Pharmacogenetics of Bleeding and Thromboembolic Events in Direct Oral Anticoagulant Users

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This study aimed to analyze associations between genetic variants and the occurrence of clinical outcomes in dabigatran, apixaban, and rivaroxaban users. This was a retrospective real-world study linking genotype data of three Finnish biobanks with national register data on drug dispensations and healthcare encounters. We investigated several single-nucleotide variants (SNVs) in the *ABCG2*, *ABCB1*, *CES1*, and *CYP3A5* genes potentially associated with bleeding or thromboembolic events in direct oral anticoagulant (DOAC) users based on earlier research. We used Cox regression models to compare the incidence of clinical outcomes between carriers and noncarriers of the SNVs or haplotypes. In total, 1,806 patients on apixaban, dabigatran, or rivaroxaban were studied. The *ABCB1* c.3435C>T (p.Ile1145=, rs1045642) SNV (hazard ratio (HR) 0.42, 95% confidence interval (CI), 0.18–0.98, *P* = 0.044) and 1236T-2677T-3435T (rs1128503-rs2032582-rs1045642) haplotype (HR 0.44, 95% CI, 0.20–0.95, *P* = 0.036) were associated with a reduced risk for thromboembolic outcomes, and the 1236C-2677G-3435C (HR 2.55, 95% CI, 1.03–6.36, *P* = 0.044) and 1236T-2677G-3435C (HR 5.88, 95% CI, 2.35–14.72, *P* < 0.001) haplotypes with an increased risk for thromboembolic outcomes in rivaroxaban users. The *ABCB1* c.2482-2236G>A (rs4148738) SNV associated with a lower risk for bleeding events (HR 0.37, 95% CI, 0.16–0.89, *P* = 0.025) in apixaban users. *ABCB1* variants are potential factors affecting thromboembolic events in rivaroxaban users and bleeding events in apixaban users. Studies with larger numbers of patients are warranted for comprehensive assessment of the pharmacogenetic associations of DOACs and their relevance for clinical practice.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Earlier studies have found associations between genetic variability and plasma levels of direct oral anticoagulants (DOACs), but it is unclear whether these associations translate into clinical outcomes.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Are variants in the *ABCG2*, *ABCB1*, *CES1*, or *CYP3A5* genes associated with bleeding or thromboembolic events in DOAC users?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ We found *ABCB1* single nucleotide variants and haplotypes to be associated with clinical outcomes in rivaroxaban and apixaban users.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This knowledge might aid in identifying individuals with suboptimal response to rivaroxaban or apixaban. Further research is warranted on the clinical impact and cost effects of pharmacogenetic variability in DOAC response.

Direct oral anticoagulants (DOACs) are increasingly used in the context of various clinical conditions and procedures, including atrial fibrillation (ischemic stroke prevention), deep vein thrombosis, and pulmonary embolism. DOACs have favorable pharmacodynamic and pharmacokinetic properties and do not require continuous therapeutic monitoring except in specific

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cases.[1–3] However, serious bleeding events and thromboembolic events of DOAC users are not uncommon.[4] Notable interindividual variation exists in the plasma levels of DOACs.[5,6]

The effect of genetic factors on the pharmacokinetics of DOACs have been investigated in several studies.[7–9] Indications of association between genotypes and drug plasma levels have been found in some studies,[10–17] but also contradictory findings have been reported.[5,18,19]

In particular, the genome-wide association substudy of the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial[13,20] shows associations of *ABCB1* and *CES1* variants with dabigatran plasma concentration and an association of one *CES1* variant with risk of bleeding. The role of genetic variants in excessive drug exposure and resulting bleeding episodes has been proposed also in some case reports.[21,22]

The aim of this retrospective real-world study was to provide more insight on the effect of genetic factors on end points associated with the use of DOACs. We have linked genotype data of three Finnish biobanks with national register data on drug dispensations and healthcare encounters to explore potential associations of genetic variants with responses to dabigatran, rivaroxaban, and apixaban, which are the three most used DOACs in Finland.[23]

METHODS

Study design

Pharmacogenomics of Antithrombotic Drugs (PreMed Study) is a retrospective cohort study linking health data from multiple sources. The data for the study consists of three subcohorts provided by Auria Biobank, Helsinki Biobank, and the Finnish Institute for Health and Welfare (THL) Biobank. Each biobank first identified subjects fulfilling the inclusion criteria of the study. Then, the biobanks formed subcohorts by linking their genomic and demographic data with healthcare encounter data from the Finnish Institute for Health and Welfare, with drug dispensation data from the Social Insurance Institution of Finland and with patient record and laboratory data from Finnish hospital districts and municipalities. The three pseudonymized subcohorts provided by the biobanks were then merged by VTT Technical Research Centre of Finland Ltd. to form the PreMed Study cohort.[24] In this article, we investigate the genetics–response associations of DOACs. Results on warfarin pharmacogenetics have been reported earlier.[25]

Patients

Individuals of at least 18 years of age at the time of the first purchase of a DOAC, with one of the inclusion diagnoses (**Table S1**) and with at least one drug dispensation as a new user of dabigatran, rivaroxaban, or apixaban in the time frame of January 2007–June 2018 were included in the analysis. Additionally, it was required that the patient's genetic variant data were available from the biobanks. We analyzed genetic variants which have been identified in earlier research to be potentially associated with bleeding or thromboembolic events in DOAC users.[7,8]

Subjects with purchases of antithrombotic drugs during the period January 1, 2005 to December 31, 2006 were excluded to ensure a washout period of at least 2 years for all patients.

Genotyping

Genotyping of patients was carried out in the framework of the ongoing FinnGen project[26] and past research projects of the Finnish Institute for Health and Welfare.[27,28] The following SNVs were included: *ABCG2* c.421C>A (p.Gln141Lys, rs2231142), *ABCB1* c.3435C>T (p.Ile1145=, rs1045642), *ABCB1* c.2677G>T (p.Ala893Ser, rs2032582), *ABCB1* c.2677G>A (p.Ala893Thr, rs2032582), *ABCB1* c.2482-2236G>A (rs4148738, intron 18), *ABCB1* c.1236C>T (p.Gly412=, rs1128503), *CYP3A5* g.6981A>G (splice defect, *CYP3A5*3*, rs776746), *CES1* c.1168-33C>T (rs2244613, intron 10) and *CES1* c.257+885T>C (rs8192935, intron 2).

We verified the quality of the genotype data by comparing the allele frequencies of the cohort with other genotyping projects.[29] In addition, we verified that all SNVs were in Hardy-Weinberg equilibrium and confirmed that the imputation quality was sufficient (info score > 0.85).[30]

Drug exposure

We estimated the drug exposure based on the data on drug dispensations including package size, product strength, and the date of dispensation. The exposure was considered to have started on the date of the first drug dispensation and was deemed to be continuous until the earliest of the following: (i) all dispensed packages of the drug were consumed and a new package was not dispensed within 30 days, (ii) another anticoagulant (**Table S2**) was purchased, or (iii) the study follow-up ended (December 31, 2018). If the subject had used more than one DOAC during the study period, we chose the first DOAC used for investigation to ensure that all patients were naïve to DOAC treatment at baseline. Subsequent periods of using other DOACs were excluded from the analysis.

Outcome events

Bleeding and thromboembolic events were observed as outcomes. We considered an outpatient or inpatient healthcare episode to be eligible as an outcome event if it occurred for the first time during the drug exposure period and if one of the defined outcome diagnosis codes listed in **Table S1** was documented for the visit. Specific consideration was applied to recurrent thromboembolic events, referring to thromboembolic events which had occurred before the drug use started and occurred again during the drug exposure and for which the same diagnosis code had been documented. We considered a recurrent thromboembolic event as an outcome only if it involved a hospitalization and if it was documented as the primary diagnosis at the admission. These conditions were deemed necessary because a diagnosis code of a past event is often documented also for a subsequent outpatient visit even when a new outcome event has not occurred.

Covariates

Participants' sex and age were available in the demographic data provided by the biobanks. Drug indication was based on the inclusion diagnosis documented for the patient closest to the initiation of the drug use. We categorized the patient as having cancer if any cancer diagnosis (International Classification of Diseases, Tenth Revision (ICD-10) code group C) was documented for an outpatient or inpatient visit during the exposure time or up to 2 years before drug exposure started. Renal function was evaluated based on the glomerular filtration rate computed from the laboratory data (creatinine test). We considered the renal function to be reduced if the glomerular filtration rate value was below 60 mL/minute/1.73 m^2.[31]

The patient was defined to be on dose reduction if a reduced drug dose was prescribed in 50% or more of the dispensations. The daily doses were for dabigatran 150 mg b.i.d. (nominal) and 110 mg b.i.d. (reduced), for rivaroxaban 20 mg q.d. (nominal) and 2.5/10/15 mg q.d. (reduced), and for apixaban 5 mg b.i.d. (nominal) and 2.5 mg b.i.d. (reduced).

We defined that the patient was a user of an interacting drug if the patient purchased a potentially interacting drug listed in **Table S2** at least once during the DOAC exposure time. The list of potentially interacting drugs was based on the Inxbase drug–drug interaction database.[32] We included 46 drugs potentially increasing the bleeding risk and three drugs potentially increasing the risk for thromboembolic events. The drugs potentially increasing the bleeding risk were further divided into antiplatelet and statin groups for the analysis of confounders (**Table S2**).

Dispensations of acetylsalicylic acid, as an over-the-counter drug, could not be extracted from the drug purchase register, which only includes prescribed medication. Instead, we extracted information of acetylsalicylic acid use from the patient records (data entered by the physician based on information given by the patient).[33]

Statistical analyses

We analyzed associations between carriers and noncarriers of SNVs or haplotypes and outcome events for each drug user group (dabigatran, apixaban, and rivaroxaban) separately.

For the SNV association analysis, we primarily used the dominant genetic model[34] comparing the noncarriers (no variant alleles) with carriers (one or two variant alleles). We repeated the analysis by using the recessive genetic model comparing homozygous subjects having two variant alleles with subjects having only one variant allele or no variant alleles. We also carried out an association analysis for the *ABCB1* haplotypes of c.1236C>T, c.2677G>T, and c.3435C>T. The haplotypes were estimated from the genotype data by using the PHASE (version 2.1.1) software package.[35] For the haplotype analysis, the dominant and recessive models were used to compare noncarriers with carriers analogously to the SNV analysis.

The occurrence of outcome events was reported as incidence rates (events per 100 patient-years). We used the Cox proportional hazards regression models to investigate the association of the SNV and haplotype groups with the occurrence of outcome events. The results were reported using hazard ratios (HRs), 95% confidence intervals (CIs) and *P* values. We investigated potential confounders using univariate Cox regression analysis. Based on the analysis, we used antiplatelet medication in the adjusted analysis of bleeding events, and we used atrial fibrillation as drug indication together with sex in the adjusted analysis of thromboembolic events. We used the Schoenfeld residuals to test the proportional hazards assumption.[36] In all analyses the follow-up time was restricted to 730 days.

We used the χ^2 test to check if the allele frequencies of variants were in Hardy–Weinberg equilibrium. We conducted all analyses with RStudio, Boston, MA, version 1.1.456 using R version 3.5.1 (July 2, 2018). Conclusions of statistical significance were based on two-sided tests with a significance level of 0.05.

Ethical aspects

This study was conducted in accordance with the Declaration of Helsinki. The study was approved by the ethics committee of the Hospital District of Helsinki and Uusimaa (HUS/513/2019) and carried out under contracts with three biobanks: Auria Biobank (study number: AB19-9833), Helsinki Biobank (study number: HBP20190038), and THL Biobank (study number: BB2019_6). After data linkage carried out by the biobanks, all data were pseudonymized. The informed consents of patients were obtained through biobanks as defined in the Finnish Biobank Act 2013 (688/2012).[37]

RESULTS

The full PreMed cohort consists of 7,005 patients. Of those, 25.8% (n = 1,806) fulfilled the eligibility criteria for the current study by having made at least one DOAC purchase (**Figure 1**). The number of study patients was highest with rivaroxaban, and atrial fibrillation was the most prevalent indication for an oral anticoagulant treatment. **Table 1** shows key characteristics of the study patients. More complete baseline data stratified by genetic variants are given in **Table S3**.

A summary of the outcome events is presented in **Table S4**. Hematuria (R31) and cerebral infarction (I63.1, I63.3, I63.4, and I63.9) were the most common bleeding and thromboembolic outcome events, respectively. The median follow-up times were 217, 128, and 290 days for the occurrence of bleeding events of dabigatran, rivaroxaban, and apixaban users, respectively. Correspondingly, the median follow-up times for thromboembolic events were 210, 128, and 298 days. During the follow-up time the incidence of bleeding events in patients treated with dabigatran, rivaroxaban, and apixaban was 4.4 (95% CI, 2.6–7.0), 5.9 (95% CI, 4.4–7.7), and 4.7 (95% CI, 3.1–6.7) per 100 patient-years, respectively. Correspondingly, the incidence of thromboembolic events for the three drugs was 2.4 (95% CI, 1.1–4.4), 3.9 (95% CI, 2.7–5.3), and 3.3 (95% CI, 2.0–5.0) per 100 patient-years.

The results of comparing noncarriers with carriers of the SNVs are shown in **Table 2** and **Figure 2**. The *ABCB1* c.3435C>T SNV was associated with a reduced risk for thromboembolic events in rivaroxaban users (HR 0.42, 95% CI, 0.18–0.98, P = 0.044). A similar trend was seen for the *ABCB1* c.2677G>T SNV (HR 0.50, 95% CI, 0.23–1.08, P = 0.077). The *ABCB1* c.2482-2236G>A SNV showed an association with a reduced risk for bleeding events (HR 0.37, 95% CI, 0.16–0.89, P = 0.025) in apixaban-treated patients.

Results of the haplotype-based analysis are shown in **Table 3** and **Figure 3**. The 1236T-2677T-3435T haplotype was associated with a reduced risk of thromboembolic events, while the 1236C-2677G-3435C and 1236T-2677G-3435C haplotypes showed associations with an increased risk of thromboembolic events in rivaroxaban users. The HRs were 0.44 (95% CI, 0.20–0.95, P = 0.036), 2.55 (95% CI, 1.03–6.36, P = 0.044), and 5.88 (95% CI, 2.35–14.72, P < 0.001), respectively, for the TTT, CGC, and TGC haplotypes. Statistically significant associations were not found for the investigated haplotypes in dabigatran or apixaban users.

When repeating the analyses using the recessive genetic model, the association of *ABCB1* c.3435C>T SNV with thromboembolic events was still found (HR 0.34, 95% CI, 0.12–1.00, P = 0.05). The results for *ABCB1* c.2677G>T and c.2482-2236G>A SNVs

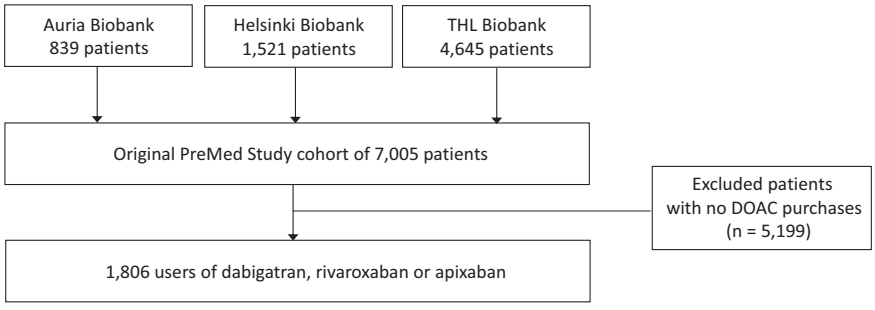

Figure 1 Cohort formation. DOAC, direct oral anticoagulant; HR, hazard ratio; THL, Finnish Institute for Health and Welfare.

Table 1 Characteristics of the study patients

	Dabigatran	Rivaroxaban	Apixaban
Number of patients	340	999	467
Sex, female, *n* (%)	162 (47.6)	498 (49.8)	239 (51.2)
Age, years, mean (SD)	69.8 (8.7)	69.6 (9.8)	72.3 (9.5)
Exposure time, days, median (IQR)	217 (60–671)	137 (57–391)	300 (202–561)
Indication for antithrombotic drug use			
Atrial fibrillation, *n* (%)	268 (78.8)	537 (53.8)	337 (72.2)
Vascular disease, *n* (%)	32 (9.4)	230 (23.0)	44 (9.4)
Pulmonary embolism, *n* (%)	10 (2.9)	66 (6.6)	34 (7.3)
Stroke, cerebral infarction, atherosclerosis, *n* (%)	16 (4.7)	44 (4.4)	27 (5.8)
Venous thrombosis, *n* (%)	14 (4.1)	122 (12.2)	25 (5.4)

IQR, interquartile range; SD, standard deviation.

as well as for 1236T-2677T-3435T and 1236C-2677G-3435C haplotypes showed a consistent trend but were not statistically significant when analyzed using the recessive genetic model. The 1236T-2677G-3435C haplotype could not be analyzed using the recessive model due to the low number of homozygote carriers.

Results on the univariate analyses for potential confounders are listed in **Table S5**. Concomitant use of acetylsalicylic acid with a DOAC was associated with an increased risk for bleeding when all three DOAC drugs were analyzed together (HR 2.27, 95% CI, 1.25–4.14, $P = 0.007$). The results show a similar trend for antiplatelets in general (HR 1.65, 95% CI, 0.92–2.96, $P = 0.091$). The analysis covering all potentially interacting drugs did not show statistically significant association with bleeding events. We also did not find association of concomitant use of statins with bleeding events.

We were not able to analyze the effect of interacting medication on the risk for thromboembolic events due to the low number of users of

Table 2 Results of SNV association analysis

SNV	Number of patients (noncarrier / heterozygote/homozygote)	Bleeding event risk hazard ratio (95% CI), *P* value	Thromboembolic event risk hazard ratio (95% CI), *P* value
Dabigatran			
ABCB1 c.3435C>T	64/148/128	1.08 (0.24–4.87), $P = 0.923$	1.16 (0.14–9.70), $P = 0.888$
ABCB1 c.2677G>T	91/172/77	2.00 (0.44–9.02), $P = 0.368$	0.88 (0.17–4.56), $P = 0.883$
ABCB1 c.2677G>A	317/23/0	n/a	2.17 (0.26–18.10), $P = 0.473$
ABCB1 c.1236C>T	93/170/77	1.27 (0.35–4.61), $P = 0.719$	0.95 (0.18–4.87), $P = 0.947$
ABCB1 c.2482-2236G>A	83/170/87	1.83 (0.41–8.25), $P = 0.432$	0.83 (0.16–4.26), $P = 0.820$
CES1 c.1168-33C>T	229/101/10	0.77 (0.24–2.51), $P= 0.665$	1.31 (0.29–5.89), $P = 0.721$
CES1 c.257+885T>C	168/150/22	0.49 (0.16–1.52), $P = 0.219$	1.09 (0.24–4.88), $P = 0.913$
Rivaroxaban			
ABCB1 c.3435C>T	170/492/337	0.84 (0.37–1.91), $P = 0.682$	0.42 (0.18–0.98), $P = 0.044$
ABCB1 c.2677G>T	282/495/222	0.88 (0.44–1.78), $P = 0.727$	0.50 (0.23–1.08), $P = 0.077$
ABCB1 c.2677G>A	928/69/2	1.06 (0.33–3.43), $P = 0.926$	n/a
ABCB1 c.1236C>T	274/509/216	1.03 (0.50–2.12), $P = 0.929$	0.82 (0.36–1.89), $P = 0.648$
Apixaban			
ABCG2 c.421C>A	400/65/2	2.30 (0.89–5.95), $P = 0.085$	0.79 (0.18–3.51), $P = 0.755$
ABCB1 c.345C>T	75/245/147	0.81 (0.27–2.40), $P = 0.699$	2.69 (0.35–20.48), $P = 0.339$
ABCB1 c.2677G>T	118/256/93	1.13 (0.41–3.08), $P = 0.815$	1.48 (0.42–5.25), $P = 0.545$
ABCB1 c.2677G>A	440/27/0	n/a	1.28 (0.17–9.79), $P = 0.810$
ABCB1 c.2482-2236G>A	107/257/103	0.37 (0.16–0.89), $P = 0.025$	0.58 (0.20–1.69), $P = 0.314$
ABCB1 c.1236C>T	113/256/98	1.46 (0.49–4.35), $P = 0.494$	1.44 (0.41–5.13), $P = 0.571$
CYP3A5 g.6981A>G[a]	7/54/406	1.49 (0.35–6.41), $P = 0.591$	0.63 (0.18–2.23), $P = 0.471$

Noncarriers (individuals with two reference alleles) were compared with carriers (heterozygous or homozygous individuals) for the variant allele.
CI, confidence interval; n/a, not available; SNV, single nucleotide variant.
[a]Patients with zero or one variant allele compared with homozygotes to enable sufficiently large patient groups.

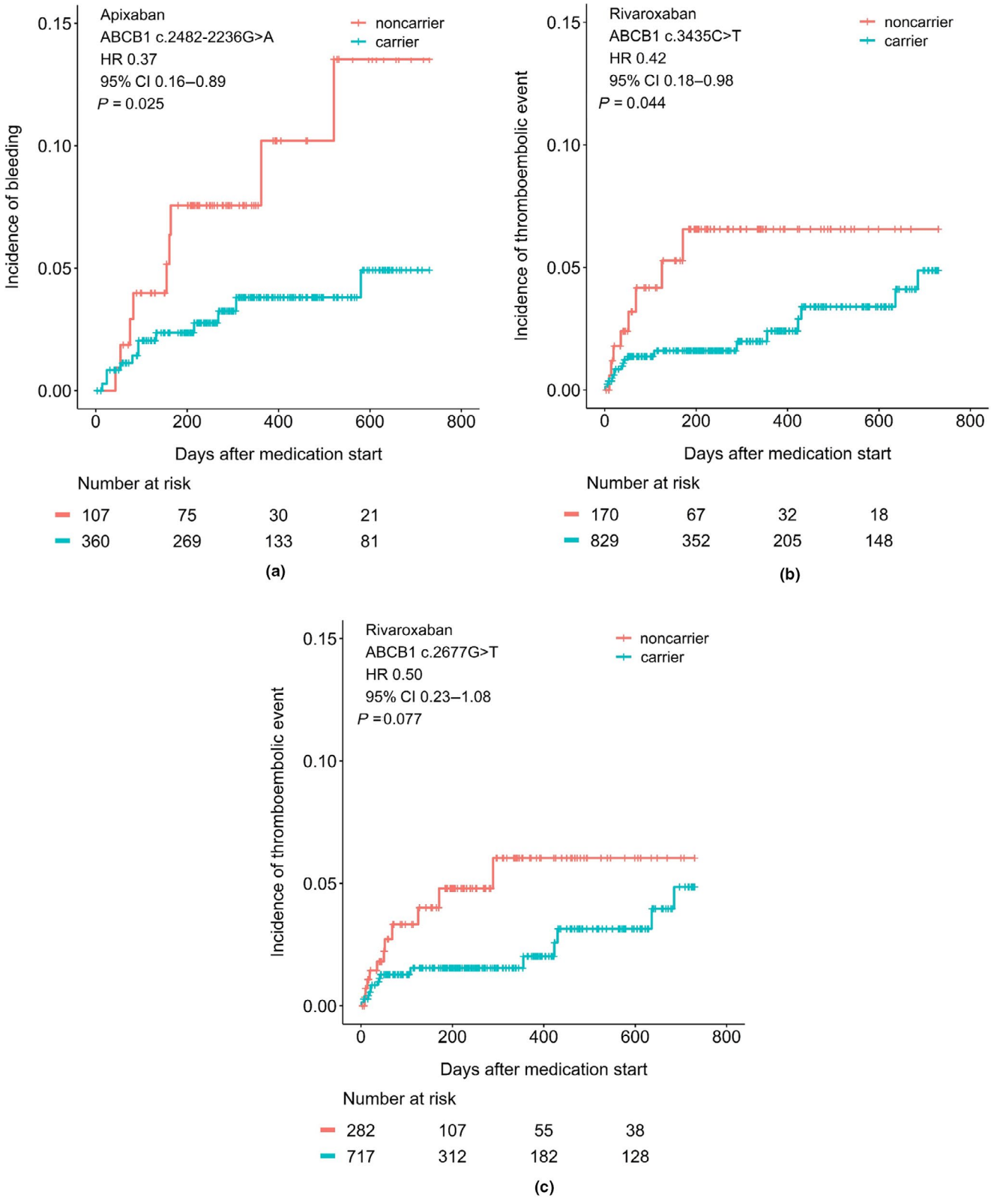

Figure 2 Incidence of bleeding and thromboembolic events for SNVs (**a**) ABCB1 c.2482-2236G>A, (**b**) ABCB1 c.3435C>T, and (**c**) ABCB1 c.2677G>T. CI, confidence interval; HR, hazard ratio; SNVs, single-nucleotide variants. [Colour figure can be viewed at wileyonlinelibrary.com]

the potentially interacting drugs. Instead, we found that female sex was associated with a decreased risk of thromboembolic events when all three drugs were analyzed together. Furthermore, patients on a DOAC and with atrial fibrillation as the drug indication were found to be at a lower risk for thromboembolic outcomes as compared with patients with other indications (HR 0.53, 95% CI, 0.29–0.96, $P = 0.035$).

Table 3 Results of haplotype-based association analysis

ABCB1 haplotype	Number of patients (noncarrier/ heterozygote/homozygote)	Bleeding event risk hazard ratio (95% CI), *P* value	Thromboembolic event risk hazard ratio (95% CI), *P* value
Dabigatran			
1236T-2677T-3435T	108/167/65	1.58 (0.44–5.76), $P = 0.484$	1.18 (0.23–6.08), $P = 0.844$
1236C-2677G-3435C	155/143/42	2.02 (0.62–6.55), $P = 0.244$	2.23 (0.43–11.50), $P = 0.338$
1236C-2677G-3435T	262/73/5	0.51 (0.11–2.33), $P = 0.388$	0.49 (0.06–4.06), $P = 0.507$
1236T-2677G-3435C	319/21/0	1.15 (0.15–8.83), $P = 0.896$	n/a
Rivaroxaban			
1236T-2677T-3435T	318/500/181	1.09 (0.54–2.19), $P = 0.808$	0.44 (0.20–0.95), $P = 0.036$
1236C-2677G-3435C	431/445/123	1.37 (0.71–2.63), $P = 0.347$	2.55 (1.03–6.36), $P = 0.044$
1236C-2677G-3435T	778/207/14	1.07 (0.52–2.20), $P = 0.847$	0.78 (0.29–2.06), $P = 0.611$
1236T-2677G-3435C	941/56/2	0.51 (0.07–3.73), $P = 0.508$	5.88 (2.35–14.72), $P < 0.001$
Apixaban			
1236T-2677T-3435T	142/247/78	1.50 (0.55–4.10), $P = 0.428$	1.99 (0.56–7.07), $P = 0.287$
1236C-2677G-3435C	194/224/49	1.08 (0.45–2.60), $P = 0.866$	0.75 (0.27–2.06), $P = 0.570$
1236C-2677G-3435T	367/94/6	0.36 (0.08–1.54), $P = 0.169$	0.81 (0.23–2.88), $P = 0.745$
1236T-2677G-3435C	431/36/0	0.69 (0.09–5.13), $P = 0.714$	1.06 (0.14–8.13), $P = 0.952$

Noncarriers (individuals without the haplotype) were compared with carriers (heterozygous or homozygous individuals) for the haplotype.
CI, confidence interval; n/a, not available.

The other investigated covariates—age, cancer, renal function, and reduced dose—did not show statistically significant association with either of the outcome events. A considerable risk of thromboembolic events in dabigatran users with reduced dose was seen; however, the result should be interpreted with caution due to the small total number of thromboembolic outcome events (seven events) in the dabigatran user group. For the analysis on kidney function, 73% of patients (those with a creatinine laboratory test available) were included.

Based on the results of the univariate analyses on potential confounders, we selected the use of antiplatelet drugs to be adjusted in the analyses on bleeding events, and atrial fibrillation as drug indication and sex to be adjusted in the analyses on thromboembolic events. The results of adjusted SNV and haplotype analysis are presented in **Tables S6** and **S7**. The adjusted results were comparable with the crude estimates presented in **Tables 2** and **3**. Reduced risk for thromboembolic events in rivaroxaban users was also seen in the adjusted analyses for ABCB1 c.3435C>T (HR 0.39, 95% CI, 0.17–0.91, $P = 0.028$), and a similar trend for c.2677G>T (HR 0.48, 95% CI, 0.22–1.04, $P = 0.062$) SNVs as well as for 1236T–2677T–3435T (HR 0.42, 95% CI, 0.20–0.92, $P = 0.029$), 1236C–2677G–3435C (HR 2.68, 95% CI, 1.07–6.68, $P = 0.035$), and 1236T–2677G–3435C (HR 5.80, 95% CI, 2.31–14.58, $P < 0.001$) haplotypes.

DISCUSSION

Association between genetic variants and clinical outcomes of DOAC users have earlier been investigated only in a few studies carried out in prospective trial settings. For our study, we adopted the real-world data approach, which enabled us to use a longer follow-up time than has been the case in the earlier studies.[38,39]

We found the *ABCB1* c.3435C>T SNV and 1236T-2677T-3435T haplotype to be associated with a lower risk and 1236C-2677G-3435C and 1236T-2677G-3435C haplotypes to be associated with a higher risk for thromboembolic outcomes in rivaroxaban users. The *ABCB1* c.3435C>T SNV has previously been identified as potentially affecting rivaroxaban and dabigatran pharmacokinetics by modulating the P-glycoprotein activity and thereby leading to increased plasma drug levels.[5,12,22] Our results on the thromboembolic outcomes are in line with this anticipated effect, although we could not see a corresponding association with bleeding risk.

We also found an association of the *ABCB1* c.2482-2236G>A SNV with a decreased bleeding risk in apixaban users. This variant has been found to be associated with bleeding in one earlier study,[10] while other studies have not been able to confirm the association.[19,34]

So far, the most extensive investigation of the pharmacogenomics of DOACs has been the genome-wide association study of Paré *et al*. focusing on dabigatran users.[13] The study found association of the *CES1* rs2244613 SNV with a decreased risk of bleeding. We could not confirm this association with bleeding in dabigatran patients.

Among the other variants investigated in our study, *ABCG2* c.421C>A and *CES1* c.257+885T>C suggested possible association with bleeding events in apixaban and dabigatran users, respectively, although statistical significance was not reached. Our results on the *ABCG2* c.421C>A SNV are in line with the results of earlier prospective studies on plasma concentration and drug clearance[11,15,40] as well as with a case report.[41] Our results on *CES1* c.257+885T>C SNV are in line with studies reporting association of the variant with drug plasma concentration.[13,16,42]

The present study revealed once again the important role of antiplatelets as interacting drugs. Antiplatelets are in many cases used in parallel with a DOAC to increase protection towards thromboembolic events. As shown in this study and others, concomitant use of antiplatelets and a DOAC increases the risk of bleeding.[43–45] One strength of the present study is that we were

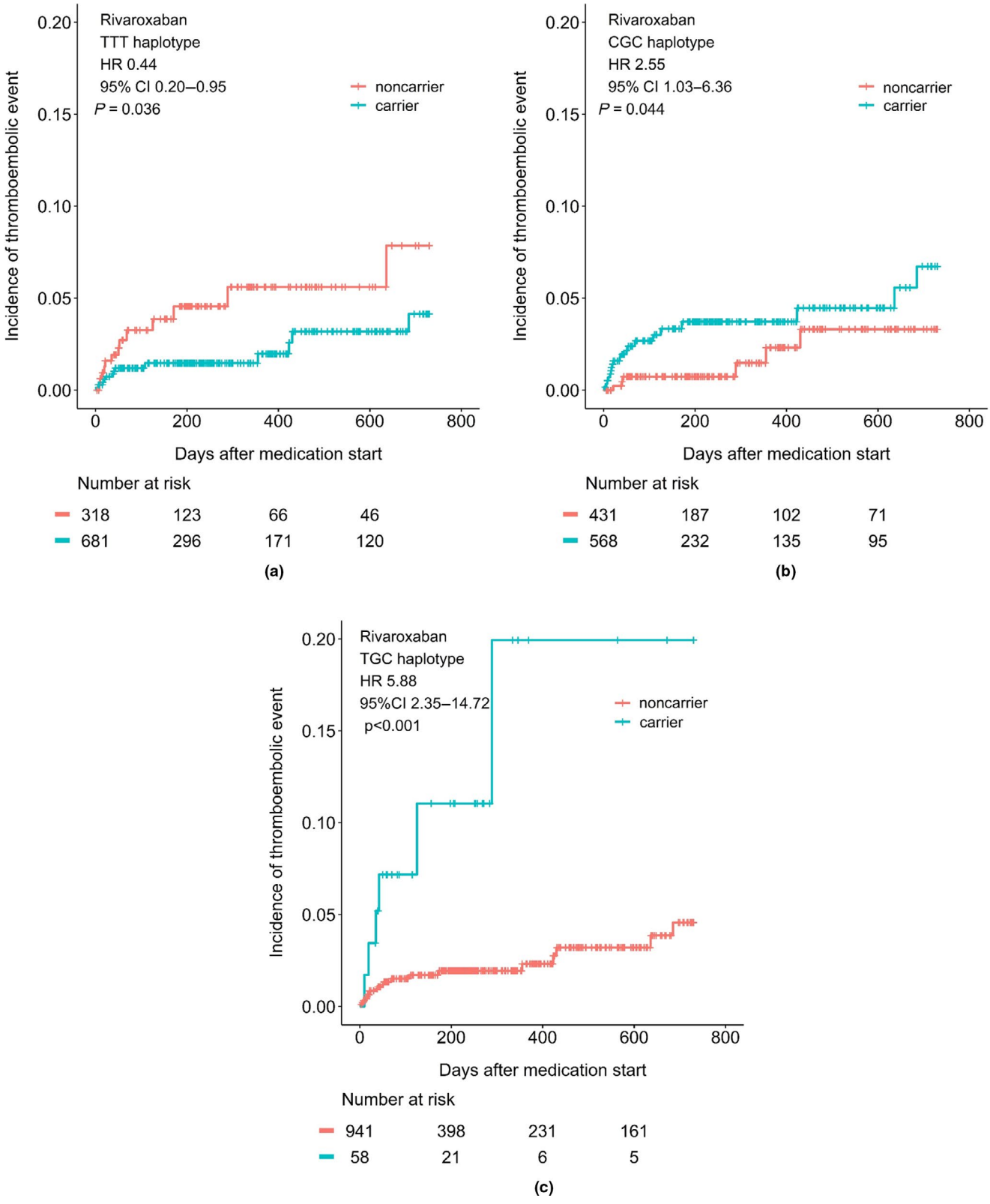

Figure 3 Incidence of bleeding and thromboembolic events for haplotypes (**a**) ABCB1 1236T-2677T-3435T, (**b**) 1236C-2677G-3435C, and (**c**) 1236T-2677G-3435C. CI, confidence interval; HR, hazard ratio. [Colour figure can be viewed at wileyonlinelibrary.com]

able to access information on acetylsalicylic acid usage via the data obtained from patient records.[33] Sex was the most significant confounder for thromboembolic events, which is in line with current knowledge. Sex differences have been reported in platelet function and coagulation factor activities as well as in the rate of cardiovascular events.[46]

The main limitation of the study is the relatively small number of participants. As DOACs are relatively new drugs, the accumulated clinical data of the drug users are still limited and, furthermore, only individuals with already existing genotype data in the biobanks could be included in the study. The small number of subjects guided us to study patients as a single group regardless of the indication for use of DOAC. The analysis of confounders revealed that the risk to atrial fibrillation patients for thromboembolic events was lower, and therefore we controlled atrial fibrillation as the drug indication in the adjusted results. The number of patients enabled us to detect only major differences in the clinical outcomes of the genotype or haplotype groups. Furthermore, due to the limited sample size, we may have missed finding the effect of some of the potential confounders. For example, the results reflected the anticipated effect of the renal function,[47] but statistical significance was not achieved. Furthermore, we could not apply stratified analysis by dividing the bleeding events into minor and major bleedings as has been done in larger studies with warfarin users.[48]

Another potential limitation is the risk of inaccuracy due to missing data. In a real-world setting the completeness of data cannot be fully guaranteed, although the Finnish registries have proven to be of high quality, especially in the registration of cardiovascular diagnoses.[49]

CONCLUSIONS

In our real-world study, we investigated *ABCB1*, *CES1*, and *ABCG2* variants potentially associated with the risk of bleeding or thromboembolic events in DOAC users. We found *ABCB1* c.3435C>T SNV and 1236T-2677T-3435T haplotype to be associated with a lower risk and the 1236C-2677G-3435C and 1236T-2677G-3435C haplotypes to be associated with a higher risk for thromboembolic outcomes in rivaroxaban users. We also found an association of the *ABCB1* c.2482-2236G>A SNV with a decreased bleeding risk in apixaban users. This is one of the first real-world studies evaluating potential associations of genetic variants with clinical end points in patients on DOACs. Further studies with larger sample sizes are warranted to verify the findings.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

M.L. is a consultant for Bristol Myers Squibb–Pfizer-alliance, Bayer, Boehringer-Ingelheim, and Merck Sharp & Dohme; and a speaker for Bristol Myers Squibb–Pfizer-alliance, Bayer, Boehringer-Ingelheim, Merck Sharp & Dohme, Terve Media, and Orion Pharma. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

J.L. and A.-L.V. wrote the manuscript. J.L., A.-L.V., J.P., K.H., M.L., M.N., and M.v.G. designed the research. J.L., A.-L.V., and J.P. performed the research. J.L., A.-L.V., and J.P. analyzed the data.

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