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ARTICLE

# *CYP2C19* loss-of-function alleles and use of omeprazole or esomeprazole increase the risk of cardiovascular outcomes in patients using clopidogrel

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**Abstract**

Our aim was to investigate in a real-life prospective patient cohort how *CYP2C19* loss-of-function (LOF) variants and CYP2C19 inhibitor omeprazole or esomeprazole influence the incidence of cardiovascular events in patients using clopidogrel. Data based simultaneously on these factors are conflicting and sparse. A cohort of prospective patients ($n=1972$) with acute coronary syndrome ($n=1302$) or symptomatic chronic coronary disease ($n=656$) was followed for 365 days after hospitalization with information on purchased prescription drugs, hospital discharge, death, and genotype for *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*8* LOF variants. The primary study outcome measurement was cardiovascular death or recurring myocardial infarction or stroke. Altogether, 608 patients (30.8%) carried *CYP2C19* LOF alleles. During the 365-day follow-up 252 patients (12.8%) had an ischemic vascular event. Cardiovascular events were significantly more frequent in carriers of *CYP2C19* LOF alleles (14.8%, 95% confidence interval [CI], 11.7–17.8) than in non-carriers (10.8%, 95% CI, 9.0–12.6, $p=0.0159$). Omeprazole or esomeprazole use was similar among LOF allele carriers ($n=131$, 21.5%) and non-carriers ($n=250$, 18.3%, $p=0.185$). Cardiovascular events were significantly more common in a composite group consisting of all *CYP2C19* LOF carriers regardless of proton pump inhibitor use status and non-carriers using omeprazole or esomeprazole than in non-carriers not using omeprazole or esomeprazole (14.8%, 95% CI, 12.2–17.3 vs. 9.9%, 95% CI, 8.0–11.9, $p=0.00173$). We observed significantly more cardiovascular events in carriers of *CYP2C19* LOF variants and in non-carriers using omeprazole or esomeprazole. For optimal patient care, both genetics and concomitant medication should be considered.

Markus Ramste and Markus Ritvos contributed equally to this work.

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**Study Highlights**

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

*CYP2C19* loss-of-function (LOF) allele carriers using clopidogrel are at increased risk of major adverse cardiac events (MACEs) as well as patients using proton pump inhibitors (PPIs) concomitantly with clopidogrel.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

The aim of this study was to investigate how *CYP2C19* LOF variants and omeprazole or esomeprazole influence the incidence of cardiovascular events in a real-life patient cohort using clopidogrel.

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

The results show phenoconversion where non-carriers of *CYP2C19* LOF alleles are converted into poor or intermediate CYP2C19 metabolizers by the effect of drug interaction between CYP2C19 genotype and PPIs and thus resulting in a modified clinical response and increased MACEs.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**

These results emphasize careful evaluation of the concomitant PPI medication when commencing clopidogrel treatment or especially if using genotype-guided treatment decisions.

## INTRODUCTION

Clopidogrel is an antiplatelet drug used for atherothrombotic event prophylaxis in patients after percutaneous coronary intervention (PCI).[1] When added to aspirin, this dual antiplatelet inhibition effectively reduces the rate of major cardiovascular events. Clopidogrel is still widely and frequently chosen due to its cost-effectiveness.[2,3] It inhibits platelet aggregation via inhibition of the $P2Y_{12}$ receptor expressed on the platelet cell surface. The conversion of clopidogrel into its active form occurs through the hepatic cytochrome P450 system. The efficacy of clopidogrel differs substantially due to interindividual variability, that arises from multiple factors, including drug–drug interactions and loss-of-function (LOF) allelic variants of the *CYP2C19* gene. Therefore, in patients carrying the *CYP2C19* LOF allele, the conversion of clopidogrel to its active metabolite is reduced, resulting in weak or absent inhibition of platelet aggregation and a greater risk of recurrent thromboembolic events.[4]

Previous studies have shown that patients carrying *CYP2C19* LOF alleles have a relative risk increase of 1.53 to 3.69 of major cardiovascular events compared with those not carrying LOF alleles.[5] Clopidogrel nonresponse has been identified in 4% to 34% of patients, depending on the method of testing and the definition of response.[6,7] Approximately 30% of White patients have an inadequate response to clopidogrel, resulting in an increased risk of recurrent major adverse cardiac events (MACEs).[4]

Patients using dual antiplatelet inhibition are prone to gastrointestinal bleedings. Proton pump inhibitors (PPIs) are thus widely prescribed for gastric protection. Certain PPIs inhibit CYP2C19 activity, therefore interacting with clopidogrel metabolism. An earlier study observed that concomitant use of a PPI and clopidogrel compared was associated with a higher rate of MACE than clopidogrel alone.[8] The extent of CYP2C19 inhibition varies between different PPIs, omeprazole and esomeprazole being the most potent and pantoprazole the least potent inhibitor.[9] Data on the effects of PPIs on MACE are, however, conflicting. Several randomized controlled trials (RCTs) have evaluated concomitant omeprazole and clopidogrel use and have not found an increase in MACE rate.[8,10–14] However, these studies have not considered the pharmacogenomics of clopidogrel. By contrast, some RCTs have investigated clopidogrel genetics but have neglected the PPI interaction.[15,16] Only a few studies on this PPI interaction based on *CYP2C19* genetics are published.[17–20] A meta-analysis showed that *CYP2C19* LOF allele noncarriers using PPIs had more MACE, but PPIs did not affect clopidogrel efficacy in *CYP2C19* LOF allele carriers.[21] However, not all relevant *CYP2C19* LOF alleles were measured in these studies.

Although RCTs are the "gold standard" for evaluating the efficacy and safety of possible therapy, there is a clear need for observational studies in a real-world setting, as they provide information of the therapy's efficacy and effectiveness in clinical practice.[22]

In this study, we assessed from a prospective registry the clinical data of patients with acute coronary syndrome (ACS) or symptomatic chronic coronary disease, their cardiovascular events, death rate, and drug

purchases, and evaluated clopidogrel metabolism by *CYP2C19* genotyping. Additionally, we were able to address the importance of phenoconversion: a phenomenon in which non-carriers of *CYP2C19* LOF alleles are converted into poor or intermediate CYP2C19 metabolizers by the effect of drug interaction between CYP2C19 genotype and PPIs, resulting in a modified clinical response. Phenoconversion as a phenomenon, contributes to the poor prognostic value of genotype-focused studies, and it should be considered when interpreting the results.[23,24]

# METHODS

## Study design

The prospective Corogene study register comprises consecutive consenting patients who underwent an angiography ($n = 5294$) at Helsinki University Central Hospital between March 2006 and March 2008. The comprehensive information gathered from all patients consists of a questionnaire incorporating medical history, current condition, cardiovascular risk factors, medications, and electrocardiogram, echocardiography, and coronary angiogram results.[26] Patients were prospectively followed by different Finnish national registries: the actual purchases and users of different medications were verified from the Finnish Prescription Registry maintained by the Social Insurance Institution of Finland, covering all patients' medication purchases between January 1, 2005, and December 31, 2015.[25] The Hospital Discharge Registry of the Finnish Institute for Health and Welfare was used for diagnosis-related hospitalizations, in this case, based on recurring ischemic vascular events and hemorrhages. The validity of the diagnosis codes has been studied previously.[27] Hemorrhages were defined according to Lamberts et al.[28] (Table S1). Causes of death were obtained from Statistics Finland and the follow-up lasted until December 31, 2015, or until the patient's death,[29] whichever occurred first. For the present study, we used only the data of above registries of a period of the first 365 days after the patients signed an informed consent form.

The Corogene registry included a total of 2409 patients using clopidogrel verified by the Finnish Prescription Registry purchases. Of these, 437 were excluded due to unavailable or incomplete genotype data. The genotype data of the 1972 patients and the Corogene and the Hospital Discharge Registry data were then combined for the analysis (Figure S1). Cardiovascular events were defined as myocardial infarction, ischemic stroke, and cardiovascular-related death.

## Statistical analyses

The analysis comprised only purchases made after inclusion in the study, containing all purchases during the follow-up period. The length of treatment was recorded and the quantity of clopidogrel was calculated by multiplying the purchases with the package size. Discontinuation of treatment is defined as a break of longer than 2 weeks. The follow-up was censored to 365 days or the end of clopidogrel treatment, whichever came first.

All statistical analyses were performed using either IBM's SPSS software, versions from 26 to 27 (SPSS) or R software, version 4.0.3. The $p$ values of $< 0.05$ was considered statistically significant using two-tailed tests. Values are shown as percentages, with continuous variables presented as a mean with standard deviation (SD). Normally distributed scale variables (body mass index [BMI], age, and cholesterol levels) were analyzed with the independent $t$-test and one-way analysis of variance (ANOVA). Fisher's exact test was used to determine whether a significant association existed between two categorical variables.

We used Cox proportional hazards models to evaluate the association of risk factors with myocardial infarction, ischemic stroke, and hemorrhage. As risk factor candidates for Cox proportional hazards models, we selected cholesterol levels, BMI, hypertension, diabetes mellitus, age, sex, and smoking. Final risk factors were chosen by the significance level of $p \leq 0.05$ (Wald test) for each factor in different regression models. Hazard ratios (HRs) with 95% confidence intervals (CIs) are shown.

To estimate the survival function between carriers and non-carriers of *CYP2C19* LOF alleles, we used Kaplan–Meier survival estimator and plotted out the curves. To further address the phenoconversion and to investigate the effect of a possible drug-phenotype interaction, we created a composite group consisting of all CYP2C19 LOF carriers and non-carriers using PPIs. The $p$ values are calculated using the log rank test.

## Genotyping

Altogether, 1671 samples at a DNA concentration of 50 ng/μL were genotyped for *CYP2C19*2* (rs4244285), **3* (rs4986893), **8* (rs41291556), and **17* (rs12248560), *CYP2B6* rs3745274 (c.516G>T, p.Q172H), and rs8192719 (c.1294 + 53C>T), *CYP2C9*3* (rs1057910), *CES1* rs71647871 (c.428G>A, p.G143E), and *PEAR1* rs12041331 allelic variants with TaqMan OpenArray system on QuantStudio 12K Flex real-time polymerase chain reaction equipment (ThermoFisher Scientific) following the manufacturer's instructions. Data were analyzed with Taqman Genotyper

software, version 1.5.0 (Applied Biosystems) for genotype clustering and calling. Genotyping was repeated for samples with undetermined results. Altogether 43 samples had at least one undetermined genotype after the second genotyping. For an additional 344 patients, all of the respective genotypes were obtained from FinnGen genotyping data, a large, harmonized genome dataset that is available for research through Finnish biobanks.[30] One or more genotypes were unavailable from the FinnGen data for 344 samples. *CYP2C19*2*, **3*, and **8* were considered LOF alleles as per the Clinical Pharmacogenetics Implementation Consortium clinical function definition.[31,32] *CYP2C19*2*, *CES1* rs71647871, *CYP2B6* rs3745274 and rs8192719, *CYP2C9*3*, and *PEAR1* rs12041331 were used to calculate a polygenic clopidogrel response score, as described previously.[33] This resulted in 1972 patients with genotyping data. Genotyping was not used to guide the therapy.

## Ethics approval

All patients provided written informed consent for the Corogene (Genetic Predisposition of Coronary Artery Disease) registry.[25] The Helsinki University Central Hospital Ethics Committee approved the research protocol for the Corogene study (registry numbers 426/E5/05, 205/E0/2007, HUS/152/2016, and HUS/1203/2016). This study also complies with the 1964 Declaration of Helsinki and subsequent revisions.

# RESULTS

## Study population

Patient characteristics are shown in Table 1. Prevalence of classical risk factors were high, as expected in this population. Of the patients, 66.7% had ACS and 33.3% had chronic coronary artery disease. Previous revascularization, either PCI or coronary artery bypass graft bypass surgery, had been performed on 25.1% of the patients. Acetylsalicylic acid was used by 92.7%, statins as lipid-lowering medication by 95.5%, and angiotensin converting enzyme inhibitor or angiotensin receptor blocker by 70.1% of patients. As majority of the patients were using statins, and thus, statistical evaluation of statin effect on the new cardiovascular events cannot reliably be done. Patients that were prescribed clopidogrel were included in this study, however, the length of the prescription varied (Figure S2) depending on diagnosis, procedure type, and physician's preference. There was no difference in the length of clopidogrel use between study groups. No significant differences emerged in patient characteristics between carriers and non-carriers of *CYP2C19* LOF alleles, except for use of beta blockers, which were more frequent in carriers. Additionally, there were no significant differences between the two groups regarding the types of coronary procedures or stents used.

## Genetics

The CYP2C19 metabolizer phenotypes were distributed as follows: ultrarapid 86 (4.4%), rapid 503 (25.5%), normal 775 (39.3%), intermediate 547 (27.7%), and poor metabolizers 61 (3.1%; Figure S3A). Pharmacogenomic polygenic clopidogrel response score (PgxRS) distribution is shown in Figure S3B. Patients carrying *CYP2C19* LOF alleles comprise of intermediate and poor metabolizer phenotypes ($n=608$, 30.8%). Ultrarapid, rapid, and normal metabolizer phenotypes ($n=1364$, 69.2%) were determined as non-carriers of *CYP2C19* LOF alleles and served as a control group for the carriers of *CYP2C19* LOF alleles.

## Cardiovascular events during the 365 days

During the 365-day study period 252 patients (12.8%) had an ischemic vascular event, that is, myocardial infarction ($n=189$, 9.6%), stroke ($n=24$, 1.2%), or cardiovascular death ($n=39$, 2.0%). Total mortality during the study period was 2.8% (55). In *CYP2C19* LOF carriers, 18 deaths (3.0%) were recorded and in the non-carrier group there were 37 deaths (2.7%; Fisher's exact test, $p=0.7667$). Of all patients, 30 sustained hemorrhages (Figure S1).

During the follow-up, new cardiovascular events were significantly more frequent among carriers of *CYP2C19* LOF alleles (14.8%, 95% CI, 11.7–17.8) than among non-carriers (10.8%, 95% CI, 9.0–12.6), with the Kaplan–Meier estimate showing that LOF carriers have a significantly higher risk of recurring events ($p=0.016$; Figure 1a). Similarly, new myocardial infarctions were more frequent in carriers of *CYP2C19* LOF alleles than in non-carriers (12.7% vs. 8.8%, $p=0.009$ for Kaplan–Meier).

## Multivariate analysis and Cox regression

The impact of classic risk factors, including smoking, obesity, hypertension, dyslipidemia, diabetes, sex, and age, was validated. Of those, smoking (HR, 1.77, 95% CI, 1.15–2.70, $p=0.009$), diabetes mellitus (HR, 1.48, 95% CI, 1.04–2.10, $p=0.029$), and age (HR, 1.04, 95% CI, 1.03–1.1, $p>0.001$) were independent risk factors for a higher risk for cardiovascular events (Figure 2a). Similarly, smoking (HR, 1.89, 95% CI, 1.28–2.8, $p=0.001$), diabetes mellitus

**TABLE 1** Baseline characteristics of the patient cohort.

| | All | Non-carriers of CYP2C19 LOF | Carriers of CYP2C19 LOF | *p* value |
|---|---|---|---|---|
| Patients | 1972 | 1364 | 608 | |
| European ancestry (%) | 1972 (100.0) | 1364 (100.0) | 608 (100.0) | NaN |
| Age (mean, SD) | 65.1 (11.4) | 65.4 (11.4) | 64.5 (11.5) | 0.095 |
| Women (%) | 600 (30.4) | 403 (29.5) | 197 (32.4) | 0.201 |
| BMI (mean, SD) | 27.5 (4.7) | 27.6 (4.7) | 27.4 (4.5) | 0.504 |
| Risk factors and prior procedures | | | | |
| Smoker (%) | 582 (29.5%) | 401 (29.4%) | 181 (29.8%) | 0.721 |
| Ex-smoker (%) | 675 (34.2%) | 476 (34.9%) | 199 (32.7%) | 0.721 |
| Hypertension (%) | 1287 (65.3%) | 882 (64.7%) | 405 (66.6%) | 0.401 |
| DM (%) | 396 (20.0%) | 280 (20.5%) | 116 (19.1%) | 0.827 |
| Dyslipidemia (%) | 1437 (72.9%) | 994 (72.9%) | 443 (72.9%) | 0.844 |
| Prior procedures | | | | |
| Prior MI (%) | 451 (22.9) | 324 (23.8) | 127 (20.9) | 0.844 |
| Prior PCI (%) | 303 (15.4) | 213 (15.6) | 90 (14.8) | 0.644 |
| Prior CABG (%) | 191 (9.7) | 125 (9.2) | 66 (10.9) | 0.241 |
| Procedures | | | | |
| PCI (%) | 1682 (85.3) | 1176 (86.2) | 516 (84.9) | 0.565 |
| DES (%) | 259 (13.3) | 171 (12.5) | 88 (14.5) | 0.565 |
| BMS (%) | 1306 (66.2) | 920 (67.4) | 386 (63.5) | 0.565 |
| DES + BMS (%) | 52 (2.6) | 37 (2.7) | 15 (2.5) | 0.565 |
| POBA only (%) | 75 (3.8) | 48 (3.5) | 27 (4.5) | 0.565 |
| Number of stents/patient (mean, SD) | 1.5 (1.0) | 1.5 (1.0) | 1.5 (1.0) | 0.584 |
| Length (mm) of stents/patient (mean, SD) | 21.3 (11.6) | 21.3 (11.7) | 21.2 (11.3) | 0.875 |
| CABG during first hospitalization (%) | 20 (1.0) | 16 (1.1) | 4 (0.6) | 0.074 |
| CABG elective (%) | 31 (1.6) | 16 (1.1) | 15 (2.5) | 0.074 |
| Vessel status | | | | |
| 1-artery disease (%) | 836 (42.4) | 599 (43.9) | 237 (39.0) | 0.124 |
| 2-artery disease (%) | 584 (29.6) | 390 (28.6) | 194 (31.9) | 0.124 |
| 3-artery disease (%) | 530 (26.9) | 359 (26.3) | 171 (28.1) | 0.124 |
| Nonsignificant (≤50%) stenosis (%) | 22 (1.1) | 16 (1.2) | 6 (1.0) | 0.124 |
| CAD types | | | | |
| Chronic CAD (%) | 656 (33.3) | 450 (33.0) | 206 (33.9) | 0.758 |
| ACS (%) | 1316 (66.7) | 914 (67.0) | 402 (66.1) | 0.758 |
| ACS types | | | | |
| STEMI (%) | 512 (25.9) | 356 (26.0) | 156 (25.6) | 0.981 |
| NSTEMI (%) | 656 (33.2) | 456 (33.4) | 200 (32.8) | 0.981 |
| UAP (%) | 134 (6.7) | 93 (6.8) | 41 (6.7) | 0.981 |
| Type II MI (%) | 14 (0.7) | 9 (0.7) | 5 (0.8) | 0.981 |
| Other diseases | | | | |
| Atrial fibrillation (%) | 115 (5.8) | 82 (6.0) | 33 (5.4) | 0.768 |
| PVD (%) | 167 (8.5) | 113 (8.3) | 54 (8.9) | 0.322 |
| CVA (%) | 211 (10.7) | 143 (10.5) | 68 (11.2) | 0.875 |
| Kidney disease (%) | 66 (3.3) | 43 (3.2) | 23 (3.8) | 0.473 |

**TABLE 1** (Continued)

| | All | Non-carriers of CYP2C19 LOF | Carriers of CYP2C19 LOF | *p* value |
|---|---|---|---|---|
| Cholesterol levels at admission (mmol/L)* | | | | |
| fP-Cholesterol (mean ± SD) | 4.58 ± 1.07 | 4.60 ± 1.07 | 4.56 ± 1.07 | 0.468 |
| fp-Triglycerides (mean ± SD) | 1.40 ± 0.89 | 1.40 ± 0.86 | 1.43 ± 0.95 | 0.465 |
| fP-LDL-C (mean ± SD) | 2.85 ± 0.93 | 2.86 ± 0.94 | 2.83 ± 0.93 | 0.645 |
| fP-HDL-C (mean ± SD) | 1.23 ± 0.34 | 1.24 ± 0.35 | 1.21 ± 0.31 | 0.102 |
| Concurrent drugs | | | | |
| Clopidogrel (%) | 1972 (100.0) | 1364 (100.0) | 608 (100.0) | NaN |
| Omeprazole or esomeprazole (%) | 381 (19.3) | 250 (18.3) | 131 (21.5) | 0.185 |
| β-blockers (%) | 1793 (90.9) | 1229 (90.1) | 564 (92.8) | 0.044* |
| ASA (%) | 1829 (92.7) | 1265 (92.7) | 564 (92.8) | 0.890 |
| ACE inhibitors (%) | 1049 (53.2) | 744 (54.5) | 305 (50.2) | 0.078 |
| ATR blockers (%) | 333 (16.9) | 227 (16.6) | 106 (17.4) | 0.654 |
| Statins (%) | 1883 (95.5) | 1297 (95.1) | 586 (96.4) | 0.150 |
| Warfarin (%) | 89 (4.5) | 61 (4.5) | 28 (4.6) | 0.890 |

*Note*: Number of patients and percentages unless otherwise specified.

Abbreviations: ACE, angiotensin-converting enzyme; ACS, acute coronary syndrome; ASA, acetylsalicylic acid; ATR, angiotensin receptor; BMI, body mass index; BMS, bare metal stent; CABG, coronary artery bypass graft; CAD, coronary artery disease; CV, cardiovascular; CVA, cerebrovascular attack; DES, drug-eluting stent; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; NSTEMI, non–ST-elevation myocardial infarction; PCI, percutaneous coronary intervention; POBA, percutaneous old balloon angioplasty; PVD, peripheral vascular disease; SD, standard deviation; STEMI, ST-elevation myocardial infarction; UAP, unstable angina pectoris.

*Cholesterol data was available for 1677 (85%) patients.

(HR, 1.45, 95% CI, 1.06–2.0, $p = 0.022$), and age (HR, 1.05, 95% CI, 1.04–1.1, $p > 0.001$) were independent risk factors for a higher risk for myocardial infarctions and cardiovascular deaths in *CYP2C19* LOF allele carriers (Figure 2b). Usage of PPI medication did not influence these risk factors (Figures S4 and S5). However, PPI use significantly increased risk of cardiovascular events in *CYP2C19* LOF non-carriers but not in *CYP2C19* LOF carriers (Figure 2c,d). Sex or BMI did not show any association with myocardial infarction. PgxRS, as defined by Lewis et al.,[33] revealed no significant association with cardiovascular events (Figure S6).

## Hemorrhages

Hemorrhagic events ($n = 30$) requiring hospitalization occurred at the earliest on day 7 and up to 365 days after angiography. Carriers of *CYP2C19* LOF, but also the composite group of all *CYP2C19* LOF carriers and non-carriers using omeprazole or esomeprazole had a tendency toward a lower risk of hemorrhagic complications than non-carriers ($p = 0.704$ and 0.375, respectively; Figure 1c,d). Interestingly, the *CYP2C19* poor metabolizer group did not have any hemorrhages and the intermediate group only a few ($n = 9$). Surprisingly, patients using PPIs had more hemorrhagic events, but due to the low number of hemorrhagic events in the cohort this result should be interpreted with caution (Figure S7).

## Interaction with PPI medication—phenoconversion

Overall, 381 patients were using PPIs, omeprazole (53.5%) was used more often than esomeprazole (46.5%). No other PPIs were used in the study cohort. Usage of these PPIs was similar among LOF allele carriers ($n = 131$, 21.5%) and non-carriers ($n = 250$, 18.3%; $p = 0.184$). Cardiovascular events within the 365-day follow-up time were significantly more common in the composite group of carriers of *CYP2C19* LOF alleles (either using or not using omeprazole or esomeprazole) and non-carriers who were using omeprazole or esomeprazole than in non-carriers of *CYP2C19* LOF alleles who were not using omeprazole or esomeprazole (14.8%, 95% CI, 12.2–17.3 vs. 9.9%, 95% CI, 8.0–11.9, $p = 0.00173$ for Kaplan–Meier; Figure 1b,d). Use of PPI alone in the whole patient cohort did not have a significant effect on major adverse cardiac events (Figure S4). Additionally, *CYP2C19* LOF non-carriers using PPIs were at higher risk for the recurring cardiovascular events than non-carriers without PPI usage ($p = 0.044$; Figure 2d).

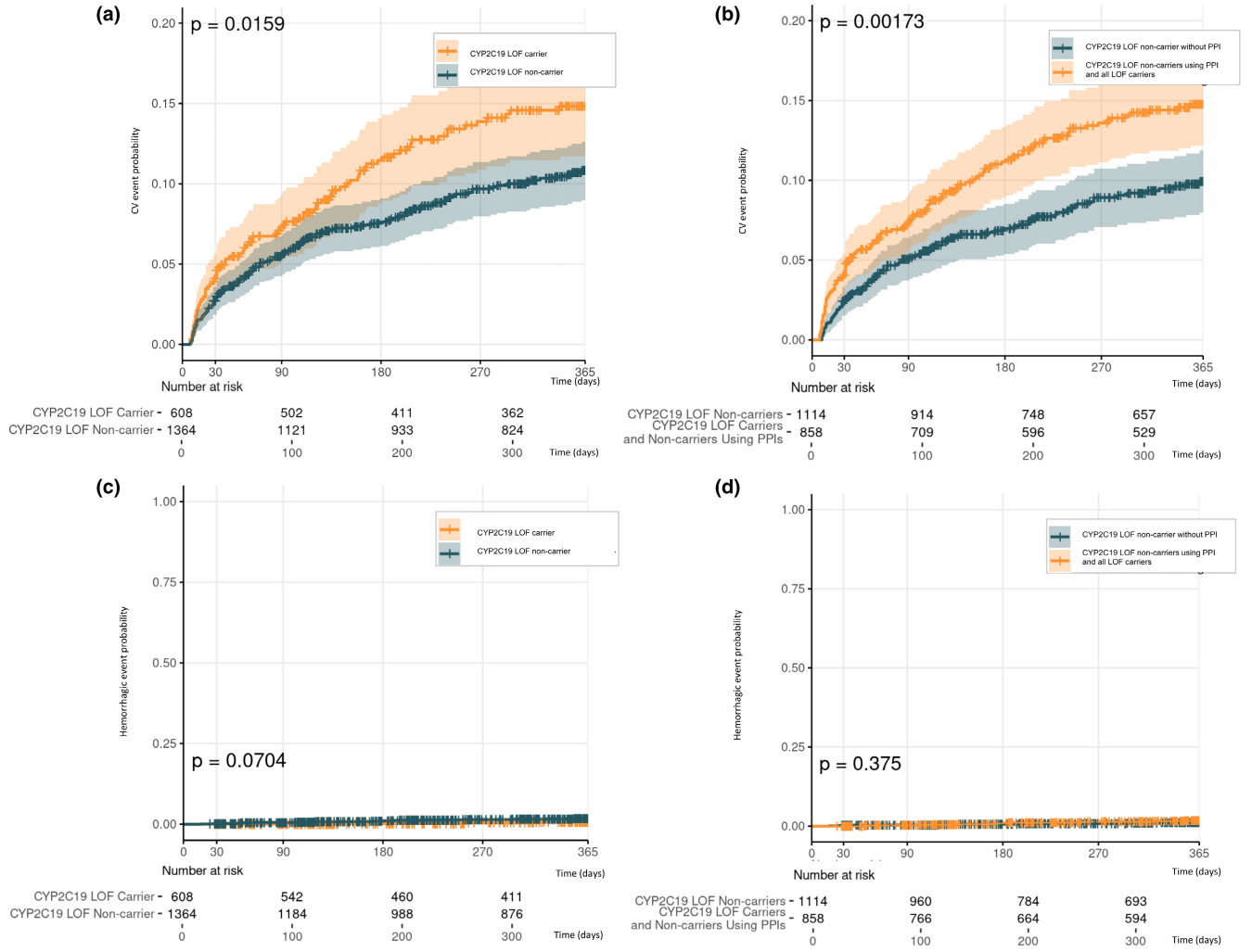


**FIGURE 1** Cardiovascular events in *CYP2C19* loss-of-function alleles carriers and non-carries and PPI-drug effect. Shadowed area represents 95% CI. (a) Cardiovascular events in *CYP2C19* LOF allele carriers and non-carriers. (b) Cardiovascular events in composite group including all *CYP2C19* LOF carriers and non-carriers with omeprazole or esomeprazole use versus non-carriers without omeprazole or esomeprazole use. (c) Hemorrhagic events in in *CYP2C19* LOF carriers and non-carriers. (d) Hemorrhagic events in composite group, including all *CYP2C19* LOF carriers and non-carriers using omeprazole or esomeprazole versus non-carriers without omeprazole or esomeprazole use. CI, confidence interval; CV, cardiovascular; LOF, loss-of-function; PPI, proton pump inhibitor.

## DISCUSSION

We studied patients with ACS and stable coronary artery disease on dual antiplatelet medication in a large prospective real-world cohort. All patients used clopidogrel as a $P2Y_{12}$ inhibitor. Our results show that cardiovascular events are significantly more common in patients carrying *CYP2C19* LOF alleles than in non-carriers. Furthermore, omeprazole or esomeprazole caused a phenoconversion phenomenon in non-carriers. In this phenomenon, non-carriers of *CYP2C19* LOF alleles are converted into poor or intermediate CYP2C19 metabolizers by the interaction between PPIs and CYP2C19 phenotype, resulting in a modified clinical response similar to that of patients carrying *CYP2C19* LOF alleles. This phenoconversion increased the number of MACEs in non-carriers of *CYP2C19* LOF alleles. When prescribing clopidogrel, both the *CYP2C19* genotype and the use of omeprazole or esomeprazole should be considered to avoid excess cardiovascular events.

*CYP2C19* genotype and clopidogrel efficacy have been investigated in several different settings. A prospective real-world study of 1815 patients with stable coronary artery disease and post-PCI ACS revealed that patients on clopidogrel with *CYP2C19* LOF alleles had a greater number of MACEs than those on ticagrelor or prasugrel.[10] A meta-analysis of nine observational studies identified an association between *CYP2C19* genotype and clopidogrel efficacy.[34,35] A randomized trial where patients with ACS were divided into standard care versus *CYP2C19* point of care testing showed that the primary end point of cardiovascular death,

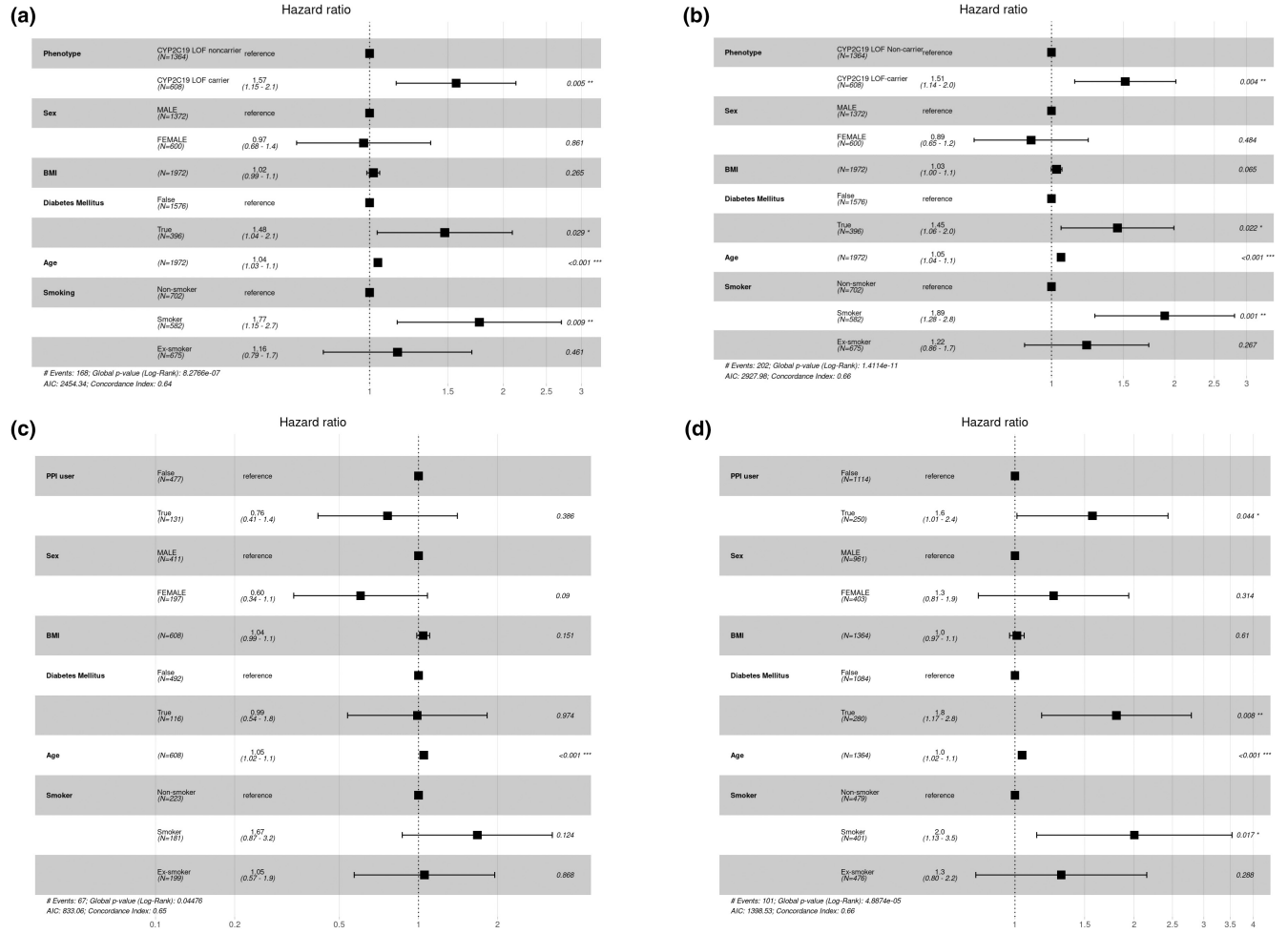


**FIGURE 2** The impact of classical risk factors on (a) cardiovascular events and (b) myocardial infarctions or cardiovascular deaths in all patients. Impact of PPI use on cardiovascular events in (c) *CYP2C19* LOF carriers and (d) non-carriers. HRs with 95% CIs are shown. ANC, absolute neutrophil count; BMI, body mass index; CI, confidence interval; HR, hazard ratio; LOF, loss-of-function; PPI, proton pump inhibitor.

myocardial infarction, stroke, and major bleeding was significantly reduced in the personalized therapy arm. However, clopidogrel was used more frequently in the standard care arm but is no longer considered up-to-date care for patients with ACS.[36] The TAILOR PCI trial showed that a CYP2C19 genotype-guided strategy for selection of oral $P2Y_{12}$ inhibitor therapy was noninferior to standard treatment with ticagrelor or prasugrel at 12 months. Patients in the genotype-guided arm did not have more thrombotic events but interestingly did have a lower incidence of bleeding.[37] All these studies show that *CYP2C19* LOF allele carriers using clopidogrel are at higher risk of MACE.

Our study is one of the largest real-world studies on clopidogrel genetics. The results are in line with earlier findings, as we also noted that carriers of *CYP2C19* LOF alleles have significantly more cardiovascular end points. We also observed a lower risk of hemorrhagic complications in carriers of *CYP2C19* LOF alleles than in non-carriers. However, this finding is somewhat inconclusive due to the low number of hemorrhages in the cohort. Omeprazole and esomeprazole were used frequently in our cohort for gastrointestinal protection, and pantoprazole or rabeprazole were not used at all.

PPIs are CYP2C19 substrates and may therefore competitively interact with clopidogrel metabolism. The extent of their inhibition on CYP2C19 varies between different PPIs and, for example, omeprazole, esomeprazole, and lansoprazole appear to be stronger CYP2C19 inhibitors than pantoprazole.[9] In fact, data from four randomized, placebo-controlled, crossover studies even suggested that for clopidogrel and pantoprazole such interaction would not exist.[38]

However, data on the effects of PPIs on MACE are conflicting. Several rigorous cohort studies have shown that concomitant PPI use resulted in a higher risk of rehospitalizations due to myocardial infarction[39] and increased mortality when PPIs were considered as a group, not

separately.[13,40] By contrast, a meta-analysis of 23 studies did not observe an increased risk of cardiovascular events or mortality in patients using clopidogrel and concomitant PPIs.[41]

Similarly, several high-quality RCTs have not shown that omeprazole increases risk of cardiovascular events compared with or without concomitant PPI and clopidogrel use.[11–14,42] However, these studies have not considered the effect of clopidogrel genetics. A few publications have evaluated PPI interaction based on *CYP2C19* genetics. A meta-analysis showed that *CYP2C19* LOF non-carriers using PPIs had more MACEs, but PPIs did not affect *CYP2C19* LOF carriers, a finding similar as ours in the present study.[21] Meta-analysis result was mainly based on the PLATO trial and the TRIUMPH cohort.[18,20,43] This finding is in line with our results, however, our *CYP2C19* genotyping is more complete.

Phenoconversion, a phenomenon in which genotypically normal metabolizers convert into phenotypic poor or intermediate metabolizers, occurs in *CYP2C19* LOF non-carriers using PPI drugs modifying their clinical response to that of *CYP2C19* LOF carriers. Interestingly, unlike in previous publications[42] examining all clopidogrel users, we observed significantly more MACEs in the composite group of all *CYP2C19* LOF allele carriers and non-carriers who were using omeprazole or esomeprazole, due to the forementioned phenoconversion. In a clinical real-life setting, this potential genotype–phenotype mismatch has turned out to be rather complex. Even a strong and reliable dataset of patient genotypes cannot be fully interpreted if the simultaneous use of drugs affecting the same cytochrome is not considered. For example, some studies do not mention the use of PPIs, even though it is likely to be relatively common.[15]

The Corogene registry is a prospective observational study that provides important information on real-world clinical practice and involves a realistic heterogeneous patient population. This study has one of the largest cohorts of patients with ACS and stabile coronary artery disease receiving clopidogrel and provides information on how *CYP2C19* genotypes and concomitant use of omeprazole or esomeprazole contribute to the number of MACEs. We also report the actual purchases of clopidogrel, reflecting adherence to therapy. However, our study design has some limitations. Clinical information may be incomplete, including indications for PPI prescription and the concomitant use of strong CYP-inducers, such as rifampicin. In addition, individual subgroups were underpowered to detect a difference between hemorrhagic complications. The hemorrhages in our study represent bleedings severe enough to be evaluated at the emergency department or requiring inpatient care. Less severe cases might well have been treated in outpatient facilities and not registered as actual bleedings and caution should be taken when interpreting the results.

## AUTHOR CONTRIBUTIONS

M.R.A., M.R.I., M.N., and J.S. wrote the manuscript. M.R.A., M.N., and J.S. designed the research. M.R.A., M.R.I., M.N., and J.S. performed the research. M.R.A., M.R.I., S.H., and J.K. analyzed the data. M.N. contributed new reagents/analytical tools.

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## CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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## SUPPORTING INFORMATION

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