Joint effect of multiple prothrombotic genotypes and mean platelet volume on the risk of incident venous thromboembolism

Running Head: Genotypes, platelets and venous thromboembolism

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Abstract

Background: A high mean platelet volume (MPV), a marker of increased platelet reactivity, is a risk factor for venous thromboembolism (VTE). Whether established prothrombotic single nucleotide polymorphisms (SNPs) further increase the VTE risk in subjects with high MPV because of biological interaction remains unknown.

Aim: To investigate the joint effect of high MPV and prothrombotic genotypes, comprising a 5-SNP genetic risk score (GRS), on the risk of VTE in a population-based case-cohort.

Methods*:* Incident VTE cases (n=653) and a subcohort (n=1774) were derived from the Tromsø Study (1994-2012). DNA was genotyped for rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2036914 (*F11*) and rs2066865 (*FGG*). Hazard ratios (HRs) for VTE with 95% confidence intervals (CIs) were estimated according to predefined MPV-strata (<8.5, 8.5-9.5, ≥9.5fL) and number of risk alleles for each individual SNP and the GRS (0-1, 2-3, ≥4 risk alleles) in models adjusted for age, sex, body mass index and platelet count.

Results*:* The combination of high MPV and risk alleles, either as individual SNPs or the GRS, had an additive effect on VTE risk. Compared with subjects with MPV <8.5fL and 0-1 risk allele, those with high MPV (≥9.5fL) and ≥4 risk alleles had HRs of 2.80 (95%CI 1.77-4.43) for overall VTE and 4.60 (95%CI 2.20-9.60) for unprovoked events, respectively, but there was no supra-additive effect on risk estimates.

Conclusion: The combination of high MPV and prothrombotic genotypes had an additive effect on VTE risk, suggesting there is no biological interaction between these risk factors in the pathogenesis of VTE.

Keywords: deep vein thrombosis; interaction; mean platelet volume; single nucleotide polymorphism; venous thromboembolism

Introduction

Venous thromboembolism (VTE), a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease affecting 1-2 per 1000 individuals annually.[1] VTE poses a major challenge to public health[2] because of its severe short- and long-term complications, including recurrence, post-thrombotic syndrome, post-PE syndrome, and death.[3,4] VTE has a multicausal nature, occurring as the result of both genetic and environmental risk factors,[5] but up to 50% of all events arise with no apparent predisposing factor.[1,6] An expanded knowledge into the mechanisms involved in the pathogenesis of VTE, including how risk factors interact biologically, is an essential step to improve risk stratification and targeted VTE prevention.

Blood cells and their indices have been previously explored as potential risk factors for VTE.[7-11] Among the blood cell indices, a high mean platelet volume (MPV) was associated with increased risk of incident VTE, particularly unprovoked events, in the Tromsø Study.[7] MPV is a measure of the average size of circulating platelets that reflects platelet function. Platelets with a higher MPV have been shown to adhere and aggregate faster *in vitro* and be more prone to expose negatively charged phospholipids (i.e. phosphatidylserine) on their membranes,[12] presumably leading to a greater prothrombotic potential. A number of acquired conditions may influence MPV, such as obesity,[13] hypertension, diabetes, and smoking.[14] Further, heritability studies in twin- and family-based cohorts have demonstrated that genetic factors are important determinants of platelet count and MPV.[15,16]

Several single nucleotide polymorphisms (SNPs) associated with VTE have been revealed over the past decades. To improve the identification of individuals at risk of incident VTE, de Haan *et al.*[17] created a genetic risk score (GRS) comprising the five SNPs (out of 31) that individually showed the strongest association with VTE. The GRS performed similarly as the total score of the 31 SNPs and included rs8176719 (non-O blood group) in *ABO*, rs6025

(factor V Leiden [FVL]) in *F5*, rs1799963 (prothrombin G20210A) in *F2*, rs2066865 in fibrinogen gamma gene (*FGG*), and rs2036914 in *F11*.[17] Features of particular interest of the aforementioned five SNPs include the fact that the mechanisms by which they affect the VTE risk are biologically plausible.[18-22] Moreover, it has recently been reported that up to 45% of all VTE events in the population can be attributed to these SNPs.[23]

As MPV is a marker of platelet reactivity,[12] we hypothesized that the combination of prothrombotic genotypes and a high MPV could have a synergistic effect on the risk of VTE because of biological interaction.[24] However, whether and to what extent established prothrombotic genotypes affect the VTE risk in the presence of a high MPV remains unknown. Clarification of this question may provide novel insights into the biology of VTE and improve the identification of individuals at a substantially high risk of VTE in the general population. In the present study, we sought to investigate the joint effect of a high MPV and prothrombotic genotypes, assessed either as individual SNPs or as a GRS, on the risk of incident VTE in a population-based case-cohort derived from the Tromsø Study.

Methods

Study population

The Tromsø Study is a unique Norwegian follow-up study with repeated health surveys.[25] All inhabitants ≥ 25 years of age living in the municipality of Tromsø were invited to participate in the fourth survey (Tromsø 4, 1994-1995). A total of 27,158 individuals participated, which corresponded to an attendance rate of 77% of those who were initially invited. All participants were followed from date of inclusion to the date of incident VTE, migration, death or end of follow-up (December 31, 2012). The Tromsø Study was approved by the Regional Committee for Medical and Health Research Ethics in Northern Norway, and all study participants provided written informed consent.

First lifetime VTE events were identified by a thorough search in the registries (i.e. diagnosis, autopsy and radiology registries) at the University Hospital of North Norway (UNN). The UNN is the only hospital in the Tromsø region, and all medical care and relevant diagnostic radiology procedures are provided by this hospital. Medical records for each potential VTE case were extensively reviewed by trained personnel, as previously described in detail elsewhere.[26] Briefly, adjudication criteria for a VTE were clinical signs and symptoms of DVT or PE combined with objective confirmation by radiologic procedures that resulted in treatment initiation (unless contradictions were specified).

During a median of 17.4 years of follow-up, participants who developed an incident VTE in our cohort were included as cases in the present study (n= 683). Even though MPV was assessed in the full cohort from Tromsø 4, a case-cohort study was conceived to reduce the costs and resources related to genotyping. To this end, a subcohort (n= 1991) was created by randomly sampling participants at baseline from the source cohort weighted for the age distribution of the cases in 5-year age groups (Figure 1). A total of 247 participants were excluded due to missing values on MPV and / or the studied SNPs. After exclusion, our final case-cohort comprised 2427 participants, of whom 653 were VTE cases and 1774 were subcohort members. In the case-cohort design, every participant in the cohort has the same probability of being selected to the subcohort, including the cases. Thus, 69 of the subjects randomly selected to the subcohort were also VTE cases.

Classification of VTE events

All events were classified as either isolated DVT or PE (with or without DVT), and as provoked or unprovoked VTE based on the presence of provoking factors at the time of diagnosis. In the Tromsø Study, the VTE events were classified as provoked if one or more of the following provoking factors were present: major surgery, trauma, or acute medical

conditions (acute myocardial infarction, ischemic stroke, or infectious disease) within 8 weeks prior to the VTE; active cancer at the time of VTE diagnosis; immobilization (bed rest for ≥3 days or confinement to wheelchair within the last 8 weeks, or long-distance travel ≥4 hours within the last 14 days prior to the VTE event); or other factors specifically described as provoking by a physician in the medical record (e.g., intravascular catheter).[26]

Baseline measurements and blood sampling

Baseline information was collected by self-administered questionnaires, physical examination and blood samples at study entry. Body weight and height were measured with participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Information regarding smoking (current, and former/never), use of hormone therapy (current, and former/never) and prior arterial cardiovascular disease (CVD) (i.e. myocardial infarction, angina, or stroke) was collected through the self-administered questionnaires. Hormone therapy was considered as estrogen supplementation or oral contraceptives. Non-fasting blood samples were collected from an antecubital vein into 5-mL vacutainer tubes containing EDTA (K_3-EDTA 40 μL, 0.37 mol/L per tube).

Assessment of MPV, platelet count and prothrombotic genotypes

For MPV and platelet count measurements, samples were analyzed within 12h of blood collection at the Department of Clinical Chemistry, UNN, on an automated blood cell counter (Coulter Counter; Coulter Electronics, Luton, UK).[7] For genotype assessment, DNA was isolated and stored at -70 °C at the national CONOR biobank. Genotyping was performed using the Sequenom platform for the SNPs in *ABO* (rs8176719)*, F5* (rs6025)*, F2* (rs1799963) and *F11* (rs2036914), and the TaqMan platform for the SNP in *FGG* (rs2066865), as

described previously.[27] Participants were considered to be carriers of a prothrombotic genotype when one or two risk alleles were present. Among the studied SNPs, rs2036914 in *F11* was the only one where the minor allele was associated with a lower risk of VTE,[22] and we therefore considered the common allele as the risk allele. To create the GRS, we summarized the number of risk alleles from the 5 sequenced SNPs and categorized the number of risk alleles into 0-1, 2-3 or ≥ 4 risk alleles. The theoretical maximum number of risk alleles in the GRS score in homozygous individual would be 10.

Statistical analysis

Stata version 16.0 (StataCorp, College Station, TX, USA) was used to perform the statistical analysis. The study participants were divided into three MPV categories according to the cutoff values previously defined in the Tromsø Study on the association between MPV and VTE risk: < 8.5, 8.5-9.5 and ≥ 9.5 fL.[7,28] Means (± standard deviation) and proportions of baseline characteristics across MPV categories were calculated using descriptive statistics. For each individual SNP, Cox proportional hazards regression models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for incident VTE across categories of MPV. Non-carriers (0 risk allele) with MPV < 8.5 fL served as the reference group. Age was used as a time scale, with the age at enrollment defined as the entry time, and the age at incident VTE or censoring defined as the exit time. Further, we utilized the non-weighted GRS described by de Haan *et al*.[17] to investigate the effect of multiple prothrombotic risk alleles across MPV categories on the risk of incident VTE. For the GRS, three categories of risk alleles were created, i.e. 0-1, 2-3, ≥4 alleles, and subjects with 0-1 risk allele and MPV <8.5 fL served as the reference group. All Cox regression models were adjusted for age (as a time scale) and sex. Given the well-known inverse correlation between platelet count and MPV,[12] and the association of BMI with MPV[13] and VTE risk,[29] the regression models were

additionally adjusted for BMI (continuous variable) and platelet count (continuous variable). For the GRS, we performed subgroup analysis according to the anatomical localization of the thrombotic event (DVT or PE ± DVT) and the presence of provoking factors (provoked and unprovoked VTE). The proportional hazard assumption was tested using Schoenfeld residuals, and no violation was observed.

The presence of biological interaction between a high MPV and the prothrombotic risk alleles was assessed on an additive scale by calculating the relative excess risk attributable to interaction (RERI) and the attributable proportion (AP) due to interaction with corresponding 95% CIs for overall VTE and subgroups.[24,30] The RERI can be interpreted as part of the total effect on the outcome that is attributable to interaction, and the AP as the proportion of cases in the combined exposure group that is due to interaction between the two exposures.[24] For interpretation, a RERI >0 and an AP >0 indicate a departure from additivity of effects, suggesting positive interaction, i.e. the effect of the joint exposure (having both risk factors) on the outcome is greater than the sum of the two separate effects.[24,30]

Results

The baseline characteristics of the study participants across MPV categories (<8.5, 8.5-9.5, ≥9.5 fL) are shown in Table 1. The proportion of men and the mean platelet count decreased across MPV categories. The mean age and BMI, and the proportion of subjects with self-reported history of arterial CVD, current smoking and hormone therapy use did not substantially differ across MPV categories. For each of the five SNPs investigated, carriers of ≥1 risk alleles were similarly distributed in MPV categories (Table 1). The distribution of individuals (%) according to the number of risk alleles in the GRS score is depicted in Figure 2. The median number of risk alleles among the study participants was 2, ranging from 0 to 6.

For each stratum of risk alleles (0 to ≥ 5 risk alleles), MPV categories were similarly distributed (Figure 2).

The risk of overall VTE according to the presence of prothrombotic risk alleles and MPV categories for each of the individual SNPs is shown in Table 2. Because the HRs adjusted for age, sex, BMI and platelet count were similar to the HRs adjusted for age and sex in all the analyses (data not shown), only the fully adjusted models are described. The analyses of rs1799963 (prothrombin G20210A) in *F2* were limited due to the low prevalence of carriers of this SNP in the study population. For the SNPs in *ABO* (non-O blood group), *F11* and *FGG,* the risk estimates were apparently highest in the combined category of a high MPV (≥ 9.5 fL) and ≥1 risk alleles. This was not observed for the SNP in *F5* (FVL). Compared with the reference category (MPV <8.5 fL and 0 risk allele), the presence of MPV ≥ 9.5 fL in combination with ≥1 risk alleles had an additive effect on the risk of overall VTE for the SNPs in *ABO*, *F5*, *F11* and *FGG*, since the thrombosis risk approximated the sum of the separate effects of a high MPV and having ≥1 risk alleles. The measures of biological interaction are described in Supplementary Table 1.

Table 3 displays the HRs for overall VTE according to MPV categories and number of risk alleles in the GRS. Within each MPV stratum (<8.5, 8.5-9.5 and ≥9.5 fL), the risk of VTE increased with an increasing number of risk alleles. Further, when subjects with a low MPV (<8.5 fL) and 0-1 risk allele were set as the reference group, the highest risk estimate for VTE was observed among those with a high MPV (≥ 9.5 fL) who were carriers of ≥4 risk alleles (HR 2.80, 95% CI 1.77-4.43). However, similar to the analysis of the individual SNPs, the combination of a high MPV and the high-risk category of the GRS (≥4 risk alleles) pointed toward an additive effect on the risk of VTE, as suggested by measures of biological interaction, with a RERI of 0.56 (95% CI -0.61 to 1.74) and an AP of 0.20 (95% CI -0.18 to 0.59) (Supplementary Table 1).

Subgroup analyses stratified by provoked or unprovoked events are described in Table 4 and by VTE location (DVT or PE) in Supplementary Table 2. For individuals in the combined category of MPV ≥ 9.5 fL and ≥ 4 risk alleles, the thrombosis risk was most pronounced for unprovoked VTE (HR 4.60, 95% CI 2.20-9.60), when compared with the reference (MPV < 8.5 fL and 0-1 risk allele). As in the overall analysis, measures of biological interaction suggested that the combination of a high MPV and the high-risk category of the GRS had an additive effect on thrombosis risk for all the subgroups (Supplementary Table 3).

Discussion

In this population-based case-cohort study, we investigated the joint effect of a high MPV and five established and common prothrombotic genotypes, assessed either as individual SNPs or as a GRS, on the risk of incident VTE. For overall VTE and subgroups (i.e. DVT, PE, provoked and unprovoked VTE), a high MPV (≥ 9.5 fL) combined with the high-risk category of the GRS (≥ 4 risk alleles) yielded the highest risk estimates for VTE. However, the combination of a high MPV and prothrombotic risk alleles pointed toward an additive effect on the risk of VTE, as the thrombosis risk in the joint category approximated the sum of the separate effects of the exposures, suggesting absence of biological interaction. Similar results were obtained for the combination of a high MPV and each of the individual SNPs that composed the GRS. Our findings suggest that platelet reactivity, as reflected by a high MPV, and common prothrombotic genotypes act independently in the biology of VTE risk.

Several studies have investigated the role of MPV as a diagnostic biomarker for acute VTE. In a recent meta-analysis, patients with acute VTE had a higher MPV than controls but the pooled estimates showed that the diagnostic accuracy of MPV appeared to be modest at most.[31] To date, Brækkan *et al*.[7] have conducted the only study on the association between

MPV and risk of future VTE in the general population. Using data from the Tromsø Study, the authors found that subjects with a high MPV (≥ 9.5 fL) had a 30% higher risk of overall VTE and a 50% higher risk of unprovoked events. In addition, MPV has been explored as a biomarker for future VTE in cancer patients.[32,33] In contrast to the Tromsø Study,[7] the Vienna Cancer and Thrombosis Study (CATS), a large cohort of cancer patients, found that an increased MPV was associated with reduced risk of symptomatic VTE.[32] In the same study, the authors found that a high MPV was associated with improved survival.

To the best of our knowledge, this is the first study to evaluate the presence of biological interaction with regards to VTE risk between a high MPV and prothrombotic SNPs, analyzed either individually or as a GRS. MPV is an especially interesting candidate biomarker for the assessment of thrombosis risk because it is a trait with a strong genetic component,[15,16] and reported to be relatively stable within an individual over time, particularly among healthy subjects.[34,35] The five SNPs investigated in this study are established risk factors for VTE[17,36] and are related to hypercoagulability through several mechanisms. For instance, non-O blood group is known to be associated with higher levels of von Willebrand factor and factor VIII,[18] FVL is associated with the prothrombotic phenotype of resistance to activated protein C,[19] and prothrombin G20210A and *F11* variation display increased levels of prothrombin and factor XI, respectively.[20,22] Further, rs2066865 in *FGG* is associated with changes in the levels of fibrinogen-γ', a product of alternative splicing of *FGG,* which in turn may affect the thrombosis risk.[21]

In this study, we hypothesized that the prothrombotic genotypes when jointly present with large platelets, as reflected by an increased MPV, could have a supra-additive effect on the risk of VTE. Large platelets have shown faster aggregation *in vitro* when stimulated with established platelet agonists, including adenosine diphosphate, epinephrine, collagen, or thrombin.[37-39] Moreover, large platelets contain higher absolute amounts of proteins involved

in platelet function (e.g. glycoprotein IaIIa and IIbIIIa complexes and glycoprotein VI)[12] and are more prone to expose phosphatidylserine, thereby providing a procoagulant surface for the hemostatic system.[39] It is worth noting that platelets are well-known to be involved in the pathogenesis of arterial thrombosis, and that growing evidence from murine models suggests that platelets also contribute to venous thrombus formation.[40-42] Although large platelets, as determined by the measurement of MPV, are more reactive than small platelets and could have a greater potential to contribute to thrombogenesis, in our study, established prothrombotic genotypes in the presence of a high MPV did not result in a supra-additive effect on VTE risk. From a mechanistic viewpoint, our findings suggest that an increased platelet reactivity and the prothrombotic genotypes studied may act through independent pathways in the biology of VTE risk.

The strengths of this study include a prospective study design, participants recruited from the general population and unselected, well-validated VTE events. The Tromsø Study has a high participation rate and a broad age range, presumably leading to a cohort representative of the general population. However, the study also has limitations that merit attention. The study is limited by a low number of VTE events for certain SNPs, especially for those with a low prevalence (e.g. the prothrombin mutation), and our risk estimates and measures of biological interaction should therefore be interpreted with caution, particularly among the subgroups. We did not stratify the GRS into more than three categories of risk alleles due to statistical power limitations. However, it is important to address that when we used the same categories of the GRS previously applied in the Tromsø Study (i.e. 0-1, 2, 3-4, and ≥ 5 alleles),[43,44] similar results were obtained with respect to the joint effect of a high MPV and the high-risk category of the GRS on VTE risk, albeit imprecision of estimates increased, especially due to fewer VTE events among those with ≥ 5 alleles (data not shown). Even though MPV is reported to be relatively stable within an individual over time,[34,35] intra-

individual variation in MPV during the long follow-up period in the parent cohort could have occurred, introducing the possibility for regression dilution bias[45] and a weakening of the results compared with the true associations with regards to the joint effect of MPV and prothrombotic genotypes on the risk of VTE. Despite the well standardized pre-analytical and analytical procedures for measurement of platelet count and MPV in Tromsø 4, we cannot rule out the occurrence of some technical variability in these procedures. However, the approach for blood collection, processing, and laboratory analysis did not differ for cases and subcohort members and was performed without the knowledge of the future case- or subcohort-member status. Therefore, any potential misclassification of MPV measurement would be nondifferential with regards to the VTE status, which could have led to an underestimation of the true associations. Finally, population stratification and hidden relatedness (i.e. the presence of unknown genetic relationships between individuals) can be sources of bias in association studies.[46] However, population stratification would be very unlikely in the present study because most of the participants were Caucasians. Moreover, as previously reported in a study derived from Tromsø 4, only 6% of the participants were related to another individual, and the relatedness between these individuals was distant.[47]

In conclusion, the combination of a high MPV and prothrombotic genotypes, assessed either as individual SNPs or as a GRS, resulted in an additive effect on the risk of VTE, suggesting there is no biological interaction between these risk factors in the pathogenesis of VTE.

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Disclosures

There are no conflicts of interest reported by any of the authors.

Author contributions

L. Jakobsen analyzed data, interpreted the results and drafted the manuscript. T. Frischmuth provided statistical support, interpreted the results, and revised the manuscript. S.K. Brækkan and J-B Hansen designed the study, organized data collection, interpreted the results, and revised the manuscript. V.M. Morelli designed the study, interpreted the results, contributed to the manuscript draft, and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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Figure legends

Figure 1 Flowchart illustrating the composition of the case-cohort study. Participants were recruited from the fourth survey of the Tromsø Study (1994-2012). MPV, mean platelet volume; SNP, single nucleotide polymorphism; VTE, venous thromboembolism.

Figure 2 Distribution (%) of individuals according to the number of risk alleles in the genetic risk score and categories of mean platelet volume (MPV).

Tables

Table 1 Baseline characteristics of the study population according to categories of mean platelet volume (MPV)

	MPV < 8.5 fL	MPV 8.5-9.5 fL	MPV ≥ 9.5 fL
Number of subjects	988	949	490
Age (years), mean ± SD	58 ± 15	59 ± 13	59 ± 14
Male sex, % (n)	47.1 (466)	43.7 (415)	42.5 (208)
Body mass index (kg/m^2), mean ± SD	25.9 ± 4.2	25.9 ± 4.1	26.6 ± 4.1
Platelet count (x 10^9/L), mean ± SD	276.6 ± 65.7	244.6 ± 49.6	215.6 ± 44.4
CVD, % (n)[a]	11.9 (118)	12.4 (118)	14.5 (71)
Smoking, % (n)	31.9 (315)	35.0 (332)	35.3 (173)
Hormone Therapy, % (n)[b]	8.6 (45)	11.4 (61)	10.6 (30)
rs8176719 (*ABO*), ≥ 1 risk alleles, % (n)	64.8 (640)	62.9 (597)	62.0 (304)
rs6025 (*F5*), ≥ 1 risk alleles, % (n)	7.9 (78)	9.4 (89)	8.8 (43)
rs1799963 (*F2*), ≥ 1 risk alleles, % (n)	0.9 (9)	2.3 (22)	1.2 (6)
rs2066865 (*FGG*), ≥ 1 risk alleles, % (n)	44.3 (438)	46.9 (445)	44.9 (220)
rs2036914 (*F11*), ≥ 1 risk alleles, % (n)	79.4 (784)	82.7 (785)	81.8 (401)

Abbreviations: CVD, cardiovascular disease; BMI, body mass index; SD, standard deviation.
[a] Self-reported history of arterial cardiovascular disease (myocardial infarction, angina pectoris, stroke).
[b] In women only.

Table 2 Hazard ratios (HRs) with 95% confidence intervals (CIs) for overall venous thromboembolism according to individual SNPs and MPV categories

Risk alleles	MPV < 8.5 fL		MPV 8.5-9.5 fL		MPV ≥ 9.5 fL	
SNP (*gene*)	Events	HR (95% CI)	Events	HR (95% CI)	Events	HR (95% CI)
rs8176719 (*ABO*)						
0	75	1 (Reference)	92	1.27 (0.93-1.73)	39	1.04 (0.70-1.55)
≥ 1	178	1.42 (1.08-1.87)	168	1.42 (1.08-1.87)	101	1.75 (1.28-2.40)
rs6025 (*F5*)						
0	218	1 (Reference)	220	1.09 (0.89-1.32)	120	1.19 (0.94-1.52)
≥ 1	35	2.38 (1.66-3.40)	40	2.22 (1.57-3.12)	20	2.22 (1.37-3.60)
rs1799963 (*F2*)						
0	249	1 (Reference)	250	1.06 (0.89-1.28)	139	1.18 (0.94-1.48)
≥ 1	4	1.66 (0.62-4.47)	10	2.08 (1.10-3.92)	1	0.65 (0.09-4.65)
rs2036914 (*F11*)						
0	52	1 (Reference)	43	1.07 (0.71-1.62)	22	1.02 (0.61-1.70)
≥ 1	201	1.05 (0.77-1.42)	217	1.13 (0.83-1.53)	118	1.25 (0.89-1.76)
rs2066865 (*FGG)*						
0	131	1 (Reference)	148	1.26 (0.99-1.60)	77	1.29 (0.96-1.73)
≥ 1	122	1.24 (0.97-1.59)	112	1.11 (0.86-1.44)	63	1.29 (0.94-1.78)

Abbreviations: MPV, mean platelet volume; SNP, single nucleotide polymorphism.
HR: adjusted for age (as a time scale), sex, body mass index (continuous variable), and platelet count (continuous variable).

Table 3 Hazard ratios (HRs) with 95% confidence intervals (CIs) for overall venous thromboembolism according to categories of the GRS and MPV

Number of risk alleles	Events	HR (95% CI)[a]	HR (95% CI)[b]
MPV <8.5 fL			
0 - 1	39	1 (Reference)	1 (Reference)
2 - 3	147	1.38 (0.97-1.96)	1.40 (0.98-1.99)
≥ 4	67	1.82 (1.22-2.71)	1.86 (1.25-2.76)
MPV 8.5-9.5 fL			
0 - 1	44	1 (Reference)	1.33 (0.86-2.07)
2 - 3	144	1.07 (0.76-1.51)	1.44 (1.01-2.07)
≥ 4	72	1.44 (0.98-2.10)	1.89 (1.28-2.81)
MPV ≥ 9.5 fL			
0 - 1	21	1 (Reference)	1.38 (0.80-2.36)
2 - 3	81	1.08 (0.66-1.75)	1.45 (0.97-2.14)
≥ 4	38	2.05 (1.20-3.51)	2.80 (1.77-4.43)

Abbreviations: GRS, genetic risk score; MPV, mean platelet volume.

[a] 0 - 1 risk allele as the reference category in each MPV category.

[b] MPV <8.5 fL and 0 - 1 risk allele as the reference category.

HR: adjusted for age (as a time scale), sex, body mass index (continuous variable), and platelet count (continuous variable).

Table 4 Hazard ratios (HRs) with 95% confidence intervals (CIs) for provoked and unprovoked venous thromboembolism (VTE) according to categories of the GRS and MPV

Number of risk alleles	Events	HR (95% CI)	Events	HR (95% CI)
	Provoked VTE		**Unprovoked VTE**	
MPV <8.5 fL				
0 - 1	27	1 (Reference)	12	1 (Reference)
2 - 3	95	1.31 (0.86-2.02)	52	1.60 (0.85-2.99)
≥ 4	31	1.27 (0.76-2.13)	36	3.12 (1.62-6.02)
MPV 8.5-9.5 fL				
0 - 1	25	1.13 (0.65-1.95)	19	1.78 (0.85-3.73)
2 - 3	81	1.20 (0.77-1.86)	63	1.98 (1.06-3.70)
≥ 4	42	1.62 (0.99-2.64)	30	2.50 (1.27-4.91)
MPV ≥ 9.5 fL				
0 - 1	13	1.29 (0.66-2.51)	8	1.60 (0.65-3.95)
2 - 3	44	1.16 (0.71-1.90)	37	2.06 (1.06-4.03)
≥ 4	18	1.96 (1.06-3.61)	20	4.60 (2.20-9.60)

Abbreviations: GRS, genetic risk score; MPV, mean platelet volume.
HR: adjusted for age (as a time scale), sex, body mass index (continuous variable), and platelet count (continuous variable).