



de Vries, P. S., Sabater-Lleal, M., Huffman, J. E., Marten, J., Song, C., Pankratz, N., Bartz, T. M., de Haan, H. G., Delgado, G. E., Eicher, J. D., Martinez-Perez, A., Ward-Caviness, C. K., Brody, J. A., Chen, M-H., de Maat, M. P. M., Frånberg, M., Gill, D., Kleber, M. E., Rivadeneira, F., ... Smith, N. L. (2019). A genome-wide association study identifies new loci for factor VII and implicates factor VII in ischemic stroke etiology. *Blood*, 133(9), 967-977.  
<https://doi.org/10.1182/blood-2018-05-849240>

Peer reviewed version

Link to published version (if available):  
[10.1182/blood-2018-05-849240](https://doi.org/10.1182/blood-2018-05-849240)

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## **A genome-wide association study identifies new loci for Factor VII and implicates Factor VII in the etiology of ischemic stroke.**

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Abstract word count: 248

Main text word count: 3,944 including acknowledgements, funding, and disclosures

Number of Tables: 2

Number of Figures: 4

Number of Supplementary Tables: 4

Number of Supplementary Figures: 11

**Abstract (n=248 words, limit=250)**

Factor VII (FVII) is an important component of the coagulation cascade. Few genetic loci regulating FVII activity and/or levels have been discovered to date.

We conducted a meta-analysis of nine genome-wide association studies of plasma FVII levels (seven FVII activity and two FVII antigen) among 27,495 participants of European and African ancestry.

Each study performed ancestry-specific association analyses. Inverse variance weighted meta-analysis was performed within each ancestry group and then combined for a trans-ancestry meta-analysis. Our primary analysis included the seven studies that measured FVII activity, and a secondary analysis included all nine studies. We provided functional genomic validation for newly identified significant loci by silencing candidate genes in a human liver cell line (HuH7) using siRNA and then measuring *F7* mRNA and FVII protein expression. Lastly, we used meta-analysis results to perform Mendelian randomization analysis to estimate the causal effect of FVII activity on coronary artery disease, ischemic stroke, and venous thromboembolism.

We identified two novel (*REEP3* and *JAZF1-AS1*) and six known loci associated with FVII activity, explaining 19.0% of the variance. Adding FVII antigen data to the meta-analysis did not result in the discovery of further loci. Silencing *REEP3* in HuH7 cells upregulated FVII, while silencing *JAZF1* downregulated FVII. Mendelian randomization analyses suggest that FVII activity has a positive causal effect on the risk of ischemic stroke.

Variants at *REEP3* and *JAZF1* contribute to FVII activity by regulating *F7* expression levels. FVII activity appears to contribute to the etiology of ischemic stroke in the general population.

## **Introduction**

As the initiator of the extrinsic coagulation pathway, coagulation factor VII (FVII) plays a central role in fibrin formation. FVII and tissue factor activate factor X, which then converts prothrombin to thrombin, and in turn converts fibrinogen into fibrin. Plasma levels of FVII are associated with several clinical outcomes. For example, FVII deficiency is a rare bleeding disorder associated with hemorrhagic complications,<sup>1</sup> while elevated levels of FVII have been associated with arterial thrombosis and venous thromboembolism (VTE).<sup>2-5</sup>

FVII activity and levels have a substantial heritable component, with estimates of the heritability of FVII activity ranging from 0.40 to 0.52.<sup>6,7</sup> The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium previously conducted genome-wide association studies (GWASs) with data on over 2 million common single nucleotide polymorphisms (SNPs) in European-ancestry participants, identifying four new candidate genes for FVII in addition to the protein-coding locus, *F7*.<sup>8,9</sup> The lead variants at these known loci explain 7.7% of the variance in FVII, implying that further heritability remains to be uncovered.<sup>8</sup>

To discover additional genetic variants associated with FVII, we performed an expanded GWAS with data on over 10 million common and low-frequency SNPs and insertion-deletions in 27,495 participants across nine studies, including 3,420 African American participants. Gene silencing in a human liver cell line was used to validate the genomic function of significantly associated loci. Lastly, we performed Mendelian randomization analyses to estimate the causal effects of FVII activity on atherosclerotic and thrombotic diseases by leveraging our GWAS results in Mendelian randomization analyses.

## **Methods**

### *Study design and Participating Cohorts*

This study was organized within the CHARGE Consortium Hemostasis Working Group.<sup>9</sup> Nine studies were included: the Atherosclerosis Risk in Communities (ARIC) study,<sup>10</sup> the Cardiovascular Health Study (CHS),<sup>11</sup> the Coronary Artery Risk Development In young Adults (CARDIA) study,<sup>12</sup> the Genetic Analysis for Idiopathic Thrombophilia 2 (GAIT2) study, the Framingham Heart Study

(FHS),<sup>13</sup> the Ludwigshafen Risk and Cardiovascular Health (LURIC) study,<sup>14</sup> the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study, the Precocious Coronary Artery Disease (PROCARDIS) study,<sup>15</sup> and the Rotterdam Study (RS).<sup>16</sup> Descriptions and ancestry composition of participating cohorts are found in the Supplementary Material (**Supplementary Methods** and **Supplementary Table 1**). Seven studies (ARIC, CHS, CARDIA, GAIT2, LURIC, MEGA, RS) including 23,434 participants measured FVII activity (% or IU/ml\*100) and two studies (FHS, PROCARDIS) including 4,061 participants measured FVII antigen (% or IU/ml\*100).

#### *Genotyping and imputation*

All participating cohorts performed genome-wide genotyping using commercial platforms available from Illumina or Affymetrix. Each study performed standard pre-imputation quality control checks and imputed autosomal and X-chromosome variants from the 1000 Genomes Project (1000G) Phase I version 3 reference panel using available imputation methods.<sup>17-20</sup> Genotyping, pre-imputation quality control, and imputation procedures are described in **Supplementary Table 2**.

#### *Cohort-specific association analyses*

Natural-log-transformed FVII was analyzed within each cohort. Participants with values 3 standard deviations above or below the population mean were removed prior to cohort-level analysis and any individuals on anticoagulant therapy were also excluded. Ancestry-stratified, study-specific regression analyses using an additive genetic model were performed between genome-wide 1000G imputed variant dosages and phenotype levels, adjusted for age, sex, ancestry-informative principal components, and study-specific variables, such as center. Analyses of the X-chromosome were stratified by sex, with variants in males coded as 0/2. The covariates used in each of the studies are shown in **Supplementary Table 2**. Quality control assessment of ancestry-specific results files from each study was conducted prior to meta-analysis using the EasyQC software package.<sup>21</sup> Quality control procedures are further described in the **Supplementary Methods**.

#### *Trans-ancestry meta-analysis*

The discovery trans-ancestry meta-analysis was conducted in two steps. First, METAL was used to perform ancestry-specific inverse-variance weighted meta-analysis.<sup>22</sup> We then used the same method to meta-analyze the ancestry-specific results. As suggested by Huang et al.<sup>23</sup>, we adopted a genome-wide significance threshold of  $P$ -value  $< 2.5 \times 10^{-8}$ . Compared with the traditional genome-wide significance threshold of  $5 \times 10^{-8}$ , this stricter threshold additionally corrects for the low-frequency variants that were not included in the initial generation of GWASs.<sup>24</sup> Finally, a locus was defined as +/- 1Mb from the variant with the lowest  $P$ -value.

In order to reduce heterogeneity the primary trans-ancestry meta-analysis included the seven studies that measured FVII activity, and not the two studies (FHS and PROCARDIS) that measured FVII antigen. In a secondary meta-analysis we added results from the two studies that measured FVII antigen.

#### *Post-discovery analyses*

Newly identified loci were validated and characterized by using siRNA to silence candidate genes in human liver HuH7 cells, and measuring F7 mRNA levels and release of FVII protein levels. These functional validation steps are described in detail in the **Supplementary Methods**.

To identify additional independent signals at the associated loci, an approximate method implemented in GCTA was used for conditional and joint analysis using meta-analysis summary statistics from the trans-ancestry meta-analysis of FVII activity.<sup>25,26</sup> Further details on the conditional analysis are provided in the **Supplementary Methods**.

Mendelian randomization analyses were used to investigate the causal effect of FVII activity on coronary artery disease (CAD), ischemic stroke (IS), and VTE. We used two-sample methods that rely on summary statistics (beta coefficients with standard errors from GWASs).<sup>27</sup> We obtained summary statistics for CAD from the CARDIoGRAMplusC4D consortium (<http://www.cardiogramplusc4d.org/data-downloads/>),<sup>28</sup> summary statistics for IS from the MEGASTROKE consortium,<sup>29</sup> and summary statistics for VTE from the INVENT consortium.<sup>30</sup> The methods used to perform Mendelian randomization can be found in the **Supplementary Methods**. In

brief, we used four techniques to obtain causal effect estimates based on the lead variants at the genome-wide significant loci: 1) inverse-variance weighted meta-analysis (primary analysis), 2) Egger regression,<sup>31</sup> and 3) weighted median estimator,<sup>32</sup> 4) restriction of the analysis to the lead variant at the *F7* locus. Given that the lead variant at the *F7* locus is located in the gene that encodes the FVII protein, it may be less likely to influence clinical outcomes through pathways that do not involve FVII.

## Results

### *Baseline characteristics*

In total, 20,014 European-ancestry and 3,420 African-ancestry subjects from seven studies were included in the meta-analysis of FVII activity and an additional 4,061 European-ancestry subjects were included in the combined meta-analysis of FVII activity and antigen. Baseline characteristics are shown in **Supplementary Table 1**. The mean age across the studies was 57.2 years, and 52.2% of the participants were women.

### *Trans-ancestry meta-analysis*

After quality control, 10,044,948 variants across the autosomal and X chromosomes were examined in the trans-ancestry meta-analysis of FVII activity. Of these variants 9,316,598 were SNPs and 728,350 were insertions-deletions. The genomic inflation factors that were used to apply genomic control correction to each of the included studies were all  $< 1.05$  and are shown in **Supplementary Table 2**. A QQ plot and Manhattan plot are shown in **Supplementary Figures 1 and 2**, respectively.

Genome-wide significant results are presented in **Table 1**. Briefly, 1,637 variants located in eight loci exceeded the genome-wide significance level of  $P$ -value  $< 2.5 \times 10^{-8}$ . Among the associated regions, loci containing *F7*, *PROC*, *GCKR*, *MS4A6A*, *ADH4*, and *TSKU* represented replications of previously described loci (**Supplementary Figures 3-8**),<sup>8</sup> whereas two loci were novel: *REEP3* and *JAZF1-AS1*. The most significant variant at the *REEP3* locus was an intronic variant, rs10761784 (Beta = 0.013;  $P$ -value =  $6.7 \times 10^{-10}$ ) in *REEP3* (**Figure 1**). At the second novel locus the lead variant, rs498475, was located within the noncoding RNA *JAZF1-AS1* (Beta = 0.012;  $P$ -value =  $1.5 \times 10^{-8}$ ;



**Figure 2).** Lead variants at PROCR and GCKR were identical to previously reported lead variants, whereas the lead variants at the remaining known loci differed from previously reported lead variants (**Supplementary Table 3**).

No additional genome-wide significant loci emerged when adding data from two additional studies in the combined analysis of FVII activity and antigen, but variants at the *TSKU* and *JAZF1-AS1* loci were no longer genome-wide significant (**Supplementary Table 4**). A QQ plot and Manhattan plot for the combined analysis of FVII activity and antigen are shown in **Supplementary Figures 9 and 10** respectively. The lead variants at *TSKU* and *JAZF1-AS1* had opposing effect directions on FVII activity and antigen, but lead variants at the remaining six loci had relatively similar effects on FVII activity and antigen (**Supplementary Figure 11**). The variance in FVII activity explained by the lead variants at the eight significant loci was 17.6%. The variance explained by each of the lead variants individually is shown in **Table 1**.

#### *Conditional analysis*

Conditional analysis identified four independent signals at the *F7* locus as well as two independent signals at the *PROCR* locus. The conditional analysis of the trans-ancestry meta-analysis of FVII activity is shown in **Table 2**. Among the independently associated variants at the *F7* locus was a low-frequency variant (minor allele frequency = 0.02) with the second largest effect size discovered by GWASs thus far (joint beta = 0.08; joint *P*-value =  $8.7 \times 10^{-20}$ ). By considering these independent signals, the variance in FVII activity explained by the *F7* locus increased from 13.9% to 15.2%, while the variance explained by the *PROCR* locus increased from 1.6 to 1.8%. The total variance explained therefore increased from 17.6% to 19.0%.

#### *Functional validation of novel loci*

The s47939 and s37271 silencing siRNAs both suppressed expression of *REEP3* mRNA by 88% compared with the scramble siRNA (negative control). Experiments were repeated three times with consistent results, showing that silencing of *REEP3* resulted in upregulation of *F7* mRNA (*P*-value =

0.0001 for s47939;  $P$ -value  $> 0.05$  for s37271; **Figure 3**) and a corresponding increase in FVII protein levels ( $P$ -value =  $9.1 \times 10^{-5}$  for s47939;  $P$ -value = 0.0003 for s37271; **Figure 3**).

At the *JAZF1-AS1* locus we targeted the *JAZF1* gene for silencing rather than the antisense noncoding RNA in which the lead variant was located. The s225897 silencer reduced expression of *JAZF1* mRNA by 68%, whereas the s48121 silencer reduced *JAZF1* mRNA expression by 75%. As shown in **Figure 3**, silencing of *JAZF1* resulted in downregulation *F7* mRNA ( $P$ -value = 0.02 for s225897;  $P$ -value  $< 2 \times 10^{-6}$  for s48121) and a corresponding decrease in FVII protein expression: silencing experiments showed no effect on FVII protein in the media of cells silenced with s225897, but a significant decrease upon silencing with s48121 ( $P$ -value =  $1.1 \times 10^{-6}$ ).

#### *Mendelian randomization*

**Figure 4** contains forest plots showing causal effect estimates of FVII activity on A) CAD, B) IS, and C) VTE. Causal effect estimates are given as odds ratios (ORs) per 1 unit increase in natural-log-transformed FVII activity (% or IU/ml\*100). Effect estimates were obtained from single variants associated with FVII activity and meta-analyzed to produce combined causal effect estimates. Heterogeneity was detected among the causal effect estimates ( $P_{\text{heterogeneity}} < 0.05$ ), and the variant at the *PROCR* locus, rs867186, was removed from all analyses due to outlying causal effect estimates for CAD, IS, and VTE. No further heterogeneity was detected after excluding this variant ( $P_{\text{heterogeneity}} > 0.05$ ).

A significant causal effect of FVII activity on IS was detected (OR<sub>I<sub>VW</sub></sub> = 1.37; 95% confidence interval (CI<sub>95</sub>) = 1.14-1.65). Given that the SD of natural-log-transformed FVII activity ranged from 0.18 to 0.26 across our studies, the causal effect estimate corresponds to an approximate OR of 1.06 to 1.09 per SD change in natural-log-transformed FVII activity. Results were consistent across sensitivity analyses, including the use of Egger regression, the weighted median estimator, and restriction of the analysis to the rs561241 variant at the *F7* locus, indicating that pleiotropy is unlikely to have biased the causal estimate. Causal effect estimates for CAD (OR<sub>I<sub>VW</sub></sub> = 1.14; CI<sub>95</sub> = 0.97-1.34) and VTE (OR<sub>I<sub>VW</sub></sub> = 1.22; CI<sub>95</sub> = 0.80-1.85) were more modest and failed to reach statistical

significance. Nevertheless, the magnitude of these effect estimates were consistent across sensitivity analyses, including when the rs561241 variant at the *F7* locus was examined in isolation ( $OR_{CAD} = 1.14$ ;  $CI_{95} = 0.96-1.36$ ;  $OR_{VTE} = 1.31$ ;  $CI_{95} = 0.84-2.07$ ).

## Discussion

In this GWAS of circulating FVII levels, we identified the six previously known FVII loci as well as two new loci: *REEP3* and *JAZF1-ASI*. In total, the eight loci associated with FVII activity explained 19.0% of the variance. For each new discovery, we showed functional impact *in vitro* of candidate genes on *F7* mRNA and FVII protein expression: *REEP3* gene silencing in liver cells increased *F7* mRNA and FVII protein expression, whereas *JAZF1* gene silencing decreased *F7* mRNA and FVII protein expression.

*REEP3* encodes Receptor Accessory Protein 3. Although this protein has not been widely studied, there is evidence that an absence of this protein leads to defects in mitosis and a proliferation of intranuclear membranes derived from the nuclear envelope.<sup>33</sup> The *REEP* gene family may also be involved in shaping the membrane of the endoplasmic reticulum and the trafficking of G-protein coupled receptors.<sup>34</sup> Given that FVII is processed in the endoplasmic reticulum, this may explain the association with FVII levels.<sup>35</sup> The locus containing *REEP3* has been previously associated to several other coagulation phenotypes, namely circulating fibrinogen levels,<sup>36,37</sup> mean platelet volume,<sup>38,39</sup> and platelet aggregation,<sup>40</sup> as well as to liver enzyme concentrations.<sup>41,42</sup> For many of these phenotypes, the gene that was reported is not *REEP3* but *JMJD1C*, with missense variants localized in the *JMJD1C* being associated with mean platelet volume.<sup>38</sup> Functional studies in zebrafish indicate that *JMJD1C* has a major role in hematopoiesis.<sup>43</sup> Although we did not examine the consequences of *JMJD1C* silencing on *F7* expression and FVII release, our experiments implicate *REEP3* as a causal gene for FVII. These results are consistent with tissue-specific pleiotropic effects at this locus, with *JMJD1C* being involved in hematopoiesis and *REEP3* being of functional relevance in the liver, although further research is needed to confirm this hypothesis.

*JAZF1-ASI* is a non-coding RNA that may regulate the adjacent *JAZF1* gene, which encodes a transcriptional repressor. Variants at the *JAZF1-ASI* locus were associated with FVII activity, but

their effect on FVII was attenuated when we included studies that measured FVII antigen. A possible explanation is that variants at the *JAZF1-ASI* loci affect FVII activity independent of circulating FVII protein levels. However, silencing of *JAZF1* in liver cells resulted in lower *F7* mRNA and FVII protein expression, suggesting that the mechanism underlying the genetic association is likely to involve FVII levels.

Apart from *REEP3* and *JAZF1-ASI* we identified six known loci: *F7*, *PROCR*, *GCKR*, *MS4A6A*, *ADH4*, and *TSKU*. The results of this study may aid in the identification of causal variants at these loci. For example, lead variants in *PROCR* and *GCKR* were both missense variants leading to amino acid substitutions (Ser219Gly in *PROCR* and Pro446Leu in *GCKR*). These variants were also the lead variants in their respective loci in the previous GWAS of FVII,<sup>8</sup> and have been associated with other hemostatic phenotypes.<sup>44,45</sup> In contrast, the lead variants that we identified at the *F7*, *MS4A6A*, *ADH4*, and *TSKU* loci differ from those published in the previous GWAS and may be in higher linkage disequilibrium with the true causal variant.<sup>8</sup>

Using the genetic association results generated in this study, we performed Mendelian randomization analyses to estimate the causal effect of FVII activity on CAD, IS, and VTE. These analyses suggest that variation in FVII activity in the general population influences the risk of IS. These results warrant further etiological research on the role of FVII in IS, as well as translational research on potential clinical applications involving FVII. Potential clinical applications that should be investigated include the reduction of FVII activity through lifestyle or pharmaceutical interventions for the prevention of IS, as well as the restriction of off-label use of recombinant FVII given the increased risk of IS that this may result in.<sup>46-48</sup>

Our results do not exclude the possibility of additional causal effects of FVII activity on CAD and VTE. In fact, when using the rs561241 variant at the *F7* locus in isolation as an instrumental variable, the estimate of the causal effect on VTE was equal to the estimate of the effect on IS. Lower statistical power for the Mendelian randomization analysis of VTE may explain the lack of a statistically significant causal effect of FVII activity on this outcome: the GWAS from which we obtained the effect of the variants on IS was comprised of 60,341 cases and 454,450 controls,<sup>29</sup> while

the GWAS on VTE consisted of 7,507 cases and 52,632 controls.<sup>30</sup> Our results are thus consistent with causal effects of FVII activity on CAD and VTE, albeit more modest effects than on IS. Further research using larger sample sizes will be necessary to detect or rule out these effects.

Our GWAS included 27,495 participants, providing a 78% increased sample size when compared with the largest previous GWAS of FVII levels.<sup>8</sup> Other strengths include the functional validation of newly identified loci by silencing candidate genes in human liver cell lines, as well as the use of Mendelian randomization in leveraging our newly generated data for insights into disease etiology. However, these approaches also have limitations. In the gene silencing experiments, we silenced a single gene at each locus. Because an effect of FVII levels was observed in both cases, we did not pursue further experiments involving other genes at these loci. As such we cannot exclude the possibility that other genes at these loci also influence FVII. In turn, the validity of Mendelian randomization can be threatened by the presence of pleiotropic effects among FVII variants. In order to minimize the impact of pleiotropy on our results, we excluded variants with heterogeneous effects on disease outcomes from the analyses. This led to the removal of the variant at the *PROCR* locus from all analyses. Furthermore, we performed three alternative Mendelian randomization approaches, Egger regression, weighted median estimation, and restricting the analysis to the rs561241 variant at the *F7* locus, that are, to some degree, robust to pleiotropy.<sup>31,32</sup> The estimate of the causal effect of FVII activity on IS was consistent across these sensitivity analyses, as were the estimates of the causal effects of FVII activity on CAD and VTE.

To conclude, this study identifies two novel loci associated with FVII activity and functional studies suggest that *REEP3* and *JAZF1* are the causal genes within these loci. Mendelian randomization analyses indicate that FVII activity is causally involved in the development of IS and possibly CAD and VTE, with high FVII activity being associated with an increased risk of these clinical outcomes.

## **Acknowledgements**

We thank the staff and participants of each of the participating studies. We are grateful to Alexandra Bäcklund, Fariba Foroogh and Angela Silveira for helpful advice in the cell culture and mesoscale experiments.

## **Funding**

This work was supported by American Heart Association Grant #17POST33350042 (Paul S. de Vries) and National Heart, Lung, and Blood Institute (NHLBI) grant R01HL134894 (Nicholas L. Smith). Maria Sabater-Lleal is recipient of the EHA-ISTH fellowship and is partially supported by the Swedish Heart-Lung Foundation (20160290). Infrastructure for the CHARGE Consortium is supported in part by the National Heart, Lung, and Blood Institute grant R01HL105756.

The Atherosclerosis Risk in Communities (ARIC) study is carried out as a collaborative study supported by the NHLBI contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. Funding support for “Building on GWAS for NHLBI-diseases: the U.S. CHARGE consortium” was provided by the NIH through the American Recovery and Reinvestment Act of 2009 (ARRA) (5RC2HL102419).

Cardiovascular Health Study (CHS) research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, HHSN268200960009C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants U01HL080295, R01HL085251, R01HL087652, R01HL105756, R01HL103612, R01HL120393, and R01HL130114 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at [CHS-NHLBI.org](http://CHS-NHLBI.org). The provision of genotyping data was supported in

part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center.

The Coronary Artery Risk Development in Young Adults Study (CARDIA) is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with the University of Alabama at Birmingham (HHSN268201300025C & HSN268201300026C), Northwestern University (HHSN268201300027C), University of Minnesota (HHSN268201300028C), Kaiser Foundation Research Institute (HHSN268201300029C), and Johns Hopkins University School of Medicine (HHSN268200900041C). CARDIA is also partially supported by the Intramural Research Program of the National Institute on Aging (NIA) and an intra-agency agreement between NIA and NHLBI (AG0005). Genotyping was funded as part of the NHLBI Candidate-gene Association Resource (N01-HC-65226) and the NHGRI Gene Environment Association Studies (GENEVA) (U01-HG004729, U01-HG04424, and U01-HG004446). This manuscript has been reviewed and approved by CARDIA for scientific content.

Framingham Heart Study (FHS) was partially supported by the National Heart, Lung, and Blood Institute's (NHLBI's) Framingham Heart Study (Contract No. N01-HC-25195) and its contract with Affymetrix, Inc. for genotyping services (Contract No. N02-HL-6-4278). A portion of this research utilized the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. The analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHARe) project. JEH, CS, MHC, ADJ and CJO were supported by National Heart, Lung and Blood Intramural Research Funds. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the U. S. Department of Health and Human Services.

Genetic Analysis for Idiopathic Thrombophilia 2 (GAIT2) project was supported partially by grants PI-11/0184, PI-14/0582 and Red Investigación Cardiovascular RD12/0042/0032 from the Instituto

Carlos III (Fondo de Investigación Sanitaria–FIS), and 2014SGR-402 Consolidated Research Group of the Generalitat de Catalunya.

The LUDwigshafen RIsk and Cardiovascular Health (LURIC) study was supported by the 7th Framework Program RiskyCAD (grant agreement number 305739) of the European Union. The work of W.M. and M.E.K. is supported as part of the Competence Cluster of Nutrition and Cardiovascular Health (nutriCARD) which is funded by the German Federal Ministry of Education and Research.

The Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study was supported by Netherlands Heart Foundation (NHS 98.113), the Dutch Cancer Foundation (RUL 99/1992), the Netherlands Organisation for Scientific Research (912–03–033| 2003), and partially by the GenMed LABEX (ANR-10-LABX-0013). We would like to thank all colleagues from the French Centre National de Génotypage for the genotyping contribution.

The Precocious Coronary Artery DISease (PROCARDIS) study project was funded with 10 million Euros through the 6th Framework Program of the European Union (LSH-2005-2.1.1-1). It started in Apr 2007 and funding lasted until Sept 2011. After the funding ended, PROCARDIS is still actively doing research in the field of coronary artery disease genetics.

The generation and management of GWAS genotype data for the Rotterdam Study (RS) is supported by the Netherlands Organisation of Scientific Research NWO Investments (no. 175.010.2005.011, 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) project no. 050-060-810. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.

## **Disclosures**



Winfried März reports grants and personal fees from AMGEN, BASF, Sanofi, Siemens Diagnostics, Aegerion Pharmaceuticals, Astrazeneca, Danone Research, Numares, Pfizer, Hoffmann LaRoche: personal fees from MSD, Alexion; grants from Abbott Diagnostics, all outside the submitted work. Winfried März is employed by Synlab Holding Deutschland GmbH.

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## Tables

**Table 1:** Lead variants at additional loci associated with FVII activity when excluding studies that measured FVII antigen from the trans-ancestry meta-analysis.

| Variant<br>rsID | Chr:Pos      | Alleles | Freq | $\beta$ | Standard<br>Error | <i>P</i> -value        | Variance<br>Explained | Closest<br>Gene  | Annotation | Status |
|-----------------|--------------|---------|------|---------|-------------------|------------------------|-----------------------|------------------|------------|--------|
| rs569557        | 13:113769917 | G/A     | 0.89 | 0.157   | 0.003             | $6.4 \times 10^{-600}$ | 13.9%                 | <i>F7</i>        | Intronic   | Known  |
| rs867186        | 20:33764554  | G/A     | 0.10 | 0.057   | 0.003             | $3.3 \times 10^{-64}$  | 1.6%                  | <i>PROCR</i>     | Missense   | Known  |
| rs1260326       | 2:27730940   | T/C     | 0.39 | 0.024   | 0.002             | $2.3 \times 10^{-30}$  | 0.7%                  | <i>GCKR</i>      | Missense   | Known  |
| rs7935829       | 11:59942815  | G/A     | 0.39 | 0.018   | 0.002             | $6.3 \times 10^{-18}$  | 0.4%                  | <i>MS4A6A</i>    | Intronic   | Known  |
| rs6532796       | 4:100042242  | G/A     | 0.70 | 0.016   | 0.002             | $2.6 \times 10^{-13}$  | 0.3%                  | <i>ADH4</i>      | Downstream | Known  |
| rs1149616       | 11:76498369  | T/C     | 0.17 | 0.017   | 0.003             | $1.7 \times 10^{-10}$  | 0.2%                  | <i>TSKU</i>      | Intronic   | Known  |
| rs10761784      | 10:65308750  | A/T     | 0.53 | 0.013   | 0.002             | $6.7 \times 10^{-10}$  | 0.2%                  | <i>REEP3</i>     | Intronic   | Novel  |
| rs498475        | 7:28256240   | G/A     | 0.42 | 0.012   | 0.002             | $1.5 \times 10^{-8}$   | 0.2%                  | <i>JAZF1-AS1</i> | ncRNA      | Novel  |

The Chr:Pos column shows the chromosome and position (Build 37). The Alleles column shows the FVII-increasing allele / FVII-decreasing allele. The Freq column shows the frequency of the FVII-increasing allele. The variance explained shown in this table was calculated using the sample size weighted mean variance of log-transformed FVII and the betas and frequencies from the trans-ancestry meta-analysis summary statistics.

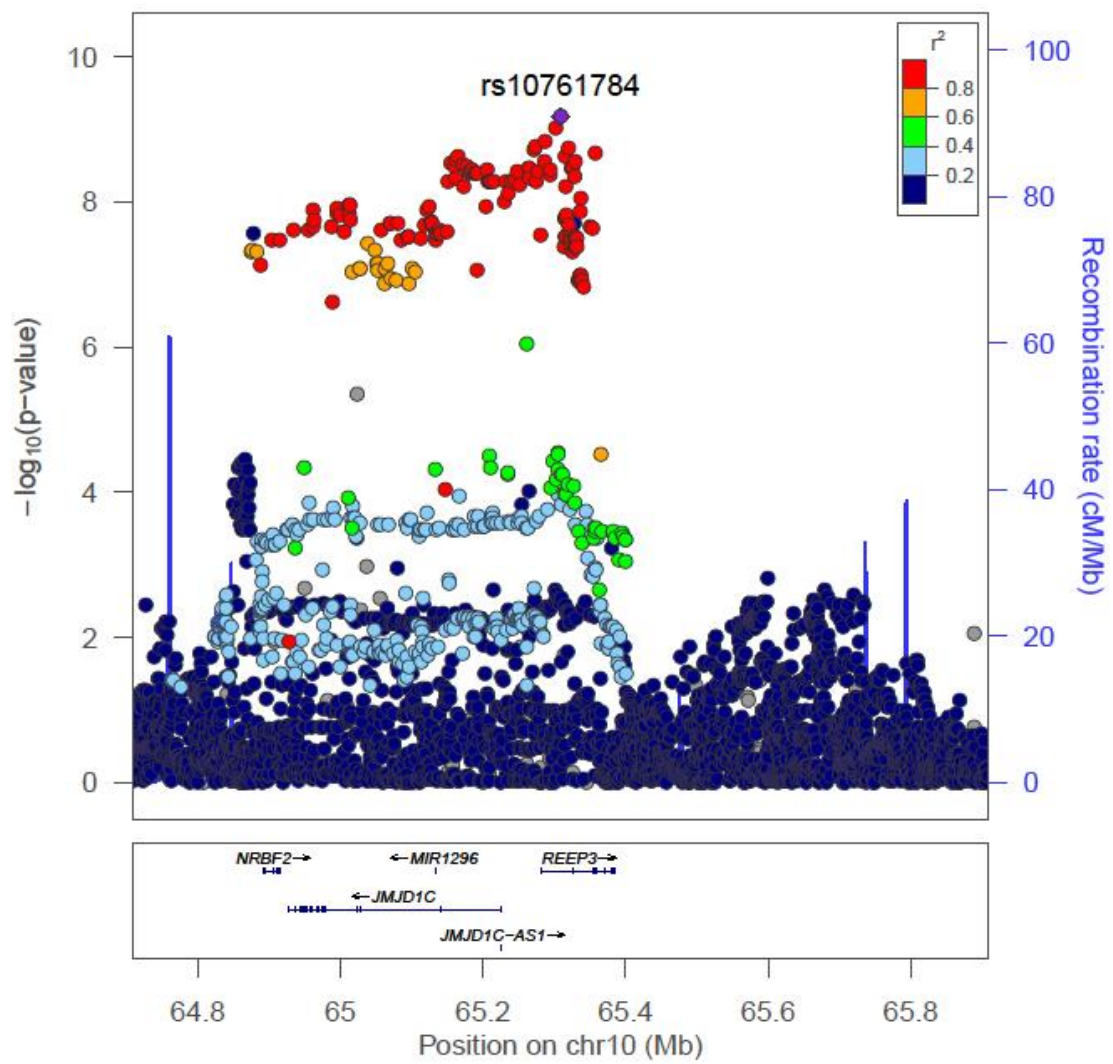
**Table 2:** Conditional analysis results for FVII activity using the trans-ancestry meta-analysis results.

| rsID         | Chr:Pos      | Alleles | Freq | $\beta$ | <i>P</i> -value        | Joint $\beta$ | Joint <i>P</i> -value   | Variance Explained |
|--------------|--------------|---------|------|---------|------------------------|---------------|-------------------------|--------------------|
| <i>F7</i>    |              |         |      |         |                        |               |                         |                    |
| rs117989138  | 13:113697671 | A/G     | 0.02 | 0.086   | $3.6 \times 10^{-22}$  | 0.081         | $8.7 \times 10^{-20}$   | 0.6%               |
| rs36086577   | 13:113728498 | C/A     | 0.87 | 0.035   | $2.2 \times 10^{-19}$  | 0.031         | $7.5 \times 10^{-15}$   | 0.6%               |
| rs71446935   | 13:113734376 | A/G     | 0.31 | 0.035   | $5.5 \times 10^{-38}$  | 0.032         | $5.1 \times 10^{-31}$   | 1.2%               |
| rs1046205    | 13:113752057 | A/T     | 0.79 | 0.121   | $3.9 \times 10^{-573}$ | 0.121         | $<1.0 \times 10^{-320}$ | 12.1%              |
| <i>PROCR</i> |              |         |      |         |                        |               |                         |                    |
| rs6119569    | 20:33672371  | G/A     | 0.78 | 0.022   | $8.8 \times 10^{-17}$  | 0.019         | $3.9 \times 10^{-13}$   | 0.3%               |
| rs867186     | 20:33764554  | G/A     | 0.10 | 0.057   | $3.3 \times 10^{-64}$  | 0.055         | $8.1 \times 10^{-59}$   | 1.4%               |

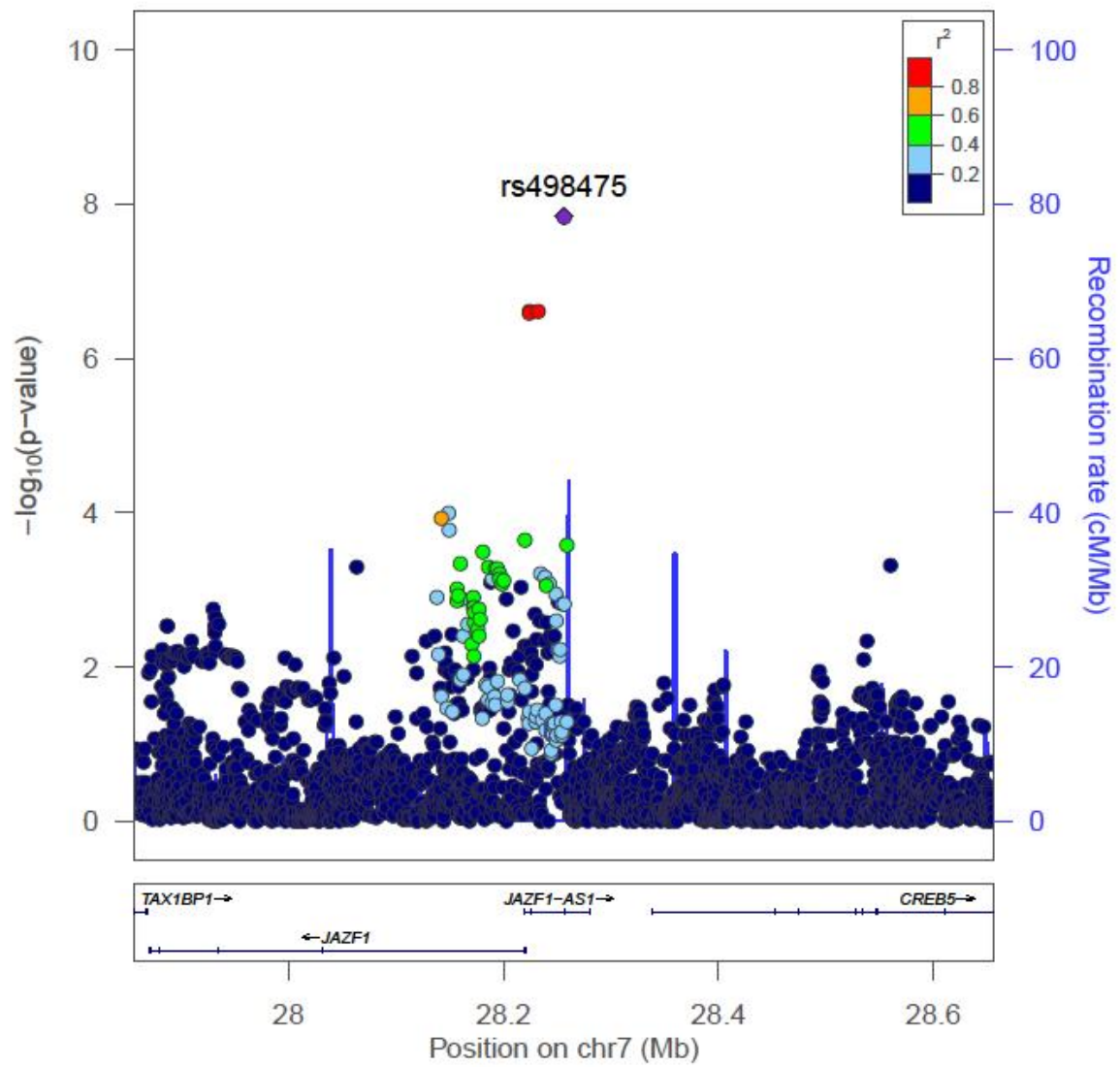
The Chr:Pos column shows the chromosome and position (Build 37). The Alleles column shows the FVII-increasing allele. The Freq column shows the frequency of the FVII-increasing allele. The variance explained shown in this table was calculated using the sample size weighted mean variance of log-transformed FVII and the betas and frequencies from the trans-ancestry meta-analysis summary statistics. The  $\beta$  and *P*-value columns are based on the association of each variant in isolation, while the Joint  $\beta$  and Joint *P*-value columns are based on the association of each variant test conditioned on the other variants. The Variance Explained column is based on the joint analysis.

**Figures**

**Figure 1:** Regional FVII association plot of the *REEP3* locus in the trans-ancestry meta-analysis of FVII activity.

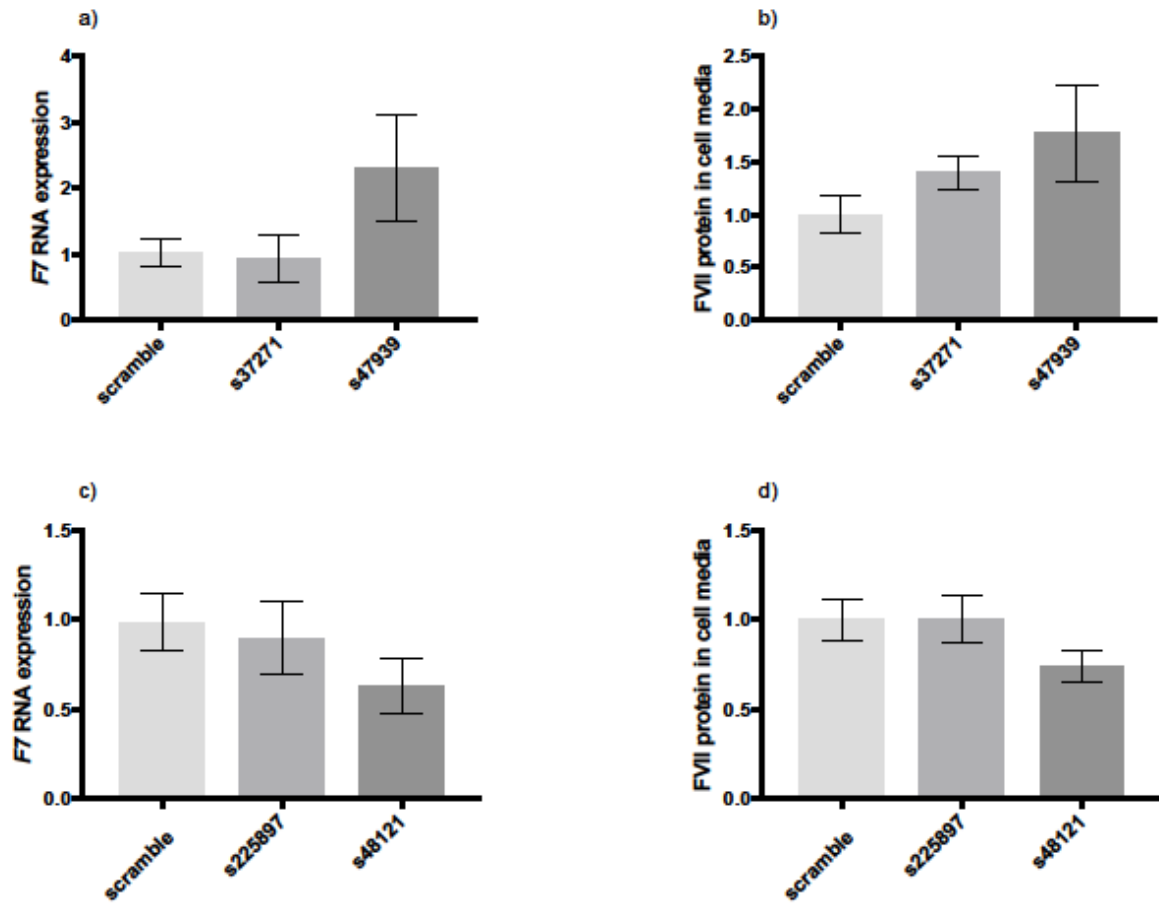


**Figure 2:** Regional association plot for the *JAZF1* locus in the trans-ancestry meta-analysis of FVII activity.

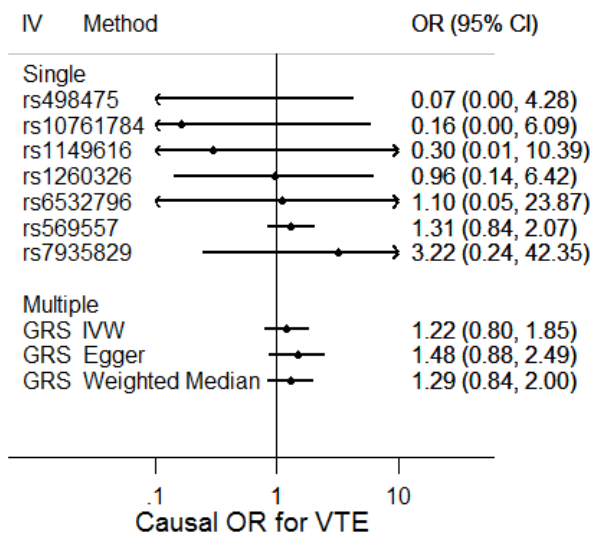
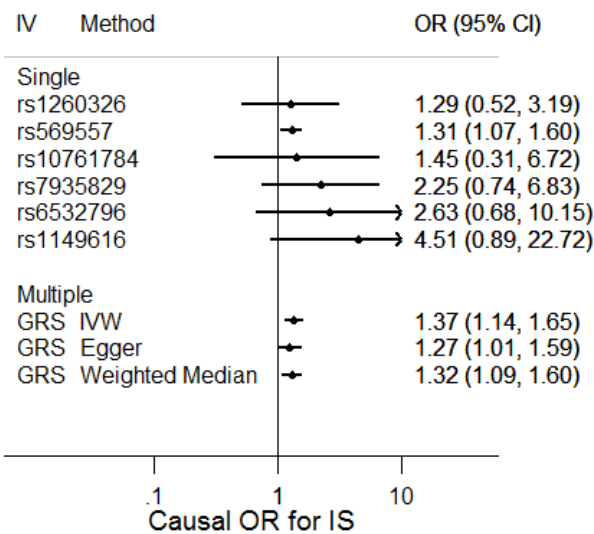
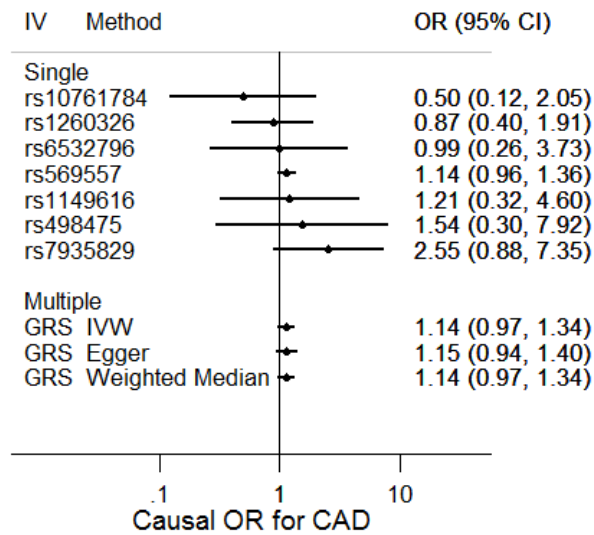




**Figure 3:** a) *F7* RNA expression after silencing *REEP3*, b) FVII protein levels in cell media after silencing *REEP3*, c) *F7* RNA expression after silencing *JAZF1*, d) FVII protein levels in cell media after silencing *JAZF1*.



**Figure 4:** Causal effect estimates of FVII activity on coronary artery disease (CAD), ischemic stroke (IS), and venous thromboembolism (VTE) using Mendelian randomization.



Causal effect estimates are shown as odds ratios (OR) and 95% confidential intervals per every higher standard deviation change in FVII activity. Causal estimates based on single variant instrumental variables (IVs) are shown, as well as causal estimates based on the combination of these variants using inverse variance weighted (IVW) meta-analysis, Egger regression, and weighted median estimation.