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SHORT COMMUNICATION

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*CYP3A4*22* may increase bleeding risk in ticagrelor users

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1 | INTRODUCTION AND BACKGROUND

The conventional treatment for acute coronary syndromes (ACS) includes percutaneous coronary intervention with stent placement requiring dual antiplatelet therapy with acetylsalicylic acid (ASA) and an antiplatelet drug (clopidogrel, prasugrel or ticagrelor). Ticagrelor outweighs some of the limitations of clopidogrel and prasugrel: It does not need metabolic activation and binds rapidly and reversibly to the $P2Y_{12}$ receptors.[1] Compared with clopidogrel, ticagrelor has greater and more consistent inhibition of platelet aggregation, and its effect does not appear to be influenced by single-nucleotide variants (SNV) in *CYP2C19* or *ABCB1*.[1]

Ticagrelor is primarily metabolized in the liver by *CYP3A4* and *CYP3A5* enzymes to its active and approximately equipotent metabolite AR-C124910XX,[1] suggesting that genetic variation in these enzymes may affect ticagrelor metabolism. In patients with ACS, *SLCO1B1* (rs113681054), *CYP3A4* (rs62471956, rs56324128) and *UGT2B7* (rs61361928) variants were associated with

ticagrelor pharmacokinetics but not with clinical outcomes.[2] Results on effects of *CYP4F2* rs3093135 variant are contradicting.[3,4] There is no established explanation on how *CYP4F2* rs3093135 variant might affect platelet function during antiplatelet therapy.[3] *CYP4F2* enzyme is involved in synthesis of 20-hydroxyeicosatetraenoic acid which may have an antiplatelet effect.[3] It also participates in metabolism of vitamin K and might, thus, affect synthesis of vitamin K dependent coagulation factors.[5] In healthy Chinese males, *SLCO1B1* (rs113681054, rs4149056), *CYP3A4*1G* (rs2242480) or *CYP3A5*3* (rs776746) variants did not affect ticagrelor pharmacokinetics or pharmacodynamics.[6] Another study in healthy volunteers found that *CYP3A4*22* (rs35599367) carriers had elevated plasma concentrations of ticagrelor and AR-C124910XX as well as a more pronounced platelet inhibition than non-carriers, whereas the *CYP3A5*3* (rs776746) allele was not associated with ticagrelor pharmacokinetics.[7]

This study utilized Finnish biobanks and national health registries to investigate the association of *CYP3A4*22* (rs35599367), *CYP3A5*3* (rs776746) and *CYP4F2* rs3093135 variants with incidence of bleeding events in ticagrelor users.

2 | MATERIALS AND METHODS

2.1 | Study design

This study was part of the Pharmacogenomics of Antithrombotic Drugs (PreMed) study, which was a retrospective cohort study linking data from national health registries and three Finnish biobanks (Auria Biobank, Helsinki Biobank and the Finnish Institute for Health and Welfare [THL] Biobank).[8] The biobanks formed a study cohort by merging (1) their genomic and demographic data with (2) healthcare encounter data from THL, (3) drug dispensation data from the Social Insurance Institution of Finland and (4) patient record and laboratory data from the Finnish hospital districts and municipalities. The data were retrieved from January 1 2007 to June 30 2018, and the subjects were followed until December 31 2018.

Inclusion criteria were as follows: (1) ≥18 years of age at the time of the first ticagrelor purchase; (2) *CYP3A4*22* (rs35599367), *CYP3A5*3* (rs776746) and *CYP4F2* (rs3093135) genotype information available; (3) a diagnosis of an ischemic heart disease (Table 1); (4) at least one ticagrelor purchase during the study period. To ensure a 2-year washout period, we excluded subjects with antithrombotic drug purchases between January 1 2005 and December 31 2006. Genotyping and determination of sample size are described in Appendix S1.

TABLE 1 Inclusion and outcome diagnoses.

Diagnosis	ICD-10
Inclusion diagnoses	
Ischemic heart diseases	I20-I25
Outcome diagnoses	
Bleeding events	D50.0, D62, D68.3, I60-I62, I69.0-I69.2, I85.0, J94.2, K22.1, K22.3, K22.6, K25.0, K25.2, K25.4, K25.6, K26.0, K26.2, K26.4, K26.6, K27.0, K27.2, K27.4, K27.6, K28.0, K28.2, K28.4, K28.6, K29.0, K62.5, K63.1, K63.3, K92.0-K92.2, N02, R04, R31, R58, S06.2-S06.6, S06.8

Abbreviation: ICD-10, International Classification of Diseases 10th Revision.

2.2 | Ticagrelor exposure

We considered ticagrelor exposure to have started on the date of the first ticagrelor dispensation and deemed it as continuous until the earliest of the following: (1) all dispensed packages were consumed and no new package was dispensed within 30 days, (2) clopidogrel was purchased (none had prasugrel purchases) and (3) the study follow-up ended.

2.3 | Outcome and covariates

The primary outcome was the occurrence of a bleeding event. The bleeding event was eligible if it occurred during the ticagrelor exposure to patients with diagnosis codes in Table 1. Major bleeding was defined according to the criteria of the International Society on Thrombosis and Haemostasis: Haemoglobin level reduced more than 20 g/L or haemorrhage into a critical anatomic site. We followed subjects until the occurrence of bleeding or until the end of exposure.

We obtained age and sex from the demographic data provided by the biobanks. Kidney function was evaluated by computing glomerular filtration rate from the plasma creatinine value. Use of ASA was extracted from patient records because ASA, as an over-the-counter drug, is not recorded in the drug purchase registry.

2.4 | Statistical analyses

We compared the groups with and without bleeding events using Mann–Whitney *U* test for continuous variables and Fisher's exact test for categorical variables. To enable reasonably sized groups, we combined

heterozygotes and homozygotes for *CYP3A4*22* and *CYP4F2*. For *CYP3A5*3*, we combined non-carriers and heterozygotes, that is, *CYP3A5* expressors. The occurrence of bleeding events is presented as incidence rates per 100 patient-years. We studied the association between the SNVs and occurrence of bleeding events using Cox proportional hazards model. We performed three analyses: unadjusted, adjusted for age and sex and adjusted for age, sex and use of ASA. Missing values were not imputed. We set the significance level to 0.05. The analyses were conducted in Spyder v4.2.1 using Python v3.9.2 and packages lifelines v0.25.9 and SciPy v1.6.0 (the analysis scripts available in Appendix S2 in the supporting information).

2.5 | Ethical aspects

The study was conducted in accordance with the Declaration of Helsinki, approved by the ethics committee of the Hospital District of Helsinki and Uusimaa (HUS/513/2019) and conducted under the contracts with the biobanks. All data were pseudonymized after data linkage in the biobanks. Informed consents were obtained through the biobanks.

3 | RESULTS

The full PreMed cohort comprised 7005 patients, of whom 368 (5.3%) fulfilled the eligibility criteria for the current study. Table 2 presents characteristics of the study population. During the median follow-up time of 366 (interquartile range: 204–394) days, 22 bleeding events occurred, corresponding to 7.2 events per 100 patient-years. Patients with bleeding events were more often *CYP3A4*22* carriers and had a shorter follow-up time. Most of the bleeding events were either gastrointestinal or epistaxis, and six major bleeding events occurred (Table 3).

Table 4 and Figure 1 present the association of SNVs with the bleeding events. Carriers of *CYP3A4*22* had increased risk of bleeding compared with *CYP3A4*22* non-carriers (hazard ratio 3.74, 95% confidence interval 1.26–11.05). The results were comparable after adjusting for the covariates. The *CYP3A4*22* carriers with bleeding

TABLE 2 Characteristics of the study population.

	All subjects	No bleeding	Bleeding	*p*-value
Number of subjects	368	346	22	
Sex, male, *n* (%)	280 (76.1)	263 (76.0)	17 (77.3)	>0.999
Age, median (IQR), a	68 (60–73)	68 (60–73)	73 (65–78)	0.074
*CYP3A4*22*				0.034[a]
Non-carrier, *n* (%)	346 (94.0)	328 (94.8)	18 (81.8)	
Heterozygote, *n* (%)	22 (6.0)	18 (5.2)	4 (18.2)	
Homozygote, *n* (%)	0 (0)	0 (0.0)	0 (0.0)	
*CYP3A5*3*				0.552[b]
Non-carrier, *n* (%)	2 (0.5)	2 (0.6)	0 (0.0)	
Heterozygote, *n* (%)	53 (14.4)	51 (14.7)	2 (9.1)	
Homozygote, *n* (%)	313 (85.1)	293 (84.7)	20 (90.9)	
CYP4F2 rs3093135				0.284[a]
Non-carrier, *n* (%)	288 (78.3)	273 (78.9)	15 (68.2)	
Heterozygote, *n* (%)	75 (20.4)	69 (19.9)	6 (27.3)	
Homozygote, *n* (%)	5 (1.4)	4 (1.2)	1 (4.5)	
GFR at the initiation of ticagrelor, median (IQR), mL/min	88 (79–95) missing = 36	88 (80–95) missing = 35	86 (73–92) missing = 1	0.194
Ticagrelor exposure time, median (IQR), d	366 (226–394)	366 (226–394)	291 (212–387)	0.101
Follow-up time, median (IQR), d	366 (204–394)	366 (226–394)	102 (72–233)	<0.001
Use of ASA, *n* (%)	225 (61.1)	214 (61.8)	11 (50.0%)	0.270

Abbreviations: ASA, acetylsalicylic acid; GFR, glomerular filtration rate; IQR, interquartile range.

[a]Heterozygotes and homozygotes combined.

[b]Non-carriers and heterozygotes combined.

TABLE 3 Bleeding locations.

	All bleeding events		Minor bleeding events		Major bleeding events[a]	
Location	**ICD-10**	***N* (%)**	**ICD-10**	***N* (%)**	**ICD-10**	***N* (%)**
Other[b]	D50.0, D62, R04.0, R58	11 (50.0)	D50.0, D62, R04.0, R58	10 (62.5)	D50.0	1 (16.7)
Gastrointestinal	K92.0, K92.1, K62.5	7 (31.8)	K92.1, K62.5	3 (18.8)	K92.0, K92.1	4 (66.7)
Urogenital	R31	3 (13.6)	R31	3 (18.8)		
Intracranial	S06.4	1 (4.5)			S06.4	1 (16.7)

Abbreviation: ICD-10, International Classification of Diseases 10th Revision.
[a]Haemoglobin level reduced more than 20 g/L or haemorrhage into a critical anatomic site (criteria of the International Society on Thrombosis and Haemostasis).
[b]Other mainly included epistaxis ($n = 7$).

TABLE 4 Associations between single-nucleotide variants and bleeding events in ticagrelor users.

	Model 1: unadjusted		Model 2: adjusted for age and sex		Model 3: adjusted for age, sex and use of ASA	
Variant	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
*CYP3A4*22*[a]	3.74 (1.26–11.05)	0.017	3.67 (1.21–11.08)	0.021	3.80 (1.26–11.43)	0.017
*CYP3A5*3*[b]	1.82 (0.42–7.77)	0.421	1.79 (0.42–7.72)	0.433	1.75 (0.41–7.59)	0.452
CYP4F2 rs3093135[a]	1.57 (0.64–3.84)	0.327	1.55 (0.63–3.79)	0.342	1.60 (0.65–3.93)	0.307

Abbreviations: ASA, acetylsalicylic acid; CI, confidence interval; HR, hazard ratio.
[a]Heterozygotes and homozygotes combined.
[b]Non-carriers and heterozygotes combined.

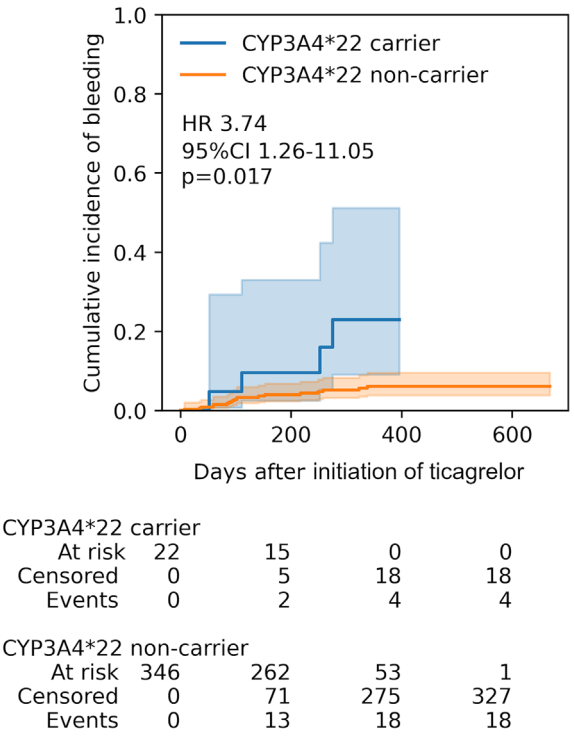

FIGURE 1 Cumulative incidence of bleeding events in *CYP3A4*22* carriers and non-carriers. The shaded area shows 95% confidence intervals. CI, confidence interval; HR, hazard ratio; censored, number of subjects whose follow-up ended without a bleeding event.

events had epidural haemorrhage, hematemesis, melena or haematuria. No statistically significant association was found between bleeding events and *CYP3A5*3* or *CYP4F2* rs3093135 carrier status. Minor allele frequencies of the SNVs are reported in the supporting information Table S1 (Appendix S1).

4 | DISCUSSION

To our knowledge, this is one of the first studies reporting that *CYP3A4*22* variant was associated with the increased risk of bleeding events in ticagrelor users. Neither *CYP3A5*3* nor *CYP4F2* rs3093135 carrier status was associated with the occurrence of bleeding events.

The *CYP3A4*22* allele has been reported to impair elimination of ticagrelor, resulting in greater ticagrelor exposure and enhanced antiplatelet effect, which might increase the risk of adverse events, like bleeding.[7] Our study supports this suggestion. However, another study found no association between *CYP3A4*22* carrier status and bleeding events in patients with ST-segment elevation myocardial infarction.[9] Our results on *CYP3A5*3* are in line with other studies; they found no association between *CYP3A5*3* variants and ticagrelor pharmacokinetics or pharmacodynamics or clinical outcomes.[6,7,9]

Studies regarding *CYP4F2* variants have provided contradicting results. One study reported carriers of *CYP4F2* rs3093135A > T variant to have lower platelet aggregation values,[4] while a later study from the same researchers did not confirm the result.[3] Instead, the T allele was found to increase odds for bleeding events. It seems that the population of the first study might be a subset of the population of the later study because both studies were conducted in the same hospital at the same time.[3,4] In contrast, we found no association between the *CYP4F2* variant and bleeding events. Different research methodologies may explain the controversy: a prospective study *versus* a register-based study, patient-reported bleeds *versus* ICD-10 codes, follow-up time (6 months *versus* median of 1 year), age of the patients (63 *versus* 68 years) and logistic regression, which is utilized for binary outcomes *versus* Cox proportional hazards model, which also takes time to event into account.

Main limitation of our study is the small sample size. Because ticagrelor is a fairly new medication, the amount of data in the registries is still limited. Furthermore, ticagrelor exposure was defined using the drug dispensation data, and we do not know if the patients used ticagrelor as prescribed. Additionally, Finnish registries have shown good quality,[10] but unprescribed pharmacy data may be missing. Especially, proportion of patients using ASA is suspiciously low (61%) as typically ticagrelor is prescribed with ASA. Use of ASA is not systematically recorded in the patient records; it is based on information given by a patient and entered by a physician into the records. Finally, the *SLCO1B1* (rs113681054), *CYP3A4* (rs62471956, rs56324128) and *UGT2B7* (rs61361928) variants were not available in the PreMed cohort and therefore not analysed. As this was an explorative study, we did not perform correction for multiple testing. Thus, larger studies are warranted to confirm that the results on *CYP3A4*22* are not caused by a chance.

In conclusion, this real-world register-based cohort study indicated that *CYP3A4*22* variant was associated with increased risk of bleeding events in ticagrelor users. Confirmatory studies with larger populations are needed to verify this exploratory finding and its clinical relevance.

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CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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