Journal of the American Heart Association

ORIGINAL RESEARCH

Factor V Leiden and the Risk of Bleeding in Patients With Acute Coronary Syndromes Treated With Antiplatelet Therapy: Pooled Analysis of 3 Randomized Clinical Trials

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BACKGROUND: Whether factor V Leiden is associated with lower bleeding risk in patients with acute coronary syndromes using (dual) antiplatelet therapy has yet to be investigated.

METHODS AND RESULTS: We pooled data from 3 randomized clinical trials, conducted in patients with acute coronary syndromes, with adjudicated bleeding outcomes. Cox regression models were used to obtain overall and cause-specific hazard ratios (HRs) to account for competing risk of atherothrombotic outcomes (ie, composite of ischemic stroke, myocardial infarction, and cardiovascular death) in each study. Estimates from the individual studies were pooled using fixed effect meta-analysis. The 3 studies combined included 17 623 patients of whom 969 (5.5%) were either heterozygous or homozygous (n=23) carriers of factor V Leiden. During 1 year of follow-up, a total of 1289 (7.3%) patients developed major (n=559) or minor bleeding. Factor V Leiden was associated with a lower risk of combined major and minor bleeding (adjusted cause-specific HR, 0.75; 95% CI, 0.56–1.00; P=0.046; I^2=0%) but a comparable risk of major bleeding (adjusted cause-specific HR, 0.93; 95% CI, 0.62–1.39; P=0.73; I^2=0%). Adjusted pooled cause-specific HRs for the association of factor V Leiden with atherothrombotic events alone and in combination with bleeding events were 0.75 (95% CI, 0.55–1.02; P=0.06; I^2=0%) and 0.75 (95% CI, 0.61–0.92; P=0.007; I^2=0%), respectively.

CONCLUSIONS: Given that the lower risk of bleeding conferred by factor V Leiden was not counterbalanced by a higher risk of atherothrombotic events, these findings warrant future assessment for personalized medicine such as selecting patients for extended or intensive antiplatelet therapy.

Key Words: acute coronary syndrome ■ antiplatelet therapy ■ bleeding ■ factor V Leiden

Factor V Leiden results from a single-point mutation in the inactivation site of the active factor V, which in turn leads to activated protein C resistance and a prothrombotic state.[1] Heterozygous factor V Leiden is 1 of the most common genetic thrombophilic defects with a prevalence of about 5% in the general population of European descent.[2] Carriers of the factor V Leiden polymorphism have a 4-fold higher risk of venous thromboembolism.[3] Whether the factor V Leiden polymorphism also increases risk of atherothrombotic events, such as myocardial infarction and stroke, is controversial.[4–10] In our recent meta-analysis of individual participant level data, factor V Leiden was not associated with elevated risk of (recurrent)

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For Sources of Funding and Disclosures, see page 9.

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CLINICAL PERSPECTIVE

What Is New?

- Factor V Leiden protects for bleeding without increasing the risk of atherothrombotic events in patients using mainly dual antiplatelet therapy.

What Are the Clinical Implications?

- If confirmed in future research, factor V Leiden could be determined for selecting patients for extended/intensive antiplatelet therapy.

Nonstandard Abbreviations and Acronyms

CURE	Clopidogrel in Unstable Angina to Prevent Recurrent Events
PLATO	Study of Platelet Inhibition and Patient Outcomes
PopGen	Popular Genetics

atherothrombotic events in patients with acute coronary syndromes.[11]

A few observational studies reported a protective effect of factor V Leiden against bleeding in patients with hemophilia and patients with venous thromboembolism using oral anticoagulants,[12,13] whereas results were inconsistent for blood loss related to cardiac surgery in patients with versus without factor V Leiden.[14,15] A multinational registry of patients with established venous thromboembolism reported a 50% lower risk of major bleeding in carriers of factor V Leiden during treatment with oral anticoagulants.[13] In contrast, atherothrombotic events are more prevalent and require life-long antithrombotic drugs use usually in the form of a single or dual antiplatelet agents depending on the underling phenotype and timing of interventions such as stent implantation.[16,17] Current guidelines recommend dual antiplatelet therapy for 12 months after acute coronary syndrome and longer in patients with low-risk for bleeding.[16,17] Use of dual antiplatelet drugs is associated with a high risk of bleeding, which depending on the definition of bleeding exceeds 10% within the first year of therapy in patients with acute coronary syndrome.[18] Whether factor V Leiden carrier status is associated with less bleeding in this high-risk population has not been studied.

To assess whether factor V Leiden is associated with less bleeding in patients with acute coronary syndrome, we pooled data from 3 randomized clinical trials with adjudicated bleeding outcomes.

METHODS

In accordance with Transparency and Openness Promotion Guidelines, the authors declare that all summary level data used for this meta-analysis are available within the article or could be made available upon request to the corresponding author. Individual participant level data for each study were not collected through the federated analysis approach and will therefore not be made available.

Participants

Randomized studies including patients with acute coronary syndrome, adjudicated bleeding events, and available data on factor V Leiden status were eligible for inclusion and were selected from the GENIUS-CHD (Genetics of Subsequent Coronary Heart Disease) consortium.[11,19] Additional PubMed search did not identify any studies eligible for inclusion. The combination of terms used for the PubMed search were the following: "Factor V Leiden" AND ("myocardial infarction" OR "acute coronary syndrome" OR stroke) AND (antiplatelet OR aspirin OR clopidogrel OR ticagrelor OR prasugrel OR antithrombotic). Detailed study protocols of the 3 included randomized clinical trials have been published previously.[18,20,21] In brief, the CURE (Clopidogrel in Unstable Angina to Prevent Recurrent Events) study was an international, multicenter, randomized, parallel-group, double-blind clinical trial of the combination of clopidogrel plus aspirin versus placebo plus aspirin in patients with acute coronary syndromes including unstable angina and non–ST-segment–elevation myocardial infarction.[20] The study involved 12 562 patients recruited between 1998 and 2000 at 482 centers in 28 countries. The PLATO (Study of Platelet Inhibition and Patient Outcomes) was an international, randomized, double-blind, double-dummy phase III trial comparing ticagrelor plus aspirin with clopidogrel plus aspirin in patients with either ST-segment–elevation myocardial infarction intended for primary percutaneous coronary intervention or with non–ST-segment–elevation acute coronary syndrome.[18] A total of 18 624 patients from 862 centers in 43 countries were enrolled between 2006 and 2008. DNA samples for genetic analyses were available from centers and patients consenting to participation in the genetics substudy. The PopGen (Popular Genetics) trial was an international, multicenter, open-label, assessor-blinded trial comparing ticagrelor or prasugrel plus aspirin with clopidogrel plus aspirin based on rapid genetic testing for clopidogrel-resistance in patients with ST-segment–elevation myocardial infarction undergoing primary percutaneous intervention.[21] A total 2476 patients from 10 centers in the Netherlands, 1 in Belgium, and 1 in Italy were enrolled between 2011 and 2018. Although the control group received ticagrelor/prasugrel plus aspirin

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therapy, in the intervention group rapid genetic testing for the presence of the *CYP2C19*2* or **3* alleles was performed, which in turn determined whether patients were prescribed clopidogrel or ticagrelor/prasugrel.[22]

In all 3 studies, patients requiring oral anticoagulants and patients with high bleeding risk such as those with recent (<24 hours) fibrinolytic therapy were excluded. Participating studies received local institutional review board approvals and included only patients who had provided informed consent at the time of enrollment.

Definitions

Factor V Leiden was defined as the presence of a single nucleotide mutation; G-to-A substitution at nucleotide 1691 in the factor V (factor V R506Q) gene (single-nucleotide polymorphism rs6025) was documented by individual genotyping assays or direct DNA sequencing using commercially available whole-genome or targeted sequencing kits.

The PLATO non–coronary bypass graft surgery (CABG)-related major and minor bleeding was defined as the composite of major and minor bleeding not related to a CABG procedure according to the previously published PLATO definition.[18] PLATO major bleeding consisted of fatal, intracranial, intrapericardial with cardiac tamponade bleed resulting in hypovolemic shock or severe hypotension that required pressers or surgery; overt bleeding associated with a decrease in hemoglobin 3 g/dL or requiring transfusion of 2 units whole blood or packed red blood cells; or other significantly disabling (eg, intraocular with permanent vision loss) bleeding. Surgical bleeding attributed to CABG was excluded. Minor bleeding was defined as bleeding not meeting the criteria for major bleeding but requiring medical intervention to stop or treat the bleeding. Although the CURE trial was conducted before the PLATO bleeding definition was established, the definition of major bleeding in the CURE trial was similar to the PLATO definition,[23] except that CABG-related bleeding events were also included as these were not classified separately. Minor bleeding in the CURE trial was defined as bleeding not meeting the criteria for major bleeding but requiring discontinuation of the study drug.[20] The primary end point of the PopGen trial was also adjudicated bleeding events using the PLATO definition of non–CABG-related major and minor bleeding.

Competing events consisted of atherothrombotic events including myocardial infarction, ischemic stroke, and cardiovascular death as classified by the adjudication committees of each study in accordance with the prevailing guidelines.[18,20,22]

Statistical Analysis

Analyses were performed in 2 stages. First, a centrally developed statistical script with harmonized definitions was shared with each study representative. This script evaluated the association of factor V Leiden with bleeding events assuming a dominant genetic model using time-to-event Cox proportional hazards regression. Single and multivariable Cox proportional hazards regression models were fitted. Adjustment variables included sex, age, hypertension, hyperlipidemia, diabetes mellitus, current smoking, history of cardiovascular event (ie, stroke or myocardial infarction), body mass index (continuous), creatinine levels (continuous), and study arm. In the CURE trial, hyperlipidemia and creatinine were not part of the adjustment variables as these were not available in the CURE trial. Given that our exposure variable (factor V Leiden) is a genetic factor, confounding by acquired factors could be questioned; however, given that some variables showed statistically significant association with factor V Leiden and the adjusted estimates were more conservative, we opted to report primarily adjusted estimates. Cause-specific hazard ratios (HRs) for bleeding events were calculated to account for competing risk of the competing event (ie, composite of myocardial infarction, ischemic stroke, and cardiovascular mortality). As sensitivity analysis, Fine and Gray subdistribution HRs were also obtained to contrast these with the cause-specific HRs. Furthermore, cause-specific cumulative incidence rates per each day of follow-up were obtained in a similar manner, whereas competing events were treated as censoring events. Crude incidence rates were calculated as the total number of events divided by the total follow-up years (occurrence of a competing event was treated as a censoring event). Differences in baseline characteristics by factor V Leiden status were assessed using a *z* value–based approach of mean/proportion differences in patients with versus without factor V Leiden in each study. Factor V Leiden association with the bleeding was also stratified on patient-level characteristics measured at baseline, including sex, age (≥70 years versus <70 years), hypertension (physician diagnosed or treated), type 2 diabetes mellitus (physician diagnosed or treated), hyperlipidemia (physician diagnosed or treated), body mass index (≥25 versus <25 kg/m^2), current smoking, and P2Y12 inhibitor (clopidogrel versus ticagrelor). Finally, adjusted cause-specific estimates of the composite atherothrombotic events were obtained.

Second, the study-specific summary estimates were shared with the study coordinator to pool the estimate using meta-analysis. Using fixed effect meta-analysis, cause-specific cumulative incidence rates and their corresponding standard errors available for each day from days 0 to 365 were pooled and plotted as cause-specific cumulative incidence rates. Study-level cause-specific HRs for the association of factor V Leiden with bleeding events in the overall study population and subgroups, and their corresponding standard

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errors, were pooled in an inverse variance weighted fixed effect meta-analysis. *P* values for the differences across the levels of the stratifying factor were calculated using a Wald test. For comparison, the cause-specific HRs of the competing event (ie, composite of atherothrombotic events) were presented. To account for the between-study heterogeneity, estimates of the random effect meta-analysis were also reported in the forest plots. Between-study variance in the random effects meta-analysis was calculated with the restricted maximum likelihood approach. Heterogeneity was quantified using a χ^2 test for heterogeneity and the I^2 statistic. In all analysis, a *P* value of <0.05 was considered statistically significant. All analyses were conducted using the R software version 3.3.3 or higher,[24] and the packages used for the main analyses were survival package 2.41.3 or higher, rms package 5.1.1 or higher, and meta package 4.9-0 or higher.

RESULTS

Clinical characteristics of the study participants in each study are summarized in the Table. A total of 5339 patients from the CURE trial, 9980 patients from the PLATO trial, and 2304 patients from the PopGen trial contributed to the analysis. The 3 studies combined included 17 623 patients of whom 969 (5.5%) were either heterozygous or homozygous (n=23) carriers of factor V Leiden. The prevalence of factor V Leiden was similar across the studies, ranging from 4.9% to 5.8%. Overall, the baseline characteristics (ie, sex, age, and traditional cardiovascular risk factors) were comparable among the studies. However, there were some major differences attributed to the inclusion criteria of each trial, whereas the CURE trial included only patients with non–ST-segment–elevation myocardial infarction and unstable angina, the PLATO trial included all patients with acute coronary syndrome including patients with ST-segment–elevation myocardial infarction, and the PopGen trial included only patients with ST-segment–elevation myocardial infarction. After stratifying by factor V Leiden status, age and sex distribution as well as the prevalence of major cardiovascular risk factors were similar, with the exception of more female carriers of factor V Leiden in the PopGen trial. The median follow-up was 304, 360, and 365 days in the CURE, PLATO, and PopGen trials, respectively.

Factor V Leiden and the Risk of Bleeding

During follow-up, a total of 53 (5.5%) patients with factor V Leiden (n=969) and 1236 (7.4%) without factor V Leiden (n=16 654) developed major or minor bleeding. Figure 1 shows the pooled cause-specific cumulative incidence of major and minor bleeding in factor V Leiden carriers compared with noncarriers. The pooled crude HR for the association of factor V Leiden with combined major or minor bleeding was 0.73 (95% CI, 0.55–0.96; *P*=0.024; I^2=0%). Pooled cause-specific HR was 0.73 (95% CI, 0.55–0.98; *P*=0.033; I^2=0%), and the Fine and Gray subdistribution HR was 0.74 (95% CI, 0.56–0.98; *P*=0.037; I^2=2%), both of which adjust for competing risk of atherothrombotic events. Further adjustment for potential confounders and the competing risk of atherothrombotic events also did not change the observed lower risk in factor V Leiden carriers (adjusted cause-specific HR, 0.75; 95% CI, 0.56–1.00; *P*=0.046; I^2=17%; Figure 2). A total of 28 (2.9%) patients with and 531 (3.2%) patients without factor V

Table . Baseline Characteristics of the Individual Studies

	CURE		PLATO		PopGen	
	FVL(+)	FVL(–)	FVL(+)	FVL(–)	FVL(+)	FVL(–)
Number of patients, n	260	5079	580	9400	129	2175
Mean age, y	62.9	63.6	62.3	62.5	62.3	62.0
Male patients, %	61.2	59.3	67.8	69.6	67.4*	75.9*
Hypertension, %	59.6	61.5	66.2	66	45.7	39.4
Diabetes mellitus, %	19.2	22.2	24.3	23	12.4	11.4
Hyperlipidemia, %	n.a.	n.a.	44.8	45.4	69.3	68.5
Current smoking, %	27.3	22.5	31.9	35.4	47.8	45.8
History of cardiovascular event, %	31.9	32.3	26.0	28.0	10.9	10.3
Mean BMI, kg/m^2	27.6	27.6	28.3	28.2	27.2	27.1
Mean creatinine, mmol/L	n.a.	n.a.	88*	86*	96*	80*

BMI indicates body mass index; CURE, Clopidogrel in Unstable Angina to Prevent Recurrent Events; FVL, factor V Leiden; n.a., not available; PLATO, Study of Platelet Inhibition and Patient Outcomes; and PopGen, Popular Genetics.

**P* value <0.05 for the difference between patients with vs without FVL.

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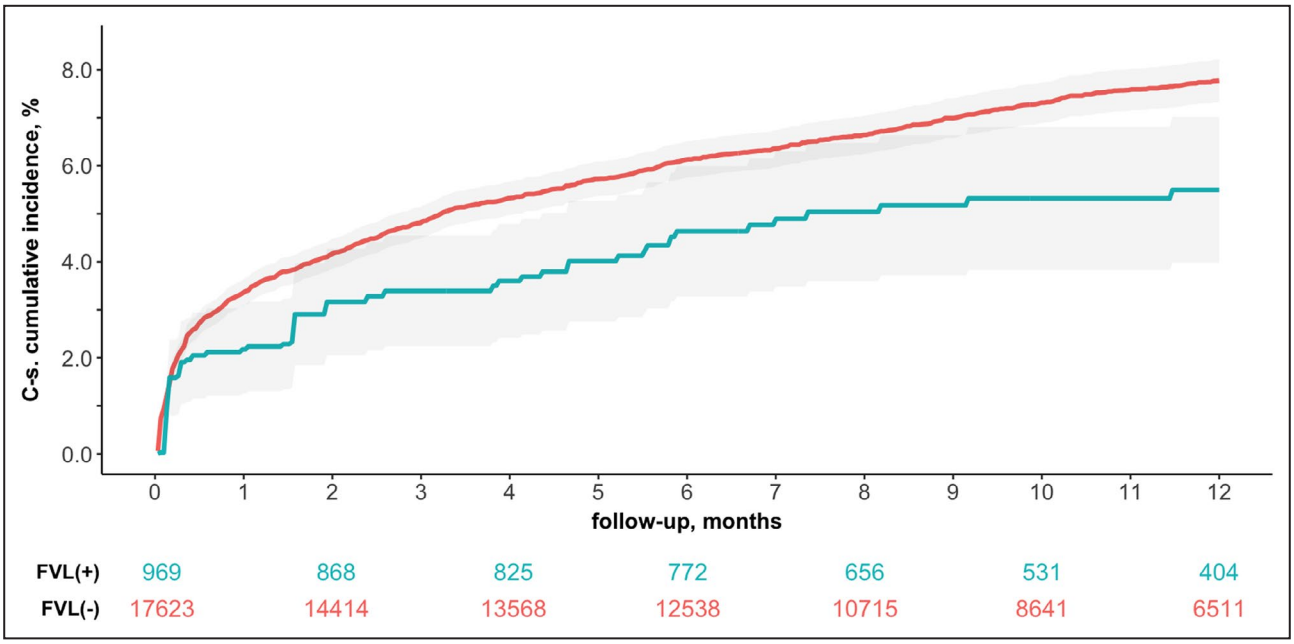

Figure 1. Pooled cause-specific cumulative incidence of combined major and minor bleeding.
The shaded gray area depicts the 95% CIs of the cumulative C-S incidence of bleeding. C-S indicates cause specific; and FVL, factor V Leiden.

Leiden developed major bleeding. The association of factor V Leiden with major bleeding was not significant (adjusted cause-specific HR, 0.93; 95% CI, 0.62–1.39; P=0.73; I^2=0%; Figure 3).

The lowest cause-specific HR for major and minor bleeding was observed in the PopGen trial, which included only patients with ST-segment–elevation myocardial infarction. The estimate for the ST-segment–elevation myocardial infarction group of the PLATO trial (adjusted cause-specific HR, 0.79; 95% CI, 0.40–1.53) was similar to the overall estimate of the PLATO trial (adjusted cause-specific HR, 0.82; 95% CI, 0.57–1.18).

In subgroup analyses, the association of factor V Leiden with bleeding was similar across levels of patients' characteristics, traditional cardiovascular risk factors, and P2Y12-inhibitor (P interaction ≥0.17 for all; Figure 4). Although not statistically significant, the lower bleeding risk associated with factor V

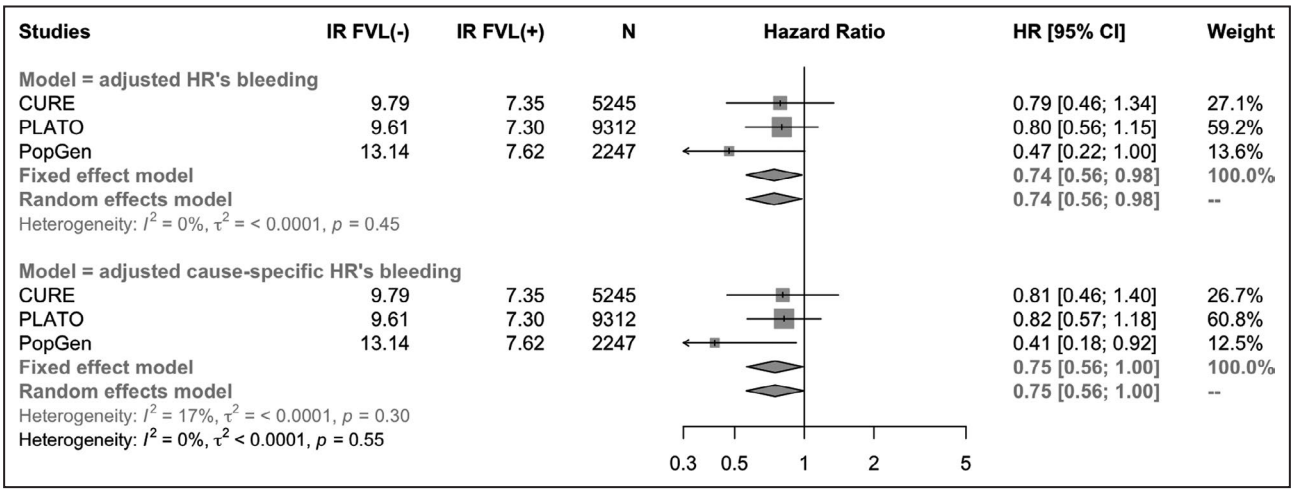

Figure 2. Overall and cause-specific HRs for the association of factor V Leiden with combined major and minor bleeding.
Adjustment variables included sex, age, hypertension, hyperlipidemia, diabetes mellitus, current smoking, history of cardiovascular event (ie, stroke or myocardial infarction), body mass index (continuous), creatinine levels (continuous), and study arm. CURE indicates Clopidogrel in Unstable Angina to Prevent Recurrent Events; HR, hazard ratio; IR FVL(–), incidence rates (per 100 person-years) of any bleeding in noncarriers of factor V Leiden; IR FVL(+), incidence rates of any bleeding in factor V Leiden carriers; PLATO, Study of Platelet Inhibition and Patient Outcomes; and PopGen, Popular Genetics.

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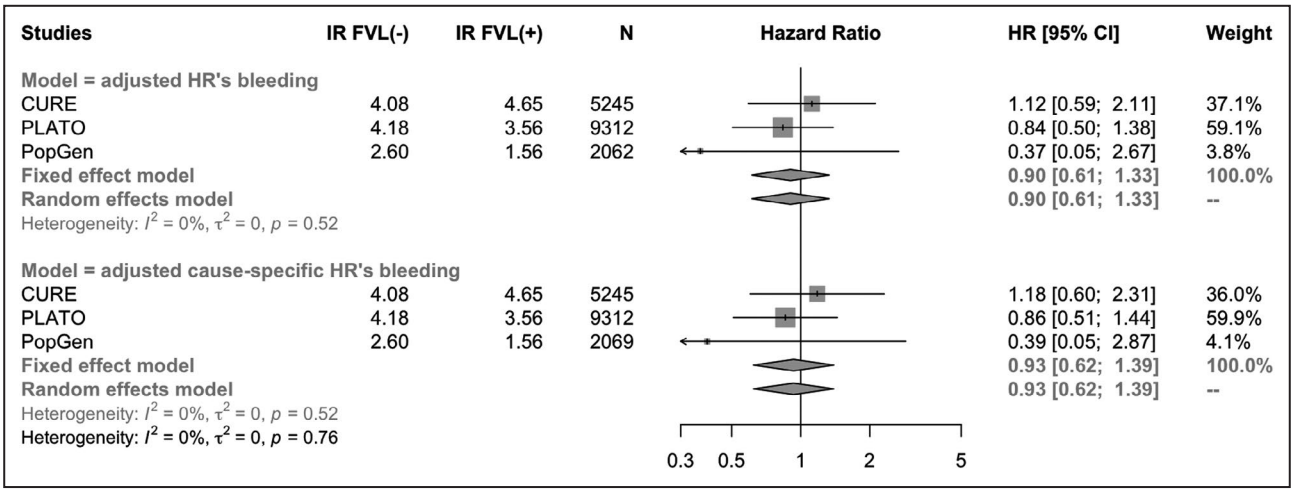

Figure 3. Overall and cause-specific HRs for the association of factor V Leiden with major bleeding.
Adjustment variables included sex, age, hypertension, hyperlipidemia, diabetes mellitus, current smoking, history of cardiovascular event (ie stroke or myocardial infarction), body mass index (continuous), creatinine levels (continuous), and study arm. CURE indicates Clopidogrel in Unstable Angina to Prevent Recurrent Events; HR, hazard ratio; IR FVL(–), incidence rates (per 100 person-years) of major bleeding in noncarriers of factor V Leiden; IR FVL(+), incidence rates of major bleeding in factor V Leiden carriers; PLATO, Study of Platelet Inhibition and Patient Outcomes; and PopGen, Popular Genetics.

Leiden was more pronounced in male patients (adjusted cause-specific HR, 0.66; 95% CI, 0.44–0.97; P=0.035; I^2=28%) compared with female patients (adjusted cause-specific HR, 0.97; 95% CI, 0.65–1.46; P=0.89; I^2=24%). This difference was driven by the estimates of the CURE trial in which the adjusted cause-specific HRs in female patients (1.50; 95% CI, 0.76–2.95) versus male patients (0.39; 95% CI, 0.15–1.06) were in opposite directions. The association of factor V Leiden with bleeding was similar in patients with ticagrelor plus aspirin versus clopidogrel plus aspirin (analysis restricted to the PLATO and PopGen trials; Figure 4). In the CURE trial, the protective of effect of factor V Leiden was limited to the aspirin-only arm (cause-specific adjusted HR, 0.50; 95% CI, 0.16–1.58) compared with the clopidogrel plus aspirin arm (cause-specific adjusted HR, 0.99; 95% CI, 0.52–1.87).

The adjusted cause-specific HR (adjusted for competing risk of major and minor bleeding and potential confounders including sex, age, hypertension, hyperlipidemia, diabetes mellitus, current smoking, history of cardiovascular event body mass index, creatinine levels, and study arm) for the association of factor V Leiden with atherothrombotic events (ie, composite of ischemic stroke, myocardial infarction, and cardiovascular death) was 0.75 (95% CI, 0.55–1.02; P=0.06; I^2=0%). Finally, the pooled adjusted cause-specific HR for the association of factor V Leiden with atherothrombotic events and major or minor bleeding combined (ie, net-clinical benefit) was 0.75 (95% CI, 0.61–0.92; P=0.007; I^2=0%; Figure 5).

DISCUSSION

The pooled analysis of the 3 randomized clinical trials including >17 000 patients with acute coronary syndromes using antiplatelet therapy, of whom nearly 1000 patients were carriers of factor V Leiden, showed that the risk of combined major or minor bleeding was lower in factor V Leiden carriers. The difference was mainly driven by the association to minor rather than major bleeding. The risk of atherothrombotic events in factor V Leiden carriers was nominally lower in factor V Leiden carriers, which was not statistically significant. Risk of any bleeding and atherothrombotic events combined was lower in factor V Leiden carriers.

Protective effect of factor V Leiden on bleeding complications in patients with venous thromboembolism using oral anticoagulants has been reported in a large multinational registry.[13] Similarly, a few observational studies reported lower bleeding risk in factor V Leiden carriers in patients with hemophilia who are prone to bleeding because of a deficiency of natural factor VIII or factor IX.[12] Corral et al reported a lower prevalence of factor V Leiden in patients with spontaneous intracranial hemorrhage compared with matched controls.[25] Results were inconsistent for blood loss related to cardiac surgery in patients with versus without factor V Leiden.[14,15] For the first time, we report a lower bleeding risk associated with factor V Leiden in patients with acute coronary syndromes using mainly dual antiplatelet therapy. In this setting, the overall bleeding incidence of both major and minor bleeding was about 7% per year and the rate of major bleeding around 3% per year. In accordance with our

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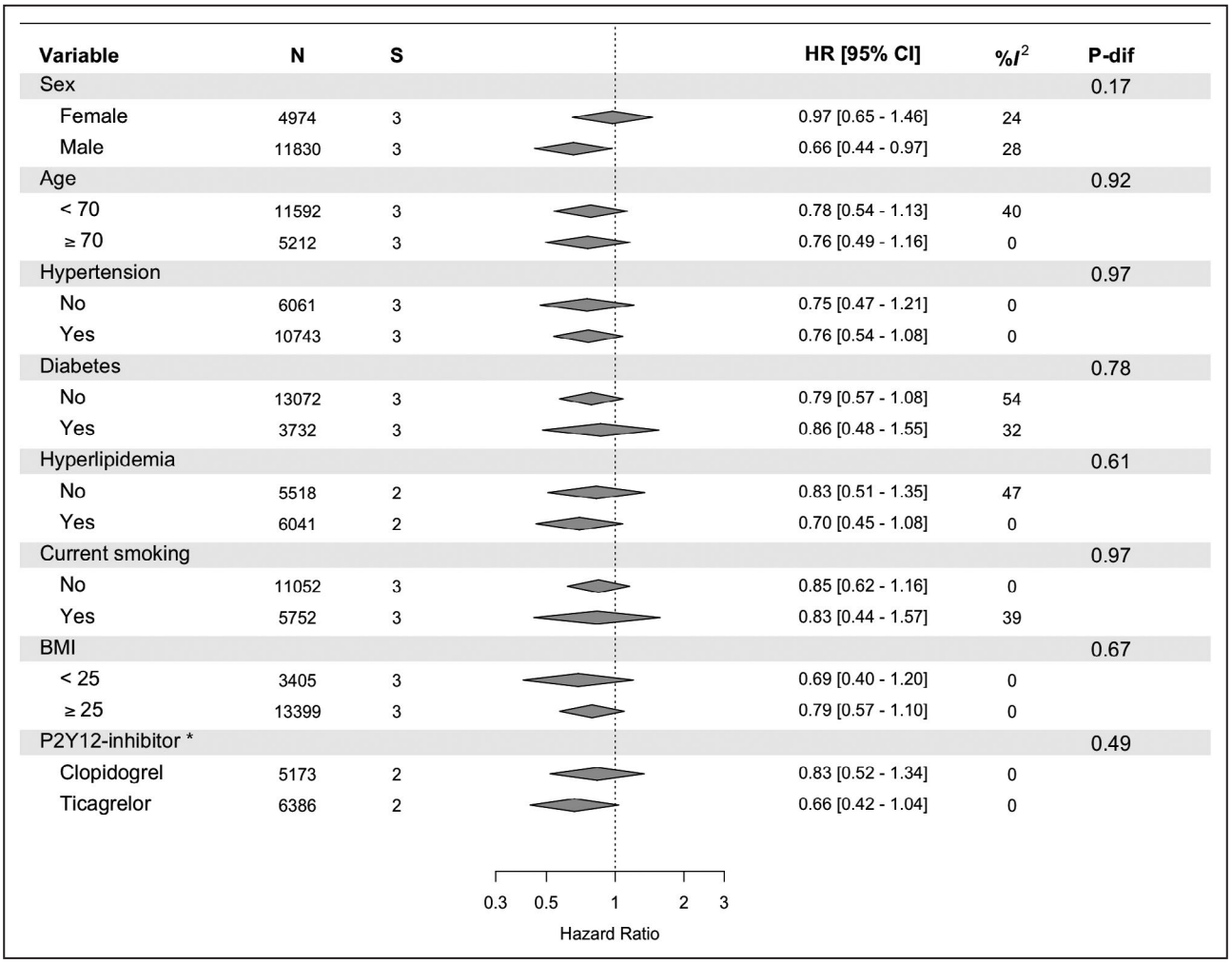

Figure 4. Pooled adjusted cause-specific associations of factor V Leiden with combined major and minor bleeding in subgroups.

Adjustment variables included sex, age, hypertension, hyperlipidemia, diabetes mellitus, current smoking, history of cardiovascular event (ie, stroke or myocardial infarction), BMI (continuous), creatinine levels (continuous), and study arm, when appropriate. Estimates are from fixed-effect meta-analyses. *Analysis restricted to PLATO and PopGen trials. In the CURE trial aspirin only arm adjusted cause-specific HR was 0.50 (95% CI, 0.16–1.58) compared with clopidogrel plus aspirin arm adjusted cause-specific HR 0.99 (95% CI, 0.52–1.87). BMI indicates body mass index; Clopidogrel in Unstable Angina to Prevent Recurrent Events; HR, hazard ratio; P-dif, interaction *P* value for the difference between the 2 strata of each variable; PLATO, Study of Platelet Inhibition and Patient Outcomes; PopGen, Popular Genetics; and S, number of studies.

recent larger meta-analysis, there was no statistically significant difference in antithrombotic events between carriers and noncarriers of factor V Leiden[11]; however, the risk of combined atherothrombotic and bleeding events was significantly lower in factor V Leiden carriers compared with noncarriers.

The factor V Leiden variant leads to activated protein C resistance and therefore a prothrombotic condition.[1] A potential interaction of factor V Leiden with antiplatelet agents may be biologically plausible given that ≈20% of human factor V is contained within platelet granules,[26] which is released upon platelet activation. Platelet-derived, not free-circulating, factor V seems to be essential in the hemostasis and thrombus formation in the arterial system.[27] However, we found no effect modification for the type of P2Y12 inhibor (clopidogrel versus more potent ticagrelor) use or aspirin-only versus dual antiplatelet therapy. Obviously reliable estimates for the aspirin-only group were hampered by the small sample size (n=2537) in our analysis. Schlachterman et al reported that factor V Leiden protects for bleeding at the microcirculation in murine hemophilia models, but not for bleeds associated with injury to major arteries.[28] Whether similar anatomical differences may explain the lack of significant risk reduction for major bleeding associated with factor V Leiden status in our study is unknown. Although there are some differences in the

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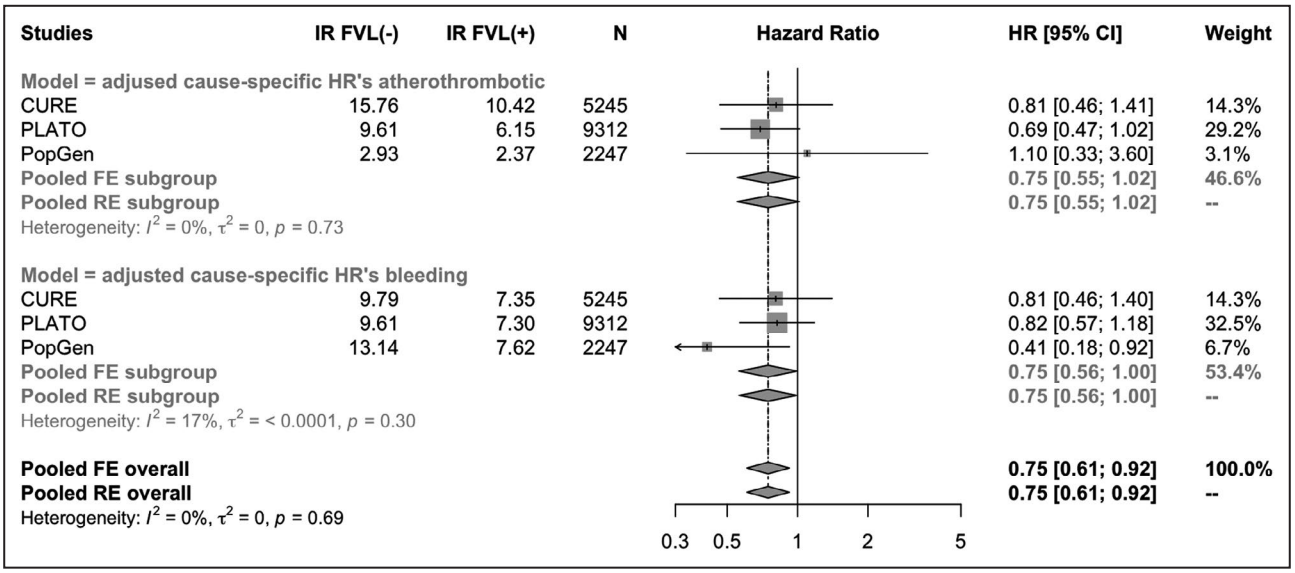

Figure 5. Adjusted cause-specific HRs for the association of factor V Leiden with atherothrombotic events and major or minor bleeding.

Adjustment variables included sex, age, hypertension, hyperlipidemia, diabetes mellitus, current smoking, history of cardiovascular event (ie, stroke or myocardial infarction), body mass index (continuous), creatinine levels (continuous), and study arm. CURE indicates Clopidogrel in Unstable Angina to Prevent Recurrent Events; FE, fixed effect; HR, hazard ratio; IR FVL(–), incidence rates (per 100 person-years) of the composite of atherothrombotic events or any bleeding in noncarriers of factor V Leiden; IR FVL(+), incidence rates of the composite of atherothrombotic events or any bleeding in factor V Leiden carriers; PLATO, Study of Platelet Inhibition and Patient Outcomes; PopGen, Popular Genetics; and RE, random effect.

various bleeding definitions, PLATO minor bleeding is roughly equivalent to the BARC (Bleeding Academic Research Consortium) 2 and the TIMI (Thrombolysis in Myocardial Infarction) minor/minimal bleeding.[23] Despite the lack of significant association of factor V Leiden with major bleeding, PLATO/CURE minor bleeding is clinically relevant as it required medical attention to stop the bleeding or discontinue dual antiplatelet therapy. Moreover, the net clinical benefit consisting of combined atherothrombotic and bleeding events was in favor of factor V Leiden carriers. From a pathophysiological point of view, the protective effect of factor V Leiden on atherothrombotic events is difficult to grasp, nevertheless several studies based on high-resolution radiological imaging modalities or pathological investigations showed that unstable plaques are more common in the presence of antithrombotic drugs use.[29–31] The plaque instability associated with antithrombotic drugs use has been ascribed to intimal neo-angiogenesis-associated microbleeds at the sites of plaques, leading to unstable plaques and subsequent plaque rupture.[29,31] Hence, a factor V Leiden–related protective effect for bleeding may also lead to a lower risk of atherothrombotic events. Finally, because a bleeding event is a known predictor of a subsequent atherothrombotic event and mortality,[32] it could be argued that the lower bleeding risk in factor V Leiden carriers could have resulted in a lower risk of atherothrombotic events. However, this could be refuted by the fact that we used competing risk analysis in which individuals encountering a bleeding event before an atherothrombotic event are censored from the risk set of atherothrombotic events.

This study has several limitations. Although definitions were harmonized across studies, residual differences such as the bleeding definition, type of acute coronary syndrome, and antithrombotic regimens inherent to the individual study designs remained. In this regard, the CURE trial in particular was different from the PLATO and PopGen trials; that is, in the CURE trial CABG-related bleeding were not excluded as these were not classified separately. Moreover, half of the CURE trial participants received single antiplatelet therapy compared with the dual antiplatelet therapy in all participants of the PLATO and PopGen trials. Subgroup analyses comparing antithrombotic regimens and acute coronary syndrome subtypes (ie, ST-segment–elevation acute coronary syndrome versus non–ST-segment–elevation acute coronary syndrome) did not show any evidence for effect modification. Despite the large overall sample size, our analyses are still underpowered for subgroups and particularly for major bleeding. For example, assuming that all 3 studies were pooled on an individual participant level (ie, ignoring the meta-analysis setting), based on the observed incidence rates and a 2-sided α level of 0.05, the pooled sample of these studies has only 61%, 56%, and 26% power to detect a 20% relative risk reduction

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conferred by factor V Leiden for any bleeding, composite atherothrombotic events, and major bleeding, respectively. In addition, because the majority (>95%) of the included patients were White patients, the results may not be applicable to other populations. In the CURE trial, which included 20% non-White patients, restricting the analysis to White patients attenuated the adjusted cause-specific HR for the combined major and minor bleeding toward 1, implying a reduced bleeding risk also in non-White factor V Leiden carriers (data not shown). Finally, unmeasured confounders or deferential selection bias leading to inclusion in these trials cannot be ruled out; therefore, declaring a causal association between factor V Leiden and bleeding is not justified based on our results. Selection bias due to the index event (i.e., baseline acute coronary syndrome) seems to have only limited impact on risk estimates of subsequent events.[33] Moreover, the prevalence of factor V Leiden in the analyzed studies was similar to the prevalence of factor V Leiden reported in the general population.[2] Finally, we were not able to adjust for history of bleeding, which is a strong predictor of subsequent bleeding. Despite these limitations, this is the first study assessing the impact of factor V Leiden on bleeding risk in patients with acute coronary syndromes using mainly dual antiplatelet therapy. If confirmed in future studies, these results may open new venues for tailored treatment such as extended dual antiplatelet therapy use in factor V Leiden carriers.

In conclusion, factor V Leiden is associated with a reduced risk of the composite of major and minor bleeding, mainly driven by a difference in minor bleeding, in patients with acute coronary syndromes on antiplatelet therapy. Given that the lower risk of bleeding conferred by factor V Leiden was not counterbalanced by a higher risk of ischemic events, these findings warrant future assessment for personalized medicine such as selecting patients for extended or intensive antiplatelet therapy.

ARTICLE INFORMATION

Received February 17, 2021; accepted July 7, 2021.

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Acknowledgments

The authors express their immense gratitude to all of the patients who participated in each of the individual studies as well as the many personnel who helped with recruitment, collection, curation, management, and processing of the samples and data.

Author contributions: Dr Mahmoodi developed the analysis script performed the analysis in Popular Genetics trial and Drs Eriksson and Ross prepared the data sets and applied the scripts to the Study of Platelet Inhibition and Patient Outcomes and Clopidogrel in Unstable Angina to Prevent Recurrent Events trials, respectively. Dr Mahmoodi performed the meta-analysis of the collected estimates and drafted the article. Drs Wallentin and ten Berg supervised the article. All authors took part in the interpretation of the data, and all authors provided critical revisions of the article for important intellectual content.

Sources of Funding

The CURE (Clopidogrel in Unstable Angina to Prevent Recurrent Events) trial was funded by Sanofi-Synthelabo and Bristol-Myers Squibb. The PLATO (Study of Platelet Inhibition and Patient Outcomes) trial was funded by AstraZeneca. Support for the analysis and the interpretation of the PLATO results was provided through funds to the Uppsala Clinical Research Center and Duke Clinical Research Institute as part of the Clinical Study Agreement. The PopGen (Popular Genetics) trial was funded by the Netherlands Organization for Health Research and Development. Dr Mahmoodi received a VENI grant from the Dutch Research Council for investigating the role of factor V Leiden in the pathogenesis of myocardial infarction. Dr Asselbergs is supported by National Institute for Health Research University College London Hospitals Biomedical Research Centre.

Disclosures

Dr Meijer reports receiving research grants from Pfizer, Bayer, and Sanquin; lecturing fees from Bayer, Sanquin, Boehringer Ingelheim, BMS, and Aspen; and consulting fees from Uniqure. All fees were paid to the institute. Dr James has received grants from AstraZeneca, The Medicines Company, Swedish Heart and Lung Foundation, Swedish Research Council, and Janssen and personal fees from Bayer. Dr Wallentin reports institutional research grants, consultancy fees, lecture fees, and travel support from Bristol-Myers Squibb/Pfizer, AstraZeneca, GlaxoSmithKline, and Boehringer Ingelheim; institutional research grants from Merck and Co. and Roche Diagnostics; and consultancy fees from Abbott and holds patent EP2047275B1 licensed to Roche Diagnostics and patent US8951742B2 licensed to Roche Diagnostics. Dr ten Berg reports receiving fees for board membership from AstraZeneca; consulting fees from AstraZeneca, Eli Lilly, and Merck; and lecture fees from Daiichi Sankyo, Eli Lilly, AstraZeneca, Sanofi, and Accumetric.

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