Journal of the American Heart Association

ORIGINAL RESEARCH

Genome-Wide Search for Nonadditive Allele Effects Identifies *PSKH2* as Involved in the Variability of Factor V Activity

Blandine Gendre, MSc; Angel Martinez-Perez, MSc; Marcus E. Kleber, MD; Astrid van Hylckama Vlieg, PhD; Anne Boland, PhD; Robert Olaso, PhD; Marine Germain, BSc; Gaëlle Munsch, PhD; Angela Patricia Moissl, MSc; Pierre Suchon, MD, PhD; Juan Carlos Souto, MD, PhD; José Manuel Soria, PhD; Jean-François Deleuze, PhD; Winfried März, MD; Frits R. Rosendaal, MD, PhD; Maria Sabater-Lleal, PhD; Pierre-Emmanuel Morange, MD, PhD; David-Alexandre Trégouët, PhD; CHARGE Hemostasis Working Group*

BACKGROUND: Factor V (FV) is a key molecular player in the coagulation cascade. FV plasma levels have been associated with several human diseases, including thrombosis, bleeding, and diabetic complications. So far, 2 genes have been robustly found through genome-wide association analyses to contribute to the inter-individual variability of plasma FV levels: structural F5 gene and PLXDC2.

METHODS AND RESULTS: The authors used the underestimated Brown-Forsythe methodology implemented in the QuickTest software to search for non-additive genetic effects that could contribute to the inter-individual variability of FV plasma activity. QUICKTEST was applied to 4 independent genome-wide association studies studies (LURIC [Ludwigshafen RIsk and Cardiovascular Health Study], MARTHA [Marseille Thrombosis Association], MEGA [Multiple Environmental and Genetic Assessment], and RETROVE [Riesgo de Enfermedad Tromboembolica Venosa]) totaling 4505 participants of European ancestry with measured FV plasma levels. Results obtained in the 4 cohorts were meta-analyzed using a fixed-effect model. Additional analyses involved exploring haplotype and gene×gene interactions in downstream investigations. A genome-wide significant signal at the *PSKH2* locus on chr8q21.3 with lead variant rs75463553 with no evidence for heterogeneity across cohorts was observed (P=0.518). Although rs75463553 did not show an association with mean FV levels (P=0.49), it demonstrated a robust significant (P=3.38x10^{-9}) association with the variance of FV plasma levels. Further analyses confirmed the reported association of *PSKH2* with neutrophil biology and revealed that rs75463553 likely interacts with two loci, *GRIN2A* and *POM121L12*, known for their involvement in smoking biology.

CONCLUSIONS: This comprehensive approach identifies the role of *PSKH2* as a novel molecular player in the genetic regulation of FV, shedding light on the contribution of neutrophils to FV biology.

Key Words: Brown-Forsythe test ■ coagulation ■ factor V plasma levels ■ non-additive genetic effects ■ parent-of-origin effects

Factor V (FV) is a central protein of the coagulation cascade. By acting as a cofactor for activated factor X, FV facilitates the conversion of prothrombin to thrombin,[1] which, in turn, converts fibrinogen into fibrin, the main component of blood clots, and also activates platelets. Mainly expressed in the liver, FV can

Correspondence to: David-Alexandre Trégouët, INSERM, Bordeaux Population Health Research Center, University of Bordeaux, UMR 1219, ELEANOR, Bordeaux, France. Email: david-alexandre.tregouet@u-bordeaux.fr

*A complete list of the CHARGE Hemostasis Working Group members can be found in the Supplemental Material.

This article was sent to Ferhaan Ahmad, MD, PhD, Senior Associate Editor, for review by expert referees, editorial decision, and final disposition.

Preprint posted on BioRxiv February 09, 2024. doi: https://doi.org/10.1101/2024.02.08.579474.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.124.034943

For Sources of Funding and Disclosures, see page 8.

JAHA is available at: www.ahajournals.org/journal/jaha

RESEARCH PERSPECTIVE

What Is New?

- We demonstrated the role of the PSKH2 locus in the genetic regulation of factor V plasma levels.
- We observed that *PSKH2* genetic variants were associated with neutrophils only in individuals exhibiting high platelet counts.

What Question Should Be Addressed Next?

- PSKH2 variant(s) responsible for the observed association remain to be identified.
- The exact role of α-granules of platelets in the relationship between *PSKH2* and factor V regulation remains to be elucidated.

Nonstandard Abbreviations and Acronyms

BF	Brown-Forsythe
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
FV	Factor V
GAIT	Genetic Analysis of Idiopathic Thrombophilia
LURIC	Ludwigshafen RIsk and Cardiovascular Health
MARTHA	Marseille Thrombosis Association
MEGA	Multiple Environmental and Genetic Assessment
POE	Parent-of-origin effect
RETROVE	Riesgo de Enfermedad Tromboembolica Venosa
VT	Venous thrombosis

also be stored and released by platelets,[2] which provide a surface for the coagulation reactions to occur and can contribute to amplify the coagulation process. There is natural variability in FV levels, and increased/decreased FV levels have been observed in several conditions including mainly bleeding[3–6] and thrombotic disorders[7,8] but also in infections,[9] inflammation,[10] pregnancy,[11,12] hormone contraceptives usage,[13] and impaired liver dysfunction.[14]

Understanding the exact sources of variability of FV levels is crucial for better identification of individuals at higher risk of clotting disorders and for better targeting appropriate preventive and therapeutic strategies. Age, sex, smoking,[15] obesity,[16] and, to lesser extent, medication use[17] are the main environmental variables known to influence FV plasma levels. Genetic factors have also been demonstrated to contribute to the inter-individual FV variability including single-nucleotide polymorphisms (SNPs) at *F5* and *PLXDC2* loci.[18] The implication of *F5* SNPs in the regulation of FV plasma levels dates back to the end of the 1 990s[19] when the HR2 haplotype tagged by rs6 027 was identified. More recently, the *F5* rs4524 was also shown to influence plasma FV levels independently of the rs6 027.[18] The first genome-wide association study (GWAS) on FV levels, based on ≈1700 individuals identified the *PLXDC2* locus as a second genetic player in FV regulation.[18] Altogether, these 3 loci explain <15% of the variability in plasma FV levels, suggesting that additional molecular determinants could be involved in its regulation. With the aim of characterizing novel genomic regulators of FV plasma levels, we deployed a large-scale agnostic genome-wide search for non-additive genetic effects associated with FV plasma levels using the Brown-Forsythe (BF) methodology implemented in the QuickTest software.[20] While initially developed for detecting parent-of-origin effects (POEs), this methodology can also detect loci prone to gene × gene or gene × environment interactions, making it a valuable tool to complement standard genome-wide association analysis. POE is a specific kind of genomic imprinting[21,22] and several studies suggest that such epigenetic mechanisms could impact key genes of the coagulation cascade,[23,24] including *PLXDC2*.[25] In this work, the BF methodology was applied to genome-wide genotype data available in 4 study populations totaling 4 505 individuals with measured FV plasma levels.

METHODS

This work builds on 4 independent study populations of unrelated individuals, all of European ancestry, that are part of the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium.[26] They include the LURIC (Ludwigshafen Risk and Cardiovascular Health) study,[27] composed of 1833 individuals among whom 100 reported venous thrombosis (VT); a subsample of 1011 participants from the MARTHA (Marseille Thrombosis Association) study,[28] which consists of 1 592 patients with VT; a subsample of 865 participants from the MEGA (Multiple Environmental and Genetic Assessment) study,[29] comprising 1289 patients with VT; and the RETROVE (Riesgo de Enfermedad Tromboembolica Venosa)[30] study, which included a sample of 398 patients with VT and 398 controls. All participants were phenotyped for plasma FV activity and genotyped for genome-wide polymorphisms using high-throughput DNA arrays. Genotype data were further imputed using

different reference panels. Detailed descriptions of the phenotype and genotype measurements are given in Table S1 together with additional details on imputation and genotype quality controls.

Research has been performed in accordance with the Declaration of Helsinki and all participating studies were approved by the respective institutional ethics committees: the ethics committee at the Medical Association of Rheinland-Pfalz (Aerztekammer Rheinland-Pfalz) for the LURIC study, the "Mediterranean I Committee for the Protection of Individuals" (reference: 12 61) for the MARTHA study, the "Medical ethics committee of the Leiden University Medical Center" for the MEGA study, and the "Institutional review board of the Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) for the RETROVE study (approval number 04/2012).

Written informed consent was obtained from all participants to be included in the genetic investigations.

Summary statistics of the POE meta-analysis are available in the GWAS catalog under accession number GCST90446466. Other data are available upon request from the corresponding author.

Statistical Analysis

The BF methodology implemented in the QuickTest program

Standard GWAS are generally performed for detecting SNPs with additive allele effects on a trait of interest. The statistical modeling can thus be expressed, in case of a quantitative trait *Y*, as:

$$E(Y \mid G) = \alpha + \beta G \quad (1)$$

with $G = \{0,1,2\}$ according to the number of tested alleles carried by an individual. This model assumes the absence of POE for the tested allele while POE would imply that the effect of the tested allele would depend on whether it has been inherited from the father or from the mother. In that case, a POE model could be written as:

$$E(Y|G) = \alpha + \frac{1}{2}(\beta_m + \beta_p)G \quad (2)$$

where β_m and β_p corresponds to the maternal and paternal effects, respectively, if they are identifiable. In the absence of family data, these effects cannot be distinguished and therefore cannot be estimated. To solve this problem, Hoggart et al.[20] proposed an appealing methodology to allow the detection of POE in genotype data of unrelated individuals only. By rewriting model (2) as:

$$E(Y \mid G = 0) = \alpha$$

$$E(Y \mid G = 1) = \alpha + \pi\beta_m + (1 - \pi)\beta_p$$

$$E(Y \mid G = 2) = \alpha + \beta_m + \beta_p$$

where π is a random variable following a Bernoulli distribution with parameter ½ (50% of alleles coming from the paternal and 50% from the maternal transmission), they observed that, in the presence of POE, the phenotypic variance in heterozygous individuals should be higher than the phenotypic variances in the 2 groups of homozygotes:

$$Var(Y \mid G = 0) = Var(Y \mid G = 2) = \sigma^2$$

$$Var(Y \mid G = 1) = \sigma^2 + \frac{1}{4}(\beta_m - \beta_p)^2$$

They then proposed to use the BF test,[31] a robust version of the Levene test, to assess whether the phenotypic variance in heterozygote carriers of a given SNP is significantly higher than the phenotypic variances observed in homozygotes. They further showed that this BF test is equivalent to performing a linear model where the absolute deviation of the phenotype from the intra-genotype median is regressed on a binary variable indicating whether an individual *i* is heterozygote at the tested *j* SNP.

$$| y_{ij} - \tilde{y}_j | = \mu + \gamma \mathbb{1}_{(\text{Individual } i \text{ is heterozygous for genotype } j)} + \varepsilon$$

A positive and significant value for the γ regression coefficient associated with this indicator variable is a sign of POE.

They implemented this BF framework in the QuickTest program (https://wp.unil.ch/sgg/program/quicktest/), which can easily be applied to large GWAS genotype data sets to detect POE acting on a quantitative trait. Of note, as highlighted by Hoggart et al, while the presence of POE can lead to a significant BF test, the inverse is not necessarily true, as a significant BF test can also be caused by other phenomena such as haplotype effects, gene × gene or gene × environment interactions.

For the present work, all SNPs with imputation quality $r^2 > 0.5$, minor allele frequency >0.005, and a number of heterozygous individuals >20 were tested through the BF methodology in relation to FV activity. Analyses were adjusted for age, sex, and main principal components derived from genome-wide genotype data to account for uncontrolled population stratification. Additional adjustment on case–control status was performed in the LURIC and RETROVE trials.

The QuickTest software was applied in each study and results were then meta-analyzed using a fixed-effect meta-analysis as implemented in GWAMA software.[32]

Heterogeneity across study populations was assessed by Cochran Q statistic and I^2 index.

Genome-wide statistical significance was considered at BF *P* values < $5x10^{-8}$.

Search for gene × gene interactions

To further investigate the possible source explaining each genome-wide significant BF signal, we sought SNPs that could modulate FV activity differentially according to the heterozygote status at the lead SNP identified by the QuickTest analysis. For this, in each contributing study population, we conducted a genome-wide interaction analysis based on a linear model where FV activity was regressed for age, sex, genetically derived principal components, heterozygote status at the lead BF SNP, any SNP, and an interaction term between the latter 2 components. All SNPs with imputation quality $r^2<0.5$ and minor allele frequency <0.005 were excluded from the analyses. These genome-wide interaction analyses were conducted using Plink2 (www.cog-genomics.org/plink/2.0/).[33]

For each tested SNP, interaction terms were then meta-analyzed across the 4 studies using a fixed-effect meta-analysis as implemented in the GWAMA software.[32]

RESULTS

In total, 4505 individuals were studied in this work. A brief description of the general characteristics of the 4 contributing studies is given in Table 1.

A total of 7,300,264 SNPs were tested in relation to FV activity through the BF framework. A Manhattan plot summarizing the statistical findings is shown in Figure. The associated Quantile-Quantile plot is given in Figure S1. One locus, chr8q21.3, reached the prespecified genome-wide statistical threshold of 5×10^{-8}. The lead SNP was rs75463553 and its POE γ coefficient was 0.129±0.022 ($P=3.38\times10^{-9}$). As shown in Table 2, the POE γ coefficients were homogeneous among the 4 contributing studies as were the allele frequencies. We observed that the variance in FV activity was higher in carriers of the G/T genotype compared with the combined groups of G/G and T/T genotypes, while no association with mean FV levels was observed (*P*=0.49 in the combined 4 studies).

Of note, no POE signal was observed at the *F5* rs6027 and rs4524 (*P*>0.95 for both) nor the *PLXDC2* rs927826 (*P*=0.68) polymorphisms previously reported to associate with plasma FV mean levels.[18] This would suggest that non-additive allele effects are unlikely to exist at these 2 loci in relation to plasma FV activity.

rs75463553 maps to an intronic region of the noncoding RNA LOC105375623, located downstream to *SLC7A13* and upstream to *ATP6V0D2*. Neither of these loci has an obvious link to the regulation of FV. rs75463553 is in strong linkage disequilibrium with 6 other nearby SNPs with genome-wide significant BF *P* values (Figure S2–Table S2). These 6 SNPs generate 3 haplotypes, with a frequency of >1%. None of them are associated with mean FV activity (Table S3), suggesting that the detected BF signal was unlikely attributable to linkage disequilibrium effects between nearby SNPs.

rs75463553 also exhibits moderate linkage disequilibrium ($r^2>0.40$, $|D'|>0.80$) with other SNPs, spanning from *PSKH2* to *WWP1* (Figure S2). *PSKH2* encoding for a protein serine kinase is a good biological candidate to contribute to FV regulation. First, the *PSKH2* locus has been implicated in the regulation of neutrophil counts,[34] whose activation has been shown to associate with increased F5 expression in individuals with inflammatory disorders.[35] Second, the release of α-granules from platelets, recognized as 1 of 2 possible sources of circulating FV,[36,37] is associated with neutrophil activation.[38–40] In addition, in the GoDMC database

Table 1. Brief Description of the Studied Populations

	LURIC	MARTHA	MEGA	RETROVE
Total, n	1833	1011	865	796
VT cases, %	5.4	100	100	50
Age (SD), y	62.3 (10.8)	47.6 (15.7)	47.6 (12.9)	54.4 (19.9)
Women, n (%)	537 (29)	633 (63)	444 (51)	406 (51)
FV, U/dL	1.13 (0.22)	1.07 (0.23)	0.95 (0.19)	0.99 (0.20)
Platelet count, 10^9/L	231.1 (66.7)	256.4 (68.9)	NA	235.3 (62.4)
Neutrophil count*	59.6 (9.46)	61.1 (8.91)	NA	58.1 (9.7)
Smoking at sampling, n (%)	342 (18)	167 (18)	NA	129 (16)

FV indicates factor V; LURIC, Ludwigshafen Risk and Cardiovascular Health Study; MARTHA, Marburg and Thompson's Atherosclerosis Study; MEGA, Multi-Ethnic Genotyping Array; NA, not available; RETROVE, Retrospective Analysis of Genetic Variants and Clinical Outcomes in Patients With Vascular Disease; and VT venous thrombosis.

*Percentage compared with total white blood cell count.

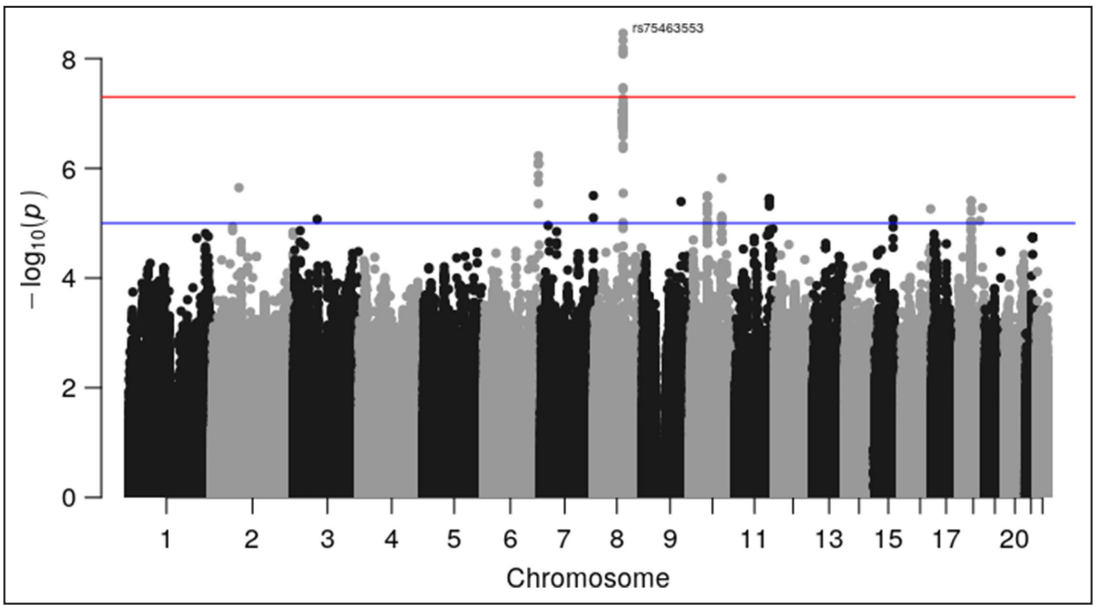

Figure. Manhattan plot of 4 studies involving unrelated European individuals for detecting parent-of-origin effect on factor V plasma levels (n=4505).

(http://mqtldb.godmc.org.uk/index),[41] rs75463553 demonstrates strong associations with whole blood DNA methylation at several CpG sites at the *PSKH2* locus (Figure S3) such as cg00001099 ($P=5.4\times10^{-291}$), cg26186954 ($P=7.54\times10^{-185}$), and cg20982735 ($P=1.13\times10^{-157}$). DNA methylation is one of the epigenetic mechanisms related to POEs,[42] and identification of such effects is an objective of the QuickTest program used in this study.

We therefore investigated the association of rs75463553 with neutrophil counts in the LURIC, MARTHA, and RETROVE studies. The pattern of associations between rs75463553 and neutrophils was homogeneous across LURIC, MARTHA, and RETROVE

Table 2. Association of rs75463553 With Phenotypic Mean and Variance of FV Activity

	LURIC	MARTHA	MEGA	RETROVE
Total, n	1833	1011	865	796
Minor allele frequency (G/T)	0.145	0.117	0.156	0.106
Imputation r^2	0.980	0.960	0.975	0.982
Number				
GG	1338	779	614	635
GT	463	222	235	153
TT	32	10	16	8
FV activity, mean				
GG	1.132	1.056	0.958	0.990
GT	1.142	1.097	0.946	0.998
TT	1.059	1.082	0.912	1.119
FV activity, SD				
GG	0.212	0.224	0.176	0.192
GT	0.228	0.264	0.217	0.220
TT	0.185	0.261	0.157	0.292
POE γ	0.098±0.033	0.181±0.049	0.152±0.050	0.115±0.055
P value	1.661×10^{-3}	1.042×10^{-4}	1.307×10^{-3}	1.913×10^{-2}

The parent-of-origin effect (POE) γ did not show any evidence of heterogeneity among cohorts ($I^2=0$, $P=0.518$).

FV indicates factor V; LURIC, Ludwigshafen Risk and Cardiovascular Health Study; MARTHA, Marburg and Thompson's Atherosclerosis Study; MEGA, Multi-Ethnic Genotyping Array; and RETROVE, Retrospective Analysis of Genetic Variants and Clinical Outcomes in Patients With Vascular Disease.

Table 3. Association of rs75463553 With Neutrophil Counts in the LURIC, MARTHA, and RETROVE Studies According Platelet Count

	All population			Platelets ≤230			Platelets >230		
	LURIC	MARTHA	RETROVE	LURIC	MARTHA	RETROVE	LURIC	MARTHA	RETROVE
GG	4.21 (1.54) N=1312	3.95 (1.43) N=675	3.93 (1.54) N=635	3.89 (1.38) N=728	3.62 (1.24) N=239	3.67 (1.39) N=318	4.60 (1.63) N=584	4.13 (1.49) N=436	4.19 (1.64) N=317
GT	4.17 (1.57) N=455	4.08 (1.59) N=186	3.93 (1.64) N=153	3.92 (1.52) N=255	3.59 (1.52) N=72	3.54 (1.33) N=73	4.49 (1.58) N=200	4.38 (1.56) N=114	4.29 (1.82) N=80
TT	4.43 (2.11) N=33	4.87 (2.79) N=10	3.75 (1.51) N=8	3.71 (0.94) N=21	4.02 (0.94) N=3	3.19 (0.37) N=6	5.68 (2.94) N=12	5.24 (3.30) N=7	5.43 (2.79) N=2
β±SE P*	β=0.26±0.27 *P*=0.343	β=0.92±0.47 *P*=0.053	β=−0.16±0.35 *P*=0.654	β=−0.18±0.32 *P*=0.571	β=0.42±0.76 *P*=0.581	β=−0.24±0.35 *P*=0.497	β=1.09±0.48 *P*=0.023	β=1.09±0.59 P=0.063	β=0.53±0.76 *P*=0.480
Combined β	β=+ 0.31±0.22 *P*=0.146			β=−0.137±0.25 *P*=0.59			β=+ 1.08±0.35 *P*=0.0023		

LURIC indicates Ludwigshafen Risk and Cardiovascular Health Study; MARTHA, Marburg and Thompson's Atherosclerosis Study; MEGA, Multi-Ethnic Genotyping Array; RETROVE, Retrospective Analysis of Genetic Variants and Clinical Outcomes in Patients With Vascular Disease; and SE, standard error.
*Association was tested using a linear model adjusted for age and sex under the assumption of recessive genetic effect β.

trials and compatible with a recessive effect of the rs75463553-T allele (Table 3). Indeed, we observed a trend for neutrophil counts being higher in homozygote carriers of the rs75463553-T allele (Table 3). However, this association was mainly observed in individuals with high platelets counts. As shown in Table 3, when the combined sample was divided according to the median of platelets observed in the global population, rs75463553-TT carriers with platelet counts above the median exhibited a significantly (*P*=0.0023) higher neutrophil count, while no association (*P*=0.59) was observed in the group of individuals with lower platelet counts. Surprisingly, we observed that the significant association was mainly restricted to smokers (Table 4). However, no such interactive effects were observed on FV activity (data not shown), suggesting that the complex relationship between rs75463553, platelets, and neutrophil count would unlikely explain the statistical BF signal observed on FV activity. Consistent with this hypothesis, the observation that the effect of rs75463553 on FV variability, as assessed by the BF methodology, remains significant in relation to platelets and smoking (Table S4), except in smokers with high platelet counts, supports this finding.

Of note, in the LURIC, MARTHA, and RETROVE trials, where neutrophil and platelet counts were measured, FV activity did not exhibit a significant correlation with either parameter (Table S5).

We then further explored whether the original detected BF signal could be explained by the interaction of rs75463553 with other SNPs. The genome-wide scan conducted in the 4 contributing studies identified one genome-wide significant ($P=2.6\times10^{-8}$) interaction (Table S6, Figure S4). In heterozygous carriers of the rs75463553-T allele, carrying the rs7190785-A allele at *GRIN2A* on chromosome 16p13.2 was associated with increased FV activity (β=0.05±0.01, $P=2.52\times10^{-8}$). By contrast, no association was observed for the rs7190785 allele in individuals with GG or TT genotypes at rs75463553 (β=−0.01±0.01, *P*=0.22). This phenomenon was consistent in the LURIC, MARTHA, and MEGA studies but not in the RETROVE trial (Table S7). It is worth noting that a second interaction signal nearly reached genome-wide significance ($P=6.27\times10^{-8}$),

Table 4. Association of rs75463553 With Neutrophil Counts According to Platelet Counts and Smoking in the LURIC, MARTHA, and RETROVE Studies Combined

	Platelets ≤230		Platelets >230	
	Non smokers	Smokers	Non smokers	Smokers
rs75463553				
GG/GT	3.59 (1.30) N=945	4.04 (1.47) N=741	4.1 (1.52) N=1044	4.77 (1.65) N=688
TT	3.49 (0.83) N=16	3.81 (0.92) N=14	4.19 (1.5) N=11	6.97 (3.44) N=10
β±SE *P* value*	β=− 0.032±0.323 *P*=0.921	β=− 0.227±0.394 *P*=0.564	β=+ 0.084±0.457 *P*=0.853	β=+ 2.17±0.538 $P=6.07\ 10^{-5}$

LURIC indicates Ludwigshafen Risk and Cardiovascular Health Study; MARTHA, Marburg and Thompson's Atherosclerosis Study; MEGA, Multi-Ethnic Genotyping Array; RETROVE, Retrospective Analysis of Genetic Variants and Clinical Outcomes in Patients With Vascular Disease; and SE, standard error.
*Association was tested using a linear model adjusted for age, sex, and cohort under the assumption of a recessive TT genetic effect.

mapping to the *POM121L12* locus on chr7p12.1, which, like *GRIN2A*, has been observed in several GWAS to be associated with smoking phenotypes.[43–45]

DISCUSSION

This work was motivated by the search of non-additive genetic effects that could contribute to the inter-individual variability of FV plasma activity. To achieve this objective, we used an underestimated methodology with great potential, that leverages existing GWAS data in a very efficient and quick manner, as it was implemented in the easy-to-use QuickTest software.[20] Even if the method was initially proposed to detect POE effects, it also has potential to detect non-additive genetic effects that could be caused by gene × gene or gene × environment interactions. Its application here provides evidence for the presence of gene × smoking interaction in the modulation of FV plasma activity.

The application of this methodology to 4505 individuals phenotyped for FV activity and with GWAS data identified SNPs at the 8q21.3 locus significantly associated with the variability of FV activity. Using publicly available resources, we observed that the lead SNP at this locus, rs75463553, was associated with DNA methylation levels at several CpG sites in the *PSKH2* gene. *PSKH2* encodes a protein serine kinase, about which little is known. Some genetic studies have linked *PSKH2* SNPs with neutrophil[34,46] and myeloid leukocyte[34] counts, whose roles in coagulation and thrombotic pathways have been highly discussed in the literature[38,39,47]. In our work, we observed an association between rs75463553 and neutrophil counts, but this association could not explain the genome-wide signal we detected. We sought to investigate whether this signal could be caused by POEs in a family study, but we were only able to assess this hypothesis in a sample of 21 families from the GAIT1 (Genetic Analysis of Idiopathic Thrombophilia) study[48] Unfortunately, only 26 informative meioses were available to test for a differential paternal–maternal effect of the rs75463553 and no statistical association was observed (Table S8). Of note, *PSKH2* was not detected to be prone to POE using an alternative methodology based on sequencing data and applied to several phenotypes, including neutrophil counts, from the UK Biobank.[49] This would suggest that the signal we detected using the QuickTest software could be attributable to other phenomena rather than POE. In line with this hypothesis are the candidate gene × gene interactions we identified with 2 loci, *GRIN2A* and *POM121L12*, which have been proposed to be involved in smoking phenotypes in previous GWAS.[43–45] Unfortunately, smoking status at the time of blood sampling was not available in all contributing studies and therefore it was not possible to assess whether the observed signal was attributable to complex interactions between several polymorphisms and smoking. Similarly, our studies had limited information about additional environmental covariates, which prevented us from performing more exhaustive gene × environment interaction analyses and from determining whether the *PSKH2* locus statistical signal could underline such epistasis phenomena. Another limitation of this work pertains to its restriction to European-ancestry populations. It would be valuable to determine whether cross-ancestry studies could help clarify the observed association as it is sometimes the case in the context of standard GWAS.

Importantly, a significant proportion (≈20%) of circulating FV is found within the α-granules of platelets.[36,37] It would be interesting to assess whether the variability in the source of circulating FV could help explain the statistical signal we observed at the *PSKH2* locus, in particular in view of the enhanced effects we observed in individuals with high platelet counts. However, exploring this hypothesis would require the measurement of intra-platelet FV, which, unfortunately, is not possible using the biobanked platelet-free plasma.

In conclusion, this work provides strong statistical argument supporting the role of the *PSKH2* locus in the variability of FV activity. However, more in-depth investigations are now needed to characterize the exact underlying mechanisms. In addition, this work also emphasizes how to leverage existing large GWAS data sets to detect non-additive allele effects using the BF methodology, as implemented in the QuickTest program, which could explain part of the missing heritability that still exists for most complex phenotypes.

ARTICLE INFORMATION

Received February 13, 2024; accepted August 23, 2024.

Affiliations

INSERM, Bordeaux Population Health Research Center, UMR 1219, ELEANOR, University of Bordeaux, Bordeaux, France (B.G., M.G., G.M., D-A.T.); Unit of Genomics of Complex Diseases, Institut de Recerca Sant Pau (IR SANT PAU), Barcelona, Spain (A.M., J.M.S., M.S.); Centre for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain (A.M., J.C.S., J.M.S., M.S.); Department of Medicine V, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany (M.E.K., A.P.M., W.M.); SYNLAB Center of Human Genetics Mannheim, Manheim, Germany (M.E.K.); Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands (A.v.H.V., F.R.R.); CEA, Centre National de Recherche en Génomique Humaine (CNRGH), Université Paris-Saclay, Evry, France (A.B., R.O., J-F.D.); Laboratory of Excellence GENMED (Medical Genomics), Evry, France (A.B., R.O., J-F.D.); Cardiovascular and Nutrition Research Center (C2VN), INSERM, INRAE, Aix-Marseille University, Marseille, France (P.S., P-E.M.); Thrombosis and Haemostasis Unit, Hospital de la Santa Creu i Sant Pau and Institut d'Investigació Biomèdica Sant Pau (IIB-Sant Pau), Barcelona, Spain (J.C.S.); Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria (W.M.); SYNLAB Academy, SYNLAB Holding Germany, Mannheim and Augsburg, Germany (W.M.); and Cardiovascular Medicine Unit, Department of Medicine, Karolinska Institutet, Stockholm, Sweden (M.S.).

Sources of Funding

B.G. benefited from the Ecole Universitaire de Recherche Digital Public Health (EUR DPH), a PhD program supported within the framework of the Programme des Investissements d'Avenir (PIA3—project reference 17-EURE-0019). D.A.T. was supported by the Senior Chair on "A multi omics approach to tackle the epidemiology of venous thromboembolism" (EPIDEMIOM-VT) from the University of Bordeaux Initiative of Excellence (IdEX); statistical analyses benefited from the computing center of the University of Bordeaux (CBiB). The MARTHA project was supported by a grant from the Program Hospitalier de la Recherche Clinique and the Laboratory of Excellence on Medical Genomics (GENMED; ANR-10-LABX-0013), a research program managed by the Agence Nationale de la Recherche (ANR) as part of the French Investment for the Future (ANR-10-LABX-0013). MEGA study was supported by the Netherlands Heart Foundation (NHS98.113 and NHS208B086), the Dutch Cancer Foundation (RUL 99/1992), and the Netherlands Organization for Scientific Research (912–03-033|2003). The RETROVE trial acknowledges support from the Government of Catalonia and CERCA Program (2017 SGR 1736), from the Spanish National Institute of Health Carlos III (ISCIII grants PI12/000612, PI15/00269, and PI18/00434), and from the nonprofit organization "Associació Activa'TT per la Salut." Maria Sabater-Lleal is supported by a Miguel Servet contract from the ISCIII Spanish Health Institute (CPII22/00007) and co-financed by the European Social Fund. The genotyping service was performed at the genotyping National Centre (CeGEN) of the Instituto de Salud Carlos III (ISCIII) (CeGen-PRB3-ISCIII) I, and it is supported by grant PT17/0019 of the PE I+D+i 2013-2016, funded by ISCIII and the European Regional Development Fund (ERDF) grant PI21/01772 from Spanish Ministerio de Ciencia, Innovación y Universidades. The Centre for Biomedical Network Research on Rare Diseases (CIBERER) is an initiative of ISCIII, cofinanced by European Regional Development Fund (ERDF), "A way to build Europe." The LURIC trial was supported by the 7th Framework Programs AtheroRemo (grant agreement number 201668) and RiskyCAD (grant agreement number 305739) of the European Union and by the HORIZON2020 Programs TO_AITION (grant agreement number 848146) and TIMELY (grant agreement number 101017424) of the European Union.

Disclosures

None.

Supplemental Material

CHARGE Hemostasis Working Group members
Tables S1–S8
Figures S1–S4
Data S1

REFERENCES

1. Bouchard BA, Chapin J, Brummel-Ziedins KE, Durda P, Key NS, Tracy PB. Platelets and platelet-derived factor Va confer hemostatic competence in complete factor V deficiency. *Blood*. 2015;125:3647–3650. doi: 10.1182/blood-2014-07-589580
2. Camire RM, Bos MH. The molecular basis of factor V and VIII procofactor activation. *J Thromb Haemost JTH*. 2009;7:1951–1961. doi: 10.1111/j.1538-7836.2009.03622.x
3. Lippi G, Favaloro E, Montagnana M, Manzato F, Guidi G, Franchini M. Inherited and acquired factor V deficiency. *Blood Coagul Fibrinolysis Int J Haemost Thromb*. 2011;22:22–166. doi: 10.1097/MBC.0b013e3283424883
4. Huang JN, Koerper MA. Factor V deficiency: a concise review. *Haemoph Off J World Fed Hemoph*. 2008;14:1164–1169. doi: 10.1111/j.1365-2516.2008.01785.x
5. Nichols WC, Seligsohn U, Zivelin A, Terry VH, Hertel CE, Wheatley MA, Moussalli MJ, Hauri HP, Ciavarella N, Kaufman RJ, et al. Mutations in the ER-Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of coagulation factors V and VIII. *Cell*. 1998;93:61–70. doi: 10.1016/S0092-8674(00)81146-0
6. Duckers C, Simioni P, Spiezia L, Radu C, Dabrilli P, Gavasso S, Rosing J, Castoldi E. Residual platelet factor V ensures thrombin generation in patients with severe congenital factor V deficiency and mild bleeding symptoms. *Blood*. 2010;115:879–886. doi: 10.1182/blood-2009-08-237719
7. Suehisa E, Kawasaki T, Toku M, Hidaka Y. Low level of factor V is associated with development of deep-vein thrombosis in Japanese patients. *Thromb Res*. 2010;125:128–133. doi: 10.1016/j.thromres.2009.05.010
8. Kamphuisen PW, Rosendaal FR, Eikenboom JC, Bos R, Bertina RM. Factor V antigen levels and venous thrombosis: risk profile, interaction with factor V leiden, and relation with factor VIII antigen levels. *Arterioscler Thromb Vasc Biol*. 2000;20:1382–1386. doi: 10.1161/01.ATV.20.5.1382
9. Stefely JA, Christensen BB, Gogakos T, Cone Sullivan JK, Montgomery GG, Barranco JP, van Cott EM. Marked factor V activity elevation in severe COVID-19 is associated with venous thromboembolism. *Am J Hematol*. 2020;95:1522–1530. doi: 10.1002/ajh.25979
10. Esmon CT. The interactions between inflammation and coagulation. *Br J Haematol*. 2005;131:417–430. doi: 10.1111/j.1365-2141.2005.05753.x
11. Brenner B. Haemostatic changes in pregnancy. *Thromb Res*. 2004;114:409–414. doi: 10.1016/j.thromres.2004.08.004
12. Hellgren M. Hemostasis during normal pregnancy and puerperium. *Semin Thromb Hemost*. 2003;29:125–130. doi: 10.1055/s-2003-38897
13. Movérare S, Skrtic S, Lindberg MK, Dahlman-Wright K, Ohlsson C. Estrogen increases coagulation factor V mRNA levels via both estrogen receptor-alpha and -beta in murine bone marrow/bone. *Eur J Endocrinol*. 2004;151:259–263. doi: 10.1530/eje.0.1510259
14. Lefrère JJ, Conard J, Mavier P, Bettan L, Beaugrand M, Gozin D, Lerable J, Dhumeaux D, Samama M. Coagulation assays as diagnostic markers of hepatocellular carcinoma. *Thromb Haemost*. 1988;60:468–470. doi: 10.1055/s-0038-1646992
15. Csordas A, Bernhard D. The biology behind the atherothrombotic effects of cigarette smoke. *Nat Rev Cardiol*. 2013;10:219–230. doi: 10.1038/nrcardio.2013.8
16. Nagai N, Hoylaerts MF, Cleuren AC, Van Vlijmen BJM, Lijnen HR. Obesity promotes injury induced femoral artery thrombosis in mice. *Thromb Res*. 2008;122:549–555. doi: 10.1016/j.thromres.2007.12.017
17. Paret N, Dautriche A, Bera AP, Gouraud A, Millaret A, Descotes J, Vial T. Decrease in factor V activity in patients treated with azathioprine or 6-mercaptopurine. *Clin Res Hepatol Gastroenterol*. 2014;38:426–431. doi: 10.1016/j.clinre.2013.12.003
18. Thibord F, Hardy L, Ibrahim-Kosta M, Saut N, Pulcrano-Nicolas AS, Goumidi L, Civelek M, Eriksson P, Deleuze JF, le Goff W, et al. A Genome Wide Association Study on plasma FV levels identified PLXDC2 as a new modifier of the coagulation process. *J Thromb Haemost JTH*. 2019;17:1808–1814. doi: 10.1111/jth.14562
19. Lunghi B, Iacoviello L, Gemmati D, Dilasio MG, Castoldi E, Pinotti M, et al. Detection of new polymorphic markers in the factor V gene: association with factor V levels in plasma. *Thromb Haemost*. 1996;75:45–48. doi: 10.1055/s-0038-1650219
20. Hoggart CJ, Venturini G, Mangino M, Gomez F, Ascari G, Zhao JH, Teumer A, Winkler TW, Tšernikova N, Luan J', et al. Novel approach identifies SNPs in SLC2A10 and KCNK9 with evidence for parent-of-origin effect on body mass index. *PLoS Genet*. 2014;10:e1004508. doi: 10.1371/journal.pgen.1004508
21. Reik W, Walter J. Genomic imprinting: parental influence on the genome. *Nat Rev Genet*. 2001;2:21–32. doi: 10.1038/35047554
22. Lokody I. Gene expression: Consequences of parent-of-origin effects. *Nat Rev Genet*. 2014;15:145. doi: 10.1038/nrg3677
23. Berger M, Mattheisen M, Kulle B, Schmidt H, Oldenburg J, Bickeböller H, et al. High factor VIII levels in venous thromboembolism show linkage to imprinted loci on chromosomes 5 and 11. *Blood*. 2005;105:638–644. doi: 10.1182/blood-2004-05-2018
24. Fry AE, Griffiths MJ, Auburn S, Diakite M, Forton JT, Green A, Richardson A, Wilson J, Jallow M, Sisay-Joof F, et al. Common variation in the ABO glycosyltransferase is associated with susceptibility to severe Plasmodium falciparum malaria. *Hum Mol Genet*. 2008;17:567–576. doi: 10.1093/hmg/ddm331
25. Ribarska T, Bastian KM, Koch A, Schulz WA. Specific changes in the expression of imprinted genes in prostate cancer—implications for cancer progression and epigenetic regulation. *Asian J Androl*. 2012;14:436–450. doi: 10.1038/aja.2011.160
26. Psaty BM, O'Donnell CJ, Gudnason V, Lunetta KL, Folsom AR, Rotter JI, Uitterlinden AG, Harris TB, Witteman JC, Boerwinkle E, et al. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: Design of prospective meta-analyses of genome-wide association studies from 5 cohorts. *Circ Cardiovasc Genet*. 2009;2:73–80. doi: 10.1161/CIRCGENETICS.108.829747
27. Moissl AP, Delgado GE, Krämer BK, Dawczynski C, Stojakovic T, März W, Kleber ME, Lorkowski S. Alcohol consumption and mortality: The Ludwigshafen Risk and Cardiovascular Health

(LURIC) study. *Atherosclerosis*. 2021;335:119–125. doi: 10.1016/j.atherosclerosis.2021.08.014
28. Oudot-Mellakh T, Cohen W, Germain M, Saut N, Kallel C, Zelenika D, Lathrop M, Trégouët DA, Morange PE. Genome wide association study for plasma levels of natural anticoagulant inhibitors and protein C anticoagulant pathway: the MARTHA project. *Br J Haematol*. 2012;157:230–239. doi: 10.1111/j.1365-2141.2011.09025.x
29. Rietveld IM, Bos MHA, Lijfering WM, Li-Gao R, Rosendaal FR, Reitsma PH, Cannegieter SC. Factor V levels and risk of venous thrombosis: The MEGA case-control study. *Res Pract Thromb Haemost*. 2018;2:320–326. doi: 10.1002/rth2.12091
30. Llobet D, Vallvé C, Tirado I, Vilalta N, Carrasco M, Oliver A, Mateo J, Fontcuberta J, Souto JC. Platelet hyperaggregability and venous thrombosis risk: results from the RETROVE project. *Blood Coagul Fibrinolysis Int J Haemost Thromb*. 2021;32:122–131. doi: 10.1097/MBC.0000000000001006
31. Brown MB, Forsythe AB. Robust tests for the equality of variances. *J Am Stat Assoc*. 1974;69:364–367. doi: 10.1080/01621459.1974.10482955
32. Mägi R, Morris AP. GWAMA: software for genome-wide association meta-analysis. *BMC Bioinformatics*. 2010;11:288. doi: 10.1186/1471-2105-11-288
33. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience*. 2015;4:7. doi: 10.1186/s13742-015-0047-8
34. Chen M-H, Raffield LM, Mousas A, Sakaue S, Huffman JE, Moscati A, et al. Trans-ethnic and ancestry-specific blood-cell genetics in 746,667 individuals from 5 global populations. *Cell*. 2020;182:1198–1213. doi: 10.1016/j.cell.2020.06.045
35. Malengier-Devlies B, Metzemaekers M, Gouwy M, Van Nieuwenhove E, Betrains A, Cockx M, et al. Phenotypical and functional characterization of neutrophils in two pyrin-associated auto-inflammatory diseases. *J Clin Immunol*. 2021;41:1072–1084. doi: 10.1007/s10875-021-01008-4
36. Norman JC. Partial artificial hearts: mechanical cloning of the ventricle. *Artif Organs*. 1978;2:235–243. doi: 10.1111/j.1525-1594.1978.tb03459.x
37. Monković DD, Tracy PB. Functional characterization of human platelet-released factor V and its activation by factor Xa and thrombin. *J Biol Chem*. 1990;265:17132–17140. doi: 10.1016/S0021-9258(17)44879-4
38. Mereweather LJ, Constantinescu-Bercu A, Crawley JTB, Salles-Crawley II. Platelet-neutrophil crosstalk in thrombosis. *Int J Mol Sci*. 2023;24:1266. doi: 10.3390/ijms24021266
39. Lisman T. Platelet-neutrophil interactions as drivers of inflammatory and thrombotic disease. *Cell Tissue Res*. 2018;371:567–576. doi: 10.1007/s00441-017-2727-4
40. Chou ML, Babamale AO, Walker TL, Cognasse F, Blum D, Burnouf T. Blood-brain crosstalk: the roles of neutrophils, platelets, and neutrophil extracellular traps in neuropathologies. *Trends Neurosci*. 2023;46:764–779. doi: 10.1016/j.tins.2023.06.005
41. Min JL, Hemani G, Hannon E, Dekkers KF, Castillo-Fernandez J, Luijk R, Carnero-Montoro E, Lawson DJ, Burrows K, Suderman M, et al. Genomic and phenotypic insights from an atlas of genetic effects on DNA methylation. *Nat Genet*. 2021;53:1311–1321. doi: 10.1038/s41588-021-00923-x
42. Zeng Y, Amador C, Xia C, Marioni R, Sproul D, Walker RM, Morris SW, Bretherick A, Canela-Xandri O, Boutin TS, et al. Parent of origin genetic effects on methylation in humans are common and influence complex trait variation. *Nat Commun*. 2019;10:1383. doi: 10.1038/s41467-019-09301-y
43. Saunders GR, Wang X, Chen F, Jang SK, Liu M, Wang C, Gao S, Jiang Y, Khunsriraksakul C, Otto JM, et al. Genetic diversity fuels gene discovery for tobacco and alcohol use. *Nature*. 2022;612:720–724. doi: 10.1038/s41586-022-05477-4
44. Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nat Genet*. 2019;51:237–244. doi: 10.1038/s41588-018-0307-5
45. Karlsson Linnér R, Biroli P, Kong E, Meddens SFW, Wedow R, Fontana MA, et al. Genome-wide association analyses of risk tolerance and risky behaviors in over 1 million individuals identify hundreds of loci and shared genetic influences. *Nat Genet*. 2019;51:245–257. doi: 10.1038/s41588-018-0309-3
46. Vuckovic D, Bao EL, Akbari P, Lareau CA, Mousas A, Jiang T, Chen MH, Raffield LM, Tardaguila M, Huffman JE, et al. The polygenic and monogenic basis of blood traits and diseases. *Cell*. 2020;182:1214–1231. doi: 10.1016/j.cell.2020.08.008
47. Swystun LL, Liaw PC. The role of leukocytes in thrombosis. *Blood*. 2016;128:753–762. doi: 10.1182/blood-2016-05-718114
48. Souto JC, Almasy L, Borrell M, Blanco-Vaca F, Mateo J, Soria JM, Coll I, Felices R, Stone W, Fontcuberta J, et al. Genetic susceptibility to thrombosis and its relationship to physiological risk factors: the GAIT study. Genetic Analysis of Idiopathic Thrombophilia. *Am J Hum Genet*. 2000;67:1452–1459. doi: 10.1086/316903
49. Hofmeister RJ, Rubinacci S, Ribeiro DM, Buil A, Kutalik Z, Delaneau O. Parent-of-origin inference for biobanks. *Nat Commun*. 2022;13:6668. doi: 10.1038/s41467-022-34383-6