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# *VKORC1* and *CYP2C9* polymorphisms related to adverse events in case-control cohort of anticoagulated patients

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**Abstract**

Vitamin K antagonists (VKAs) are highly effective but have a narrow therapeutic index and require routine monitoring of the INR. The primary aim of pharmacogenetics (PGx) is to optimize patient care, achieving drug treatments that are personalized according to the genetic profile of each patient. The best-characterized genes involved in VKA PGx involve pharmacokinetics (*VKORC1*) and pharmacodynamics (*CYP2C9*) of VKA metabolism. The role of these genes in clinical outcomes (bleeding and thrombosis) during oral anticoagulant (OAC) therapy is controversial. The aim of the present study was to evaluate any potential association between genotype *VKORC1* and *CYP2C9* and adverse events (hemorrhagic and/or thrombotic), during initiation and long-term VKA treatment, in Caucasian patients. Furthermore, we aimed to determine if the concomitant prescription of other selected drugs affected the association between genotype and adverse events.

We performed a retrospective, matched case-control study to determine associations between multiple gene variants, drug intake, and any major adverse effects in anticoagulated patients, monitored in 2 Italian anticoagulation clinics.

Our results show that anticoagulated patients have a high risk of adverse events if they are carriers of 1 or more genetic polymorphisms in the *VKORC1* (rs9923231) and *CYP2C9* (rs1799853 and rs1057910) genes.

Information on *CYP2C9* and *VKORC1* variants may be useful to identify individualized oral anticoagulant treatment for each patient, improve management and quality of VKA anticoagulation control, and monitor drug surveillance in pharmacovigilance programs.

**Abbreviations:** AF = atrial fibrillation, *CYP2C9* = cytochrome P450 2C9, DOACs = direct oral anticoagulants, DVT = deep vein thrombosis, MHV = mechanical heart valves, MI = myocardial infarction, OAC = oral anticoagulant, PE = pulmonary embolism, PGx = pharmacogenetics, RTCs = randomized clinical trials, SNPs = single nucleotide polymorphisms, TIA = transient ischemic attack, TTR = time in therapeutic range, VKAs = vitamin K antagonists, *VKORC1* = vitamin K epoxide reductase complex subunit 1, VTE = venous thromboembolism.

**Keywords:** adverse events, amiodarone, antiplatelet drugs, CYP2C9, oral anticoagulation, statins, VKORC1

## 1. Introduction

Vitamin K antagonists (VKAs: warfarin, acenocoumarol, phenprocoumon, phenindione) are prescribed commonly for treatment of venous thromboembolism (VTE), prevention of thromboembolic complications in atrial fibrillation (AF), and after mechanical heart valve (MHV) replacement.[1] VKAs are highly effective but they have a narrow therapeutic index and they require routine monitoring of the International Normalized Ratio (INR). Over recent years, the oral anticoagulant (OAC) therapy scenario has changed as a result of the introduction of direct oral anticoagulants (DOACs)[2,3] and improvements in OAC management with VKAs, including pharmacogenetic (PGx) studies, focused on identifying genetic determinants affecting VKA dose requirements.[4–6] The best-characterized genes involved in VKA PGx are vitamin K epoxide reductase complex subunit 1 (*VKORC1*) and cytochrome P450 2C9 (*CYP2C9*). The human *VKORC1* gene (16p11.2) comprises 3 exons encoding the catalytic subunit of the vitamin K epoxide reductase complex, which is the key enzyme in the Vitamin K cycle.[7–10] A single nucleotide polymorphism (SNP) in the *VKORC1* promoter (−1639G > A, rs9923231) results in a decreased transcription of the gene and has been strongly associated with warfarin dose requirements.[11,12] The *CYP2C9* gene (10q24) encompasses 9 exons and it is highly polymorphic, as more than 60 variant alleles have been identified (http://www.cypalleles.ki.se, last access February 2016). CYP2C9 is one of the most abundant cytochrome P450 in the liver and it metabolizes approximately 15% of clinical drugs.[13,14] Allelic variants are missense, nonsense or frame shift variations, causing a reduced or a null enzyme activity. The most frequent variant alleles in Caucasian population, *CYP2C9*2* (rs1799853) and *CYP2C*3* (rs1057910), in the

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homozygous condition, reduce enzyme activity to 12% and 5%, respectively, compared to the wild-type genotype *CYP2C9* 1*1*.[15–16] The U.S. Food and Drug Administration (FDA) updated the warfarin label, providing dose recommendations based on different combinations of *VKORC1* and *CYP2C9* genotypes, in 2007 and 2010.[17] Guidelines for clinicians and genetic-based algorithms have been implemented by the International PGx Warfarin Consortium.[18] Two recent randomized clinical trials (RTCs)[19,20] aimed to assess the effect of the PGx-guided initial drug dosing on improvement of Time in Therapeutic Range (TTR). The RTCs showed contradictory results because of the differences in the study design, and stimulated a considerable debate on this matter.[21–30] Although there is robust evidence of the association of genetic variants on dose requirement, the role of these genes on the clinical outcome (bleeding and thrombosis) during OAC therapy is controversial, as outlined in contradictory results reported in recent meta-analyses.[31,32] The aim of the present study is to evaluate potential associations between genotype *VKORC1* and *CYP2C9* and adverse events (hemorrhagic and/or thrombotic) during initiation and long-term VKA treatment, in a Caucasian population. Patient monitoring occurred in 2 specialized anticoagulation clinics. Furthermore, we aimed to determine if the concomitant prescription of other selected drugs (amiodarone, HMGCo-A reductase inhibitors [simvastatin], antiplatelet medication) affected the association between genotype and adverse events.

## 2. Materials and methods

### 2.1. Design overview

We performed a retrospective, matched case-control study to examine associations among *CYP2C9*2, CYP2C9*3*, *VKORC1: c. −1639G>A* polymorphisms, drug intake, and any hemorrhagic and/or thrombotic event, in oral anticoagulated patients. Cases and controls were enrolled and monitored in 2 Italian anticoagulation clinics (Anticoagulation Centre, Brescia and Haemostasis and Thrombosis Centre, Cremona) between 2009 and 2014. Both centers are affiliated with the Italian Federation of Anticoagulation Clinics (FCSA) and are placed in hospitals in the main city.

### 2.2. Patients: eligibility criteria

In order to achieve a cohort representative, as far as possible, of real life conditions, no explicit exclusion criteria were defined, except for age and Caucasian ethnicity.

Cases included patients receiving OAC therapy with the following characteristics:

- Age greater than 18 years
- Caucasian origin
- OAC therapy use for any condition (atrial fibrillation, AF; venous thromboembolism, VTE; implanted mechanical heart valves, MHV)
- History of an adverse event (thrombotic and/or ischemic) during therapy with VKAs.

Adverse events are those indicated in the Italian FCSA guidelines[33]:

- Major hemorrhages (cerebral bleeding; extra-cerebral bleeding in a critical area or organ; a decline in hemoglobin levels by 2 g/dL and/or requiring transfusion)
- Thromboembolic events (stroke; transient ischemic attack, TIA; myocardial acute infarction, IMA; venous thromboembolism, VTE, including deep vein thrombosis, DVT, and pulmonary embolism, PE).

Minor hemorrhagic events were excluded.

The control group consisted of 120 unrelated subjects who did not experienced any adverse event and were matched to cases for age, sex, clinical indication, and duration of anticoagulation.

### 2.3. Data source and genotyping

Electronic search was performed through software *Parma*$^{GTS}$ (Instrumentation Laboratory, Bedford, MA) in Brescia Haemostasis Center and in Cremona Haemostasis and Thrombosis Centre through *TAONET* (EDP-Project, Bozen, Italy), used for the management, archiving, and referral of inpatients and outpatients to the clinic. In the Brescia Haemostasis Centre, we initially identified patients (N=458) with a history of any adverse event occurring between 2009 and 2014. We excluded patients experiencing a minor adverse event, those who died from any cause, and patients who were not of Caucasian origin. We identified 196 patients with major adverse events. We then excluded patients who interrupted OAC (N=92); did not measure INR as prescribed; or did not communicate the INR value, when measured in a different setting, on more than 3 occasions (N=28). We obtained a total of 74 effective final cases. In order to achieve the predetermined statistical power for the study, a further 46 cases were enrolled from the Cremona Haemostasis and Thrombosis Center. All controls were selected from Brescia Anticoagulation Center. For both cases and controls, only patients with complete clinical information were selected (Fig. 1). Clinical data, including patient age, sex, indication for and duration of OAC therapy, concomitant medication, medical history, and INR values, were collected at the time of enrollment. In this phase of the study, we only collected information regarding the following drugs: amiodarone, HMGCo-A reductase inhibitors (simvastatin), antiplatelet medication. Venous blood samples were collected in tubes containing sodium citrate and genomic DNA was extracted by the automated nucleic acid extraction system MagCore HF 16 (RBC Bioscience, Taiwan) using the Whole Blood Kit and following the manufacturer's instructions. Genotyping was performed using the PGX-Thrombo StripAssay (ViennaLab Diagnostics, Vienna, Austria), with slight modification to the manufacturer's instructions. The process was performed using the automated instrument ProfiBlot T48 (Tecan, Männedorf, Switzerland), which allow simultaneous processing of 48 samples. The study protocol was reviewed and approved by the Ethics Committees of the participating hospitals. Written informed consent was obtained for all patients.

### 2.4. Statistical analysis

Sample size was estimated assuming a case-control design, with a control-case ratio of 1, a significance level of 5%, and a power of at least 80%. We assumed a mutation frequency of at least 14% and an odds ratio (OR) of at least 2.5. This resulted in a sample size of 118 subjects per group. This figure was rounded up to 120, for a total of 240 cases and controls, overall. Adverse events were not separated for thrombotic or hemorrhagic events. Estimation of OR for event occurrence was performed with conditional logistic models, to account for case/control matching and assuming the additive genetic effect for rs9923231 (*VKORC1*), rs1799853 (*CYP2C9*2*), rs1057910 (*CYP2C9*3*). In this model, each genotype is a distinct group; homozygous patients for major alleles (*GG* and **1*1*) are the reference group. Data are reported

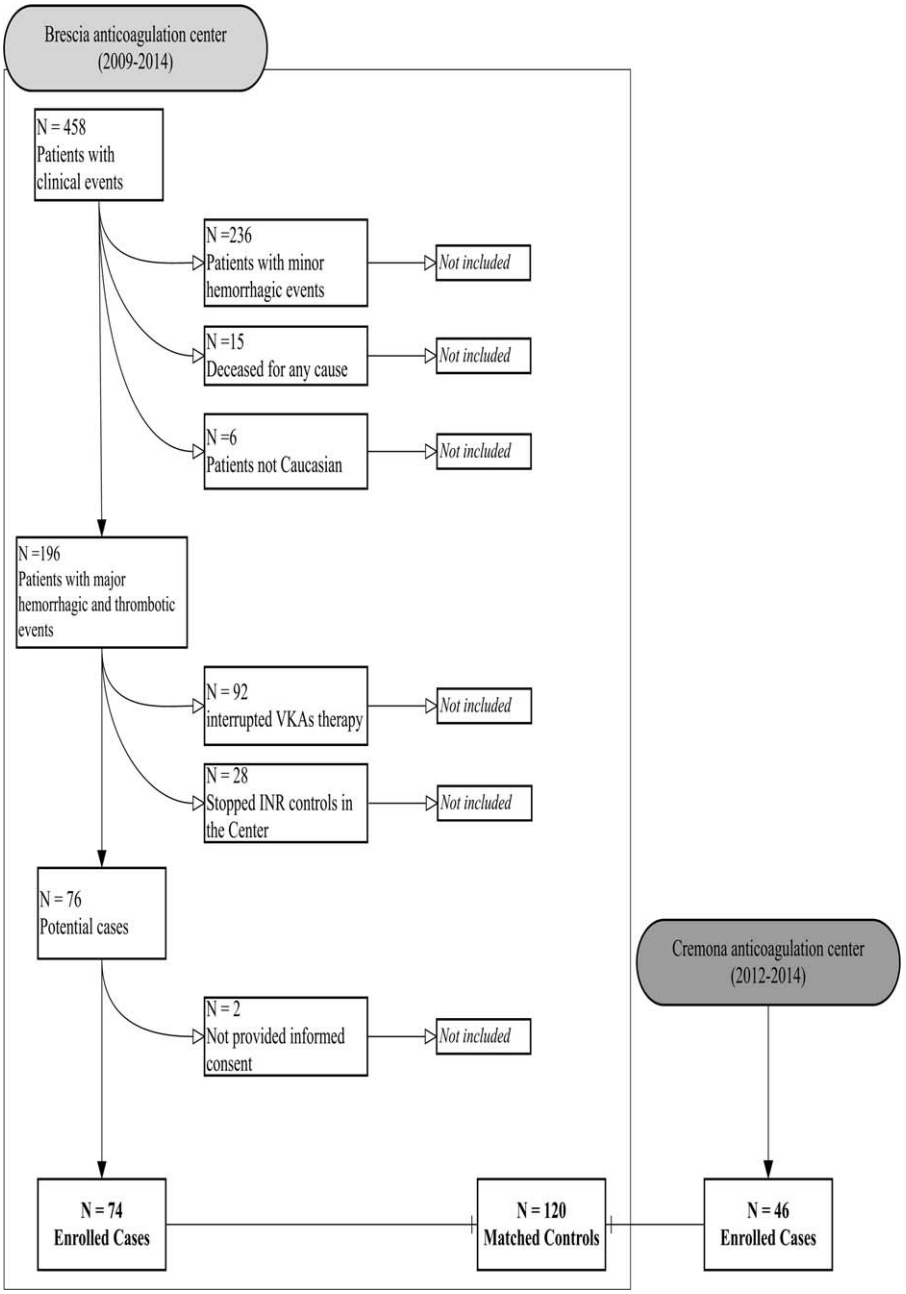


**Figure 1.** Patient-enrollment flow diagram.

as OR estimates and 95% confidence intervals. Similarly, time to event was modeled using marginal Cox models, with sandwich variance estimators to account for matching. The statistical significance level was set at $P=0.05$.

## 3. Results

### 3.1. Characteristic of the selected cohort

A total of 120 cases and 120 controls were enrolled in the study. Patient mean age was $76\pm10$ years, and 44% of subjects were female. The majority of the patients were prescribed VKAs for the following conditions: atrial fibrillation (N=134, 55.8%), mechanic heart valve replacement (N=56, 23.2%), and venous thromboembolism (N=50, 20.8%). Mean follow-up time was $8\pm6$ years. There were no statistically significant differences in comorbidity between cases and controls, except for history of hypertension, which was slightly more prevalent among controls than among cases (87.5% vs 78.3%, respectively; $P=0.047$). There were no significant differences in the use of amiodarone, simvastatin, and antiplatelet agents and no significant differences in the TTR between the 2 groups of patients (Table 1).

### 3.2. Genetic polymorphisms and adverse events association

In 65% of the selected cases, major bleeding events were observed, and in 35%, a thromboembolic event was noted (Table 2). Out of 120 cases, an adverse event occurred in 114 patients (95%) during the maintenance phase of the therapy (over the first 3 months) and in 6 patients (5%) during the induction

**Table 1**

**Demographic and clinical characteristics of patient's cohort.**

| Characteristic | Cases (N = 120) | Controls (N = 120) | OR (95% CI) | P |
|---|---|---|---|---|
| Female, N, % | 53 (44.17%) | 53 (44.17%) | Matching variables | |
| Age, y, mean ± SD | 76.9 ± 10.4 | 76.7 ± 10.3 | | |
| Indication for OAC | | | | |
| AF, N, % | 67 (55.83%) | 67 (55.83%) | | |
| MHV, N, % | 28 (23.33%) | 28 (23.33%) | | |
| VTE, N, % | 25 (20.83%) | 25 (20.83%) | | |
| Average follow-up, y, mean ± SD | 8.23 ± 5.52 | 8.51 ± 6.06 | | |
| Type of VKA, N, % | | | | |
| Warfarin | 108 (90.00%) | 104 (86.67%) | 1 | – |
| Acenocoumarol | 12 (10.00%) | 16 (13.33%) | 0.75 (0.33–1.71) | 0.49 |
| Comorbidities, N, % | | | | |
| Hypertension, yes vs no | 94 (78.33%) | 105 (87.5%) | 0.45 (0.21–0.99) | **0.047** |
| Dyslipidemias, yes vs no | 40 (33.33%) | 47 (39.17%) | 0.79 (0.45–1.37) | 0.40 |
| Chronic renal impairment,* yes vs no | 18 (15.0%) | 12 (10.0%) | 1.5 (0.67–3.34) | 0.32 |
| Diabetes mellitus, yes vs no | 17 (14.17%) | 16 (13.33%) | 1.07 (0.52–2.22) | 0.85 |
| History of cancer, yes vs no | 16 (13.33%) | 13 (10.83%) | 1.17 (0.54–2.52) | 0.70 |
| Liver disease, yes vs no | 7 (5.83%) | 9 (7.50%) | 0.78 (0.29–2.09] | 0.62 |
| Concomitant drugs, N, % | | | | |
| Statins, yes vs no | 45 (37.50%) | 43 (35.83%) | 1.40 (0.73–2.67) | 0.31 |
| Amiodarone, yes vs no | 35 (29.17%) | 30 (25.00%) | 1.26 (0.69–2.30) | 0.45 |
| Antiplatelet agents, yes vs no | 22 (18.33%) | 16 (13.33%) | 1.07 (0.64–1.77) | 0.80 |
| TTR, median, IQR† | | | | |
| 0–3 mo | 0.52 (0.37) | 0.56 (0.34) | 0.33 (0.10–1.09) | 0.07 |
| 3–12 mo | 0.69 (0.27) | 0.74 (0.26) | 0.32 (0.07–1.40) | 0.13 |
| 0–12 mo | 0.65 (0.21) | 0.70 (0.22) | 0.50 (0.10–2.57) | 0.41 |

Data are expressed as N (%), unless specified.

AF = atrial fibrillation, CI = confidence interval, IQR = interquartile range, MHV = mechanical heart valves, OAC = oral anticoagulant, OR = odds ratio, SD = standard deviation, TTR = time in therapeutic range, VKA = vitamin K antagonist, VTE = venous thromboembolism.

* Serum creatinine ≥ 1.5 mg/dL.

† OR for unit change.

phase (data not shown). The frequencies of *VKORC1* and *CYP2C9* genotypes are shown in Table 3. All 3 *VKORC1* genotypes were considered, whereas *CYP2C9* genotypes were grouped into 2 categories: wild-type *(*1*1)* and carriers of at least 1 variant *CYP2C9* allele (**1*M or *M*M*). Genotypes were in Hardy–Weinberg equilibrium for both cases and controls. The effects of 3 genetic polymorphisms on adverse events were evaluated. The variant *VKORC1: −1639 G > A* and CYP2C9 variant alleles were significantly associated with any thrombotic and/or hemorrhagic adverse event. *VKORC1: −1639 G > A* was associated with a 1.5-fold increase in the risk of adverse events (OR 1.60, 95% CI interval 1.06–2.42; *P = 0.025*). Each *CYP2C9* variant allele was associated with a 2-fold increase of the same risk (OR 2.28, 95% CI interval 1.34–3.86; *P = 0.002*). The combined *VKORC1* and *CYP2C9* genotype effect were associated with a 2-fold increase in adverse events (OR 2.37, 95% CI interval 1.37–4.10; *P = 0.002*). To take into account several confounding factors, we applied survival models to assess the association between *VKORC1* and *CYP2C9* polymorphisms and primary outcomes. Adjusting for concomitant drugs (Amiodarone, Simvastatin, and antiplatelet medication), carriers of one of the analyzed SNPs had a significant increased hazard ratio for adverse events (Table 4). *VKORC1: −1639 AA* homozygous was associated with a 2-fold increased hazard ratio of an adverse event (HR 1.97, 95% CI interval 1.13–3.43; *P = 0.02*) compared to wild-type (GG), whereas *CYP2C9*2/*3* carriers had a 1.6-fold increased risk (HR 1.58, 95% CI interval 1.14–2.35; *P = 0.008*). In *CYP2C9*2/*3* carriers (HR 1.6, 95% CI interval 1.00–2.56; *P = 0.05*), the same risk was observed for simultaneous use of antiplatelet medication (Table 4.)

**Table 2**

**Adverse events in the selected cohort.**

| Event | N (%) |
|---|---|
| Major hemorrhage | *78 (65.0%)* |
| Gastrointestinal | 21 (17.50%) |
| Epistaxis* | 19 (15.83%) |
| Intracranial | 13 (10.83%) |
| Hematuria/proctorrhagia* | 9 (7.50%) |
| Hematoma* | 9 (7.50%) |
| Other† | 7 (5.83%) |
| Thromboembolic | 42 (35.0%) |
| Transient ischemic attack | 20 (16.67%) |
| Stroke | 12 (10.00%) |
| Myocardial infarction | 7 (5.83%) |
| Venous thromboembolism‡ | 3 (2.50%) |

* Epistaxis, Hematuria, Proctorrhagia, and Hematoma with Hb reduction by ≥2 g/dL and/or requiring transfusion have been considered.

† Includes any extra-cerebral bleeding in a critical area (articular, retroperitoneal, and ocular [with blindness]).

‡ Includes deep vein thrombosis and pulmonary embolism.

## 4. Discussion

Our findings suggest that patients undergoing long-term oral anticoagulation (OAC) therapy with VKAs have a high risk of thrombotic and/or major bleeding adverse events if they are carriers of 1 or more polymorphisms in the *VKORC1* (rs9923231) and *CYP2C9* (rs1799853 and rs1057910) genes.

**Table 3**

**Genotype *VKORC1* and *CYP2C9* gene polymorphisms in the selected cohort.**

| Polymorphisms | Cases (N = 120) | Controls (N = 120) |
|---|---|---|
| *VKORC1: -1639 G>A* (rs9923231) | | |
| Genotype | | |
| GG, N (%) | 37 (30.8%) | 49 (40.8%) |
| GA, N (%) | 64 (53.33%) | 62 (51.67%) |
| AA, N (%) | 19 (15.83%) | 9 (7.50%) |
| Additive model | | |
| OR (95% CI), *P* value | | |
| (GA vs GG) | 1.47 (0.84–2.57), 0.17 | |
| OR (95% CI), *P* value | | |
| (GA vs GG) | **2.76 (1.13–6.72), 0.025** | |
| Recessive model | | |
| OR (95% CI), *P* value | | |
| (AA + GA vs GG) | **1.60 (1.06–2.42), 0.025** | |
| *CYP2C9*2* (rs1799853); *CYP2C9*3* (rs1057910)* | | |
| Genotype | | |
| **1*1, N (%)* | 60 (50.0%) | 86 (71.67%) |
| **1*M + *M*M N (%)* | 60 (50.0%) | 34 (28.33%) |
| Recessive model | | |
| (*1*M + *M*M vs *1*1) | **2.28 (1.34–3.86), 0.002** | |
| Combined *VKORC1 + CYP2C9* genotype | | |
| *GG/*1*1* | 22 (18.33%) | 33 (27.50%) |
| *GA/*1*1* | 29 (24.17%) | 47 (39.17%) |
| *AA/*1*1* | 10 (8.33%) | 6 (5.0%) |
| *GG/*1*M or GG/*M*M* | 15 (12.5%) | 16 (13.33%) |
| *GA/*1*M or GA/*M*M* | 35 (29.17%) | 15 (12.50%) |
| *AA/*1*M or AA/*M*M* | 9 (7.50%) | 3 (2.50%) |
| OR (95% CI), *P* value | | |
| (GA vs GG) | OR = 1.54 (0.86–2.75), 0.15 | |
| OR (95% CI), *P* value | | |
| (AA vs GG) | OR = **3.02 (1.18–7.78), 0.022** | |
| OR (95% CI), *P* value | | |
| (*1*M + *M*M *vs* *1*1) | OR = **2.37 (1.37–4.10), 0.002** | |

CI = confidence interval, OR = odds ratio.
* M indicates heterozygous or homozygous carrier of either *CYP2C9*2* either *CYP2C9*3* alleles.
Bold numbers: $P < 0.05$.

To the best of our knowledge, this is the first study on VKA pharmacogenetics showing an association between genetic variants and both bleeding and thrombotic events in a cohort of patients affected by a range of diseases (AF, VTE, MHV replacement) and experiencing adverse events during OAC therapy. Published data on the increased risk of side effects associated with genetic variants among VKA users is controversial, as highlighted by recently published guidelines concerning the appropriateness of genetic testing in warfarin therapy.[34] A recent meta-analysis showed that only the *CYP2C9*3* allele, but not the *CYP2C9*2* allele, was associated with a significant risk of bleeding.[35] However, another meta-analysis concluded that *CYP2C9*2* and *CYP2C9*3*, but not *VKORC1* genotypes, were associated with warfarin hemorrhagic complications.[31] A recent case-control study[36] showed that *VKORC1 1173 C>T* (rs9934438) and *CYP2C9*2/*3* polymorphisms are not associated with increases in the risk of major bleeding in long-term warfarin users. Conversely, an extensive genetic analysis of an RTC has clearly showed the influence of *VKORC1* and *CYP2C9* variants on bleeding events in patients with nonvalvular AF and treated with warfarin.[37] The patient cohort presents increased variability compared to other studies; nevertheless, our results corroborate previous findings of major bleeding and thrombotic risks in patients with *CYP2C9* and *VKORC1* variants. Tomek et al[38] showed that anticoagulated Caucasian patients carriers of 3 variant alleles of the genes *CYP2C9* and *VKORC1* exhibited a significantly higher risk of major bleeding during the initiation and maintenance phases of warfarin therapy. Kawai et al[39] showed that the *CYP2C9*3* allele could double the risk of major bleeding among patients administered warfarin for 30 or more days. However, the authors did not find any association with *VKORC1* variants and the study population largely consisted of Caucasians, although it also included Afro-Americans and others ethnic groups. Recent research identified the *VKORC1* gene as a susceptibility factor for ischemic cerebrovascular disease in a Chinese subpopulation,[40] and Wang et al[41] demonstrated the involvement of *VKORC1* in arterial vascular disease. Our results partially corroborate recent evidence on drug–drug interactions (DDIs). Santos et al[42] showed that simultaneous use of warfarin and amiodarone was not associated with adverse events in chronic OAC therapy. Also, a previous study[43] found that the combination of single antiplatelet therapy with an anticoagulant was associated with a significantly greater risk of bleeding. Sconce et al[44] showed that simvastatin reduced the mean warfarin dose in patients on chronic OAC therapy, whereas the statin effect on bleeding risk in patients on VKA is controversial.[45,46]

**Table 4**

**Cox's regression analysis.**

| Genotype | HR (95% CI) | *P* |
|---|---|---|
| *VKORC1* | | |
| GA vs GG | 1.36 (0.91–2.05) | 0.14 |
| AA vs GG | **1.97 (1.13–3.43)** | **0.02** |
| *CYP2C9** | | |
| *1*M + *M*M vs *1*1 | **1.58 (1.14–2.35)** | **0.008** |
| Antiplatelet drugs | **1.60 (1.00–2,56)** | **0.05** |
| *VKORC1 + CYP2C9* | | |
| GA vs GG | 1.38 (0.92–2.08) | 0.12 |
| AA vs GG | **1.82 (1.04–3.19)** | **0.035** |
| CYP2C9 variant vs wild type | **1.62 (1.12–2.32)** | **0.010** |
| Antiplatelet drugs | 1.56 (0.95–2.45) | 0.08 |

CI = confidence interval, HR = hazard ratio.
* M indicates heterozygous or homozygous carriers of either *CYP2C9*2* either *CYP2C9*3* alleles.
Bold numbers: $P < 0.05$.

Our study has several limitations. Some adverse events may not have been considered, for example, in patients who discontinued OAC therapy after an adverse event, patients who refused to be enrolled into the study, and patients who died before hospital admission due to any cause. Moreover, we enrolled patients monitored in anticoagulation clinics and these may not be representative of the general VKA patient population. Also, we gained information on selected interacting drugs, which are frequently used in associated comorbidities. However, we reduced potential bias by selecting cases from 2 institutions (Brescia Anticoagulation Centre and Cremona Haemostasis and Thrombosis Centre). Furthermore, controls were matched to cases for all variables (age, sex, clinical indication for therapy, and duration of OAC therapy) except for the occurrence of an adverse event. A strength of the study is that we had a homogenous population of Caucasians, important for genetic analyses, largely because *CYP2C9*2* and *CYP2C9*3* polymorphisms are primarily found in Caucasian population. Our findings may have some important implications, regarding selection and monitoring of VKA patients. A recent pharmacoeconomic study[47] showed that an indiscriminate use of DOACs in patients with atrial fibrillation is less cost-effective

than the preemptive genotyping approach. It follows that preemptive genotyping may be recommended even in warfarin-naive patients, for whom DOACs are not suitable, and this approach could improve patient care, reducing the risk of adverse events and the planned days required to achieve the target daily drug dosage by INR monitoring. Genotype determination could be useful even in long-term VKA patients, to improve the quality of OAC therapy, by increasing the frequency of INR monitoring or identifying interacting drugs with greater accuracy. These considerations are of particular relevance in the elderly and in patients with decreased or impaired renal function, as VKAs remain the mainstay of anticoagulation therapy in these patients. Finally, implementation of pharmacogenetics could improve pharmacovigilance practice, as highlighted in guidelines recently released by the European Medicines Agency.[48] VKAs are one of the most common drugs resulting in emergency department admissions[49,50] and *VKORC1* and *CYP2C9* genetic variants may represent useful predictors of adverse drug reactions. In conclusion, our results suggest that the information on *CYP2C9* and *VKORC1* variants may potentially enable clinicians to determine individualized anticoagulant treatment for each patient; improve use, management and quality of VKA anticoagulation control; and monitor drug surveillance as part of pharmacovigilance programs.

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