures of anticoagulation quality are shown in Table 3. Compared to patients without *CYP2C9* variants, patients with a *CYP2C9* variant spent less time in the therapeutic INR range (adjusted difference -3.7%; 95% CI -7.0, -0.4). Similarly, patients with *CYP2C9* variants spent more time with an INR above the therapeutic range (adjusted difference 3.3; 95% CI 0.8, 5.7), were less likely to achieve a therapeutic INR (adjusted HR 0.81; 95% CI 0.68, 0.96), and more likely to have an INR >4.5

(adjusted HR 1.42; 95% CI 1.11, 1.81). Patients with a *VKORC1* variant were more likely to have an INR >4.5 (adjusted HR 1.43; 95% CI 1.10, 1.85).

Discussion

Our results demonstrated a association between *CYP2C9* genetic variants and the composite endpoint of death, major bleeding, clinically relevant non-major bleeding, and recurrent VTE. This result was driven by a 74% higher mortality and a 39% higher risk of clinically relevant non-major bleeding in patients with *CYP2C9* variants. While patients with genetic variants, in particular *CYP2C9*, had a slightly lower anticoagulation quality, we found no relationship between genetic variants and major bleeding or recurrent VTE.

To our knowledge, our work is the first study to demonstrate a association between *CYP2C9* variants and mortality. Interestingly, the increased mortality does not appear to be driven by a higher rate of major bleeding or recurrent VTE. Several explanations for the higher mortality rate in patients with *CYP2C9* are possible. First, genetic variants may affect mortality based on mechanisms not related to anticoagulation quality. A small observational study demonstrated a higher risk of stroke and myocardial infarction in patients with *VKORC1* variants, the underlying mechanism for the increased risk of cardiovascular complications remaining unexplained.[58] Second, although we adjusted for many co-variables, we cannot fully exclude the possibility that the higher mortality in patients with *CYP2C9* variants is caused by residual confounding.

We did not observe an increased risk of major bleeding in patients with *CYP2C9* or *VKORC1* variants. This finding is consistent with previous studies focusing on short-term clinical outcomes. In a systematic review by Jorgensen et al.,

which pooled data from several smaller-sized observational studies, no association between genetic variants and major bleeding was reported.[22] Similarly, in a sub-study of the ENGAGE AF-TIMI 48 phase III clinical trial (edoxaban for stroke prophylaxis in patients with atrial fibrillation), no association between *CYP2C9/VKORC1* variants and major bleeding was found.[59] However, as demonstrated in several smaller observational studies,[60-62] we observed a association between *CYP2C9* variants and clinically relevant non-major bleeding (adjusted SHR 1.39; 95% CI 1.02, 1.89).

We did not find an association between genetic variants and recurrent VTE. This result is consistent with the findings of a small retrospective study conducted in 120 patients with mechanical heart valves, which did not report an increased risk of VTE in patients with genetic variants.[58]

In our cohort, genetic variants, in particular *CYP2C9*, were associated with a lower anticoagulation quality, but the absolute differences in anticoagulation quality measures were small. Even though prior studies have shown a consistent association between genetic variants and VKA dose requirements,[14, 61, 63-69] the evidence with regard to anticoagulation quality is much more limited. In a systematic review, the magnitude of associations between *CYP2C9* and *VKORC1* genetic variants and different measures of anticoagulation quality was moderate at best.[22] Randomized-controlled trials comparing genetic-based dosing schedules for initiation of anticoagulation to standard care did not demonstrate a relevant benefit in terms of quality of anticoagulation or clinical outcomes.[24-27] Overall, the results from our cohort of elderly patients indicate that although patients with genetic variants appear to have a somewhat lower anticoagulation quality and a higher risk of non-major bleeding (*CYP2C9*), but they do not have an increased risk of major bleeding. Thus,

the costly measurement of *CYP2C9* and *VKORC1* variants is unlikely to have a relevant beneficial effect on anticoagulation quality and clinical outcomes of elderly patients with VTE. Interestingly, associations between genetic variants and anticoagulation quality as well as clinical outcomes were much more prominent in case of *CYP2C9*. This observation suggests that fluctuations in VKA concentrations are more important than VKA sensitivity.

Our study has several strengths. In contrast to prior studies, our study had a long-term follow-up, larger sample size, and clinically relevant outcome variables. [14, 22, 24-27, 59-69] In addition, we focused on elderly patients, a population at risk for both bleeding complications and death.[70] The fact that we enrolled patients from university and non-university hospitals increases the generalizability of our findings. Because patients and managing physicians were blinded to the results of the genetic analyses, a performance bias is unlikely.

Our study has several potential limitations. First, our study included patients aged 65 years or older only. Thus, the results may not be generalizable to younger patients. Second, our study included exclusively patients with VTE and therefore, our results may not be generalizable to other populations, such as patients with atrial fibrillation. Finally, even though we adjusted our analyses for many covariates, we might have missed important predictor variables.

In conclusion, we found a association between *CYP2C9* genetic variants and long-term clinical outcomes, notably overall mortality and clinically relevant non-major bleeding. In contrast, we found no relationship between *VKORC1* variants and clinical outcomes. While genetic variants, in particular *CYP2C9*, appear to be associated with a somewhat lower anticoagulation quality, there was no relationship between genetic variants and major bleeding or recurrent VTE.

Addendum

M. Nagler, A. Limacher, A. Angelillo-Scherrer, and D. Aujesky developed the protocol and the analysis plan, conducted the analyses, and drafted the manuscript. C. Abbal conducted the genetic analysis, and intellectually reviewed the manuscript. M. Méan, A. Angelillo-Scherrer, M. Righini, B. Frauchiger, J. Osterwalder, N. Kucher, and N. Rodondi organized data collection, intellectually reviewed the manuscript, and participated in funding procedure. J. H. Beer, C. M. Matter, J. Cornuz, M. Banyai, M. Aschwanded, M. Husmann, D. Staub, L. Mazzolai, and O. Hugli organized data collection, and intellectually reviewed the manuscript. D. Aujesky was principle investigator and was responsible for planning of the study, data collection, drafting of the manuscript, and obtaining funding.

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Disclosure of Conflict of Interests

M. Nagler has received research grants or lecture fees from Bayer, Roche diagnostics, and CSL Behring. O. Hugli participated in an advisor board for Novartis. M. Husmann received lecture or travel fees from Abbott Endovascular, Medtronic, EV3, Cordis, Sanofi Aventis, Daiichi Sankyo, Bayer, Boeringer Ingelheim, and AstraZeneca. C. M. Matter reports grants from MSD, AstraZeneca, EliLilly, Bayer, and Slrtris; and personal fees from MSD and Amgen, outside the submitted work.

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Figure Legends

Figure 1. Patient flow chart. *Multiple reasons may apply.

Figure 2. Kaplan-Meier estimates of a first clinical event (death, major or clinically relevant non-major bleeding, or VTE recurrence) according to presence of CYP2C9 or VKORC1 variants. The cumulative 3-year incidence of a first clinical event was 52.3% for patients with a CYP2C9 variant vs. 41.1% for patients without variant and 47.1% for patients with a VKORC1 variant vs. 41.8% for patients without variant.

Figure 3. Cumulative incidences of (A) death, (B) major bleeding, (C) clinically relevant non-major bleeding, and (D) VTE recurrences according to the presence of *CYP2C9* or *VKORC1* variants. Compared to patients without variant, patients with a *CYP2C9* variant had a higher cumulative 3-year incidence of death (20.4% vs. 12.4%) and non-major clinically relevant bleeding (27.5% vs. 21.3%). Graphs were adjusted for the competing risk of death if appropriate.

Table 1. Baseline characteristics by presence of any genetic variant

	All (N=774)	Any genetic variant* (N=604)	No genetic variant (N=170)	Missing values
		<i>n (%) or median (IQR)</i>		<i>n (%)</i>
Age, years	75.0 (69.0; 81.0)	75.0 (69.0; 81.0)	74.5 (69.0; 81.0)	0 (0)
Female sex	355 (46)	280 (46)	75 (44)	0 (0)
BMI, kg/m ²	26.8 (24.3; 29.9)	26.8 (24.3; 29.9)	26.9 (24.3; 30.6)	4 (1)
Smoking status				2 (1)
current smoker	58 (8)	47 (8)	11 (6)	
past smoker	315 (41)	248 (41)	67 (39)	
never smoker	399 (52)	307 (51)	92 (54)	
Localization of thrombosis				0 (0)
distal DVT only	53 (7)	44 (7)	9 (5)	
proximal DVT	165 (21)	127 (21)	38 (22)	
PE	556 (72)	433 (72)	123 (72)	
Prior VTE	229 (30)	181 (30)	48 (28)	0 (0)
Family history of PE/DVT	140 (18)	112 (19)	28 (16)	7 (1)
Provoked index VTE	214 (28)	171 (28)	43 (25)	0 (0)
Active cancer [†]	82 (11)	60 (10)	22 (13)	0 (0)
Arterial hypertension	500 (65)	392 (65)	108 (64)	0 (0)
Diabetes mellitus	120 (16)	89 (15)	31 (18)	0 (0)
Chronic heart failure	91 (12)	72 (12)	19 (11)	0 (0)
Acute rheumatic disease during the last 3 months	25 (3)	21 (3)	4 (2)	0 (0)
Inflammatory bowel disease	27 (3)	19 (3)	8 (5)	0 (0)
History of major bleeding [‡]	72 (9)	52 (9)	20 (12)	1 (0)
Cerebrovascular disease (stroke, TIA)	72 (9)	59 (10)	13 (8)	0 (0)
Chronic renal disease	142 (18)	113 (19)	29 (17)	0 (0)
Chronic pulmonary disease	104 (13)	87 (14)	17 (10)	0 (0)

Chronic liver disease	11 (1)	9 (1)	2 (1)	0 (0)
Anemia [§]	267 (37)	214 (38)	53 (34)	49 (6)
Creatinine >107 µmol/L	185 (26)	146 (26)	39 (26)	63 (8)
Systolic BP <100 mm Hg	27 (4)	20 (3)	7 (4)	13 (2)
Heart rate ≥110/min.	73 (10)	214 (38)	53 (34)	15 (2)
Immobilization during the last 3 months	154 (20)	122 (20)	32 (19)	0 (0)
Antiplatelet therapy	254 (33)	189 (31)	65 (38)	0 (0)
NSAID	72 (9)	62 (10)	10 (6)	0 (0)
Polypharmacy [¶]	388 (50)	309 (51)	79 (46)	0 (0)
Duration of initial parenteral AC, days	9.0 (6.0; 16.0)	9.0 (6.0; 14.0)	12.0 (7.0; 19.0)	0 (0)
Duration of initial VKA therapy, months	9.4 (5.3; 29.1)	9.7 (5.2; 29.0)	8.4 (5.4; 29.2)	0 (0)

Abbreviations: IQR= interquartile range, BMI=body mass index, DVT= deep vein thrombosis; PE= pulmonary embolism; VTE= venous thromboembolism; TIA= transient ischemic attack; BP= blood pressure; NSAID= nonsteroidal anti-inflammatory drug; AC= anticoagulation; VKA= vitamin K antagonist

* *CYP2C9*2*, *CYP2C9*3*, or *VKORC1 G1639A* genetic variant

† Solid or hematologic cancer requiring chemotherapy, radiotherapy, surgery, and/or palliative care during the last 3 months

‡ Bleeding that led to a hospital stay or transfusions

§ Serum hemoglobin <130 g/L for men or <120 g/L for women

¶ **Pharmacotherapy with >4 different drugs**

Table 2. Associations between CYP2C9 and VKORC1 variants and clinical outcomes

Clinical outcome / genetic variant (s)	No. of events/ patients (%)	No. of events/ patients (%)	Unadjusted HR or SHR (95%-CI)	Adjusted HR or SHR (95%-CI) [§]
	With variant(s)	Without variant(s)		
Any clinical event (Death, VTE recurrence, major bleeding, or clinically relevant non-major bleeding)				
CYP2C9 variant [†]	138/ 281 (49.1)	196/ 493 (39.8)	1.29 (1.04, 1.60)	1.34 (1.08, 1.66)
VKORC1 variant [‡]	228/ 510 (44.7)	106/ 264 (40.2)	1.16 (0.92, 1.46)	1.12 (0.88, 1.41)
CYP2C9 and VKORC1 variants	99/ 187 (52.9)	235/ 587 (40.0)	1.44 (1.14, 1.81)	1.40 (1.11, 1.77)
Death				
CYP2C9 variant [†]	55/ 281 (19.6)	64/ 493 (13.0)	1.55 (1.08, 2.22)	1.74 (1.19, 2.52)
VKORC1 variant [‡]	76/ 510 (14.9)	43/ 264 (16.3)	0.90 (0.62, 1.31)	0.82 (0.55, 1.22)
CYP2C9 and VKORC1 variants	34/ 187 (18.2)	85/ 587 (14.5)	1.25 (0.84, 1.86)	1.29 (0.84, 1.96)
Major bleeding				
CYP2C9 variant [†]	37/ 281 (13.2)	63/ 493 (12.8)	1.03 (0.69, 1.54)	1.03 (0.69, 1.55)
VKORC1 variant [‡]	64/ 510 (12.5)	36/ 264 (13.6)	0.92 (0.61, 1.38)	0.93 (0.61, 1.40)
CYP2C9 and VKORC1 variants	23/ 187 (12.3)	77/ 587 (13.1)	0.93 (0.58, 1.48)	0.91 (0.57, 1.46)
Clinically relevant non-major bleeding				
CYP2C9 variant [†]	72/ 281 (25.6)	95/ 493 (19.3)	1.38 (1.01, 1.87)	1.39 (1.02, 1.89)
VKORC1 variant [‡]	119/ 510 (23.3)	48/ 264 (18.2)	1.32 (0.95, 1.85)	1.32 (0.94, 1.85)
CYP2C9 and VKORC1 variants	58/ 187 (31.0)	109/ 587 (18.6)	1.81 (1.32, 2.50)	1.78 (1.30, 2.44)
VTE recurrence				
CYP2C9 variant [†]	35/ 281 (12.5)	65/ 493 (13.2)	0.93 (0.62, 1.40)	0.95 (0.62, 1.44)
VKORC1 variant [‡]	66/ 510 (12.9)	34/ 264 (12.9)	1.00 (0.66, 1.51)	1.11 (0.72, 1.70)
CYP2C9 and VKORC1 variants	25/ 187 (13.4)	75/ 587 (12.8)	1.03 (0.65, 1.62)	1.15 (0.72, 1.84)

Abbreviations: HR= hazard ratio; CI= confidence interval; SHR= sub-hazard ratio

* Death, VTE recurrence, major bleeding, or clinically relevant non-major bleeding

† *CYP2C9*2* and *CYP2C9*3* genetic variants

‡ *VKORC1 G1639A* genetic variant

§ Adjusted for age, sex, active cancer, provoked VTE, prior VTE, heart failure, chronic pulmonary disease, overt PE, history of major bleeding, heart rate ≥ 110 /min, systolic BP < 100 mm Hg, anemia, creatinine > 107 $\mu\text{mol/L}$, antiplatelet treatment, and periods of anticoagulation as time-varying covariate

¶ Adjusted for age, sex, active cancer, immobilization, heart failure, chronic pulmonary disease, overt PE, history of major bleeding, heart rate ≥ 110 /min, systolic BP < 100 mm Hg, anemia, creatinine > 107 $\mu\text{mol/L}$, and periods of anticoagulation as a time-varying covariate

** Adjusted for age, sex, active cancer, history of major bleeding, overt PE, anemia, creatinine > 107 $\mu\text{mol/L}$, antiplatelet treatment, and periods of anticoagulation as time-varying covariate

†† Adjusted for age, sex, active cancer, provoked VTE, prior VTE, and periods of anticoagulation as time-varying covariate

Table 3. Associations between CYP2C9 and VKORC1 variants and quality of anticoagulation

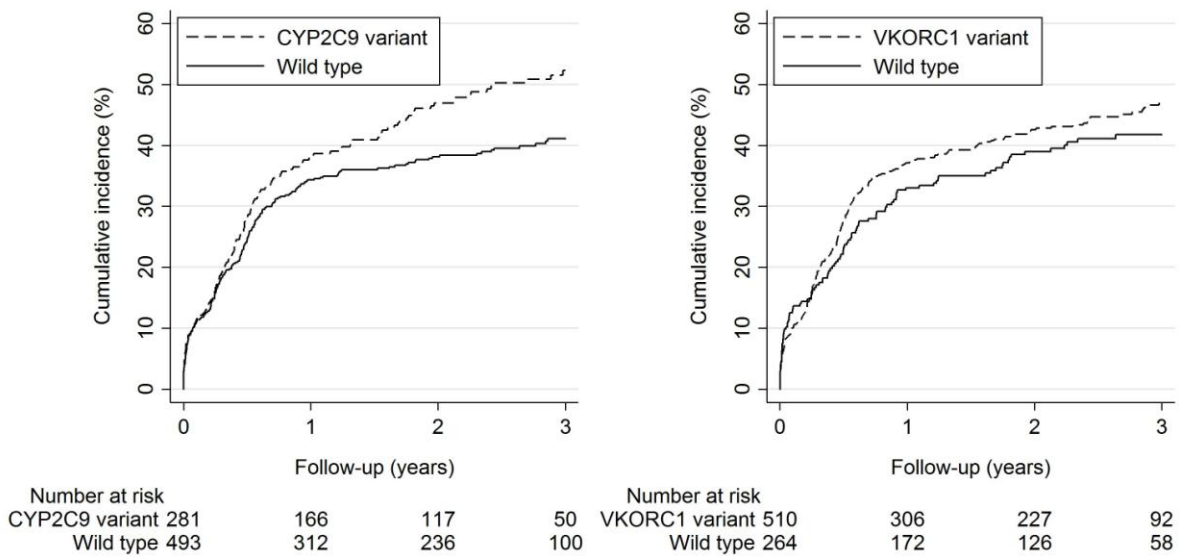
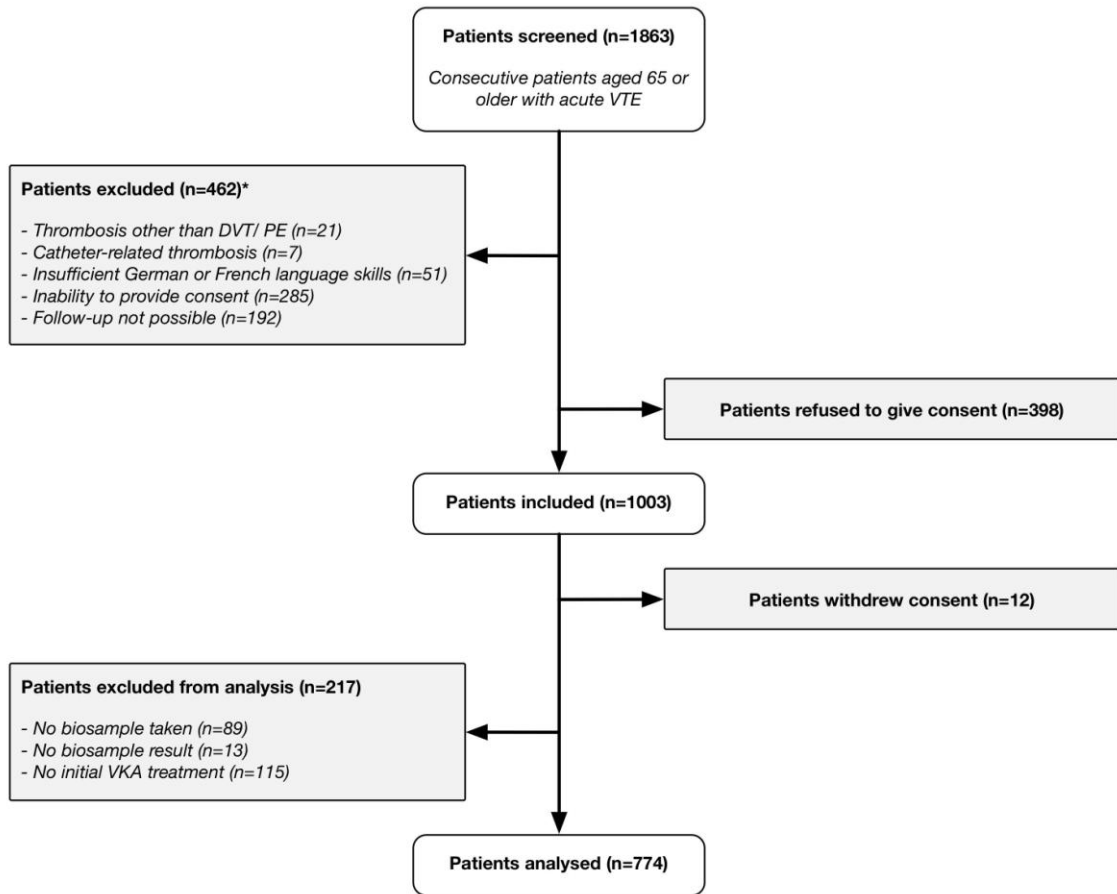
Genetic variants	Percentage of time in the therapeutic INR range (2.0-3.0)		Percentage of time above therapeutic INR range (>3.0)		Time to a first therapeutic INR (≥2.0)	Time to first INR >4.5
	Mean (SD)	Adjusted difference (95% CI) ‡	Mean (SD)	Adjusted difference (95% CI) ‡	Adjusted hazard ratio (95%-CI) ‡	Adjusted hazard ratio (95%-CI) ‡
CYP2C9 variant*						
No (n=481)	61.1 (22.8)		13.8 (15.3)		1 (reference)	1 (reference)
Yes (n=274)	57.4 (23.7)	-3.7 (-7.0, -0.4)	17.1 (18.7)	3.3 (0.8, 5.7)	0.81 (0.68, 0.96)	1.42 (1.11, 1.81)
VKORC1 variant†						
No (n=257)	58.4 (23.7)		13.5 (15.6)		1 (reference)	1 (reference)
Yes (n=498)	60.4 (22.9)	2.4 (-1.0, 5.7)	15.8 (17.1)	2.3 (-0.2, 4.8)	1.01 (0.85, 1.20)	1.43 (1.10, 1.85)
CYP2C9 and VKORC1 variant						
No (n=571)	60.2 (23.2)		13.9 (15.4)		1 (reference)	1 (reference)
Yes (n=184)	58.3 (23.4)	-1.5 (-5.2, 2.2)	18.4 (19.7)	4.4 (1.7, 7.2)	0.80 (0.65, 0.97)	1.47 (1.13, 1.93)

Abbreviations: INR= international normalized ratio; CI= confidence interval

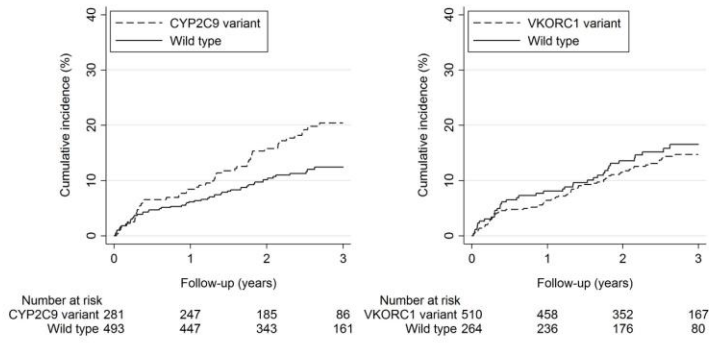
* CYP2C9*2 and CYP2C9*3 genetic variants

† VKORC1 G1639A genetic variant

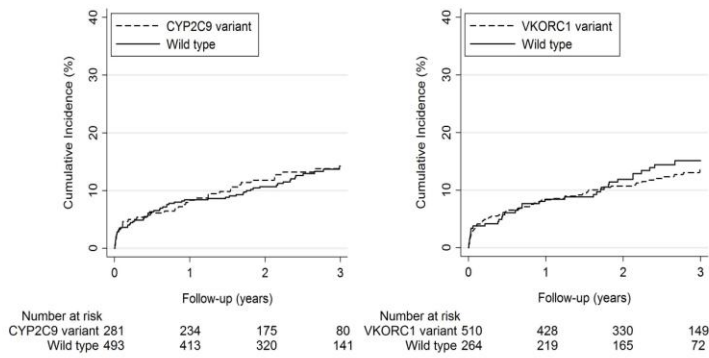
‡ Adjusted for age, sex, provoked VTE, active cancer, diabetes, heart failure, chronic pulmonary disease, chronic liver disease, severe renal failure (GFR <30 ml/min.), body mass index, hypertension, and smoking status



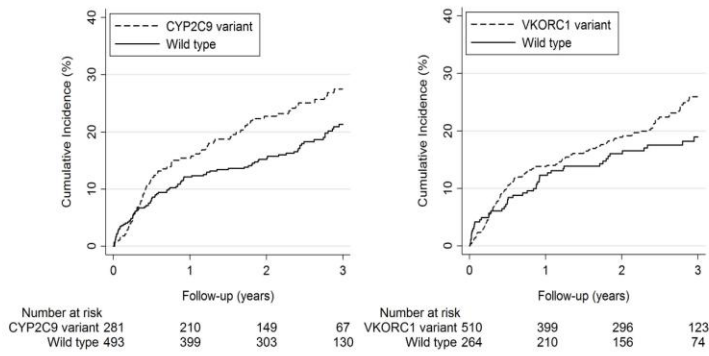
A Death



B Major bleeding



C Clinically relevant non-major bleeding



D VTE recurrence

