Circulation

Genome-Wide Association Transethnic Meta-Analyses Identifies Novel Associations Regulating Coagulation Factor VIII and von Willebrand Factor Plasma Levels

BACKGROUND: Factor VIII (FVIII) and its carrier protein von Willebrand factor (VWF) are associated with risk of arterial and venous thrombosis and with hemorrhagic disorders. We aimed to identify and functionally test novel genetic associations regulating plasma FVIII and VWF.

METHODS: We meta-analyzed genome-wide association results from 46 354 individuals of European, African, East Asian, and Hispanic ancestry. All studies performed linear regression analysis using an additive genetic model and associated ≈35 million imputed variants with natural log–transformed phenotype levels. In vitro gene silencing in cultured endothelial cells was performed for candidate genes to provide additional evidence on association and function. Two-sample Mendelian randomization analyses were applied to test the causal role of FVIII and VWF plasma levels on the risk of arterial and venous thrombotic events.

RESULTS: We identified 13 novel genome-wide significant ($P \leq 2.5 \times 10^{-8}$) associations, 7 with FVIII levels (*FCHO2/TMEM171/TNPO1*, *HLA*, *SOX17/RP1*, *LINC00583/NFIB*, *RAB5C-KAT2A*, *RPL3/TAB1/SYNGR1*, and *ARSA*) and 11 with VWF levels (*PDHB/PXK/KCTD6*, *SLC39A8*, *FCHO2/TMEM171/TNPO1*, *HLA*, *GIMAP7/GIMAP4*, *OR13C5/NIPSNAP*, *DAB2IP*, *C2CD4B*, *RAB5C-KAT2A*, *TAB1/SYNGR1*, and *ARSA*), beyond 10 previously reported associations with these phenotypes. Functional validation provided further evidence of association for all loci on VWF except *ARSA* and *DAB2IP*. Mendelian randomization suggested causal effects of plasma FVIII activity levels on venous thrombosis and coronary artery disease risk and plasma VWF levels on ischemic stroke risk.

CONCLUSIONS: The meta-analysis identified 13 novel genetic loci regulating FVIII and VWF plasma levels, 10 of which we validated functionally. We provide some evidence for a causal role of these proteins in thrombotic events.

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Clinical Perspective

What Is New?

- Plasma coagulation factor VIII and von Willebrand factor concentrations are associated with risk of cardiovascular disease, but the genetic factors that control their levels are not fully understood.
- Using a multiethnic meta-analysis of genome-wide association studies, we identified 7 genome-wide significant novel associations for factor VIII and 11 for von Willebrand factor.

What Are the Clinical Implications?

- We evaluated the effect of genetic variants with coronary artery disease, ischemic stroke, and venous thrombosis through mendelian randomization analyses and found evidence of a causal effect of factor VIII activity levels on venous thrombosis and coronary artery disease risk, and a causal effect of plasma von Willebrand factor levels on stroke risk.
- Our findings suggest that factor VIII and von Willebrand factor may be potential therapeutic targets to prevent thrombotic events.

Factor VIII (FVIII) and its carrier protein von Willebrand factor (VWF) regulate hemostasis and thrombosis, and higher plasma levels of these factors have been associated with risk of arterial and venous thrombosis, whereas lower levels are associated with hemorrhagic disorders[1–4] and with reduced risk of thrombotic events.[5] Previously published genetic association studies have investigated the contribution of nucleotide variation to plasma levels of these factors using genome-wide and exome-wide markers.[6–8] These studies identified and replicated 8 genetic loci associated with plasma VWF levels (*STXBP5*, *SCARA5*, *ABO*, *STAB2*, *STX2*, *VWF*, *TCN2* and *CLEC4M*), 5 of which were also associated with FVIII levels (*STXBP5*, *SCARA5*, *ABO*, *STAB2*, and *VWF*). These discoveries have broadened our understanding of the regulation of hemostasis through follow-up functional investigations.[9,10]

The causal effect of these factors on bleeding is well established; severe FVIII and VWF deficiencies lead to the bleeding disorders hemophilia A and von Willebrand disease, respectively. Although it is currently unclear whether FVIII and VWF levels causally influence the risk of thrombotic diseases, some genetic and observational evidence suggest an effect of these proteins on thrombotic disease. Genetic variants in the *F8* gene and in 3 VWF-associated genes (*ABO*, *STXBP5*, and *VWF*) are robustly associated with risk of venous thrombosis, but no causal association has been established.[11–13]

The aim of this investigation was to identify new genetic associations that influence plasma levels of FVIII and VWF by expanding the size and ancestral diversity of the discovery sample from previous genome-wide association studies (GWASs) and by improving coverage of the genome through the use of 1000 Genomes imputed data and the inclusion of chromosome X variants.[14] For discoveries that reached genome-wide significance, we conducted first-pass functional characterization of the candidate loci both to provide additional evidence of association and to better understand the biology regulating plasma levels of these coagulation phenotypes. Finally, by applying our genetic findings as instrument variables, we characterized the causal effect of plasma FVIII and VWF levels on clinical cardiovascular events using Mendelian randomization (MR) analyses.

METHODS

Because of patient confidentiality agreements and to comply with the study participants' consent, the original data and study materials cannot be made available to other researchers for purposes of reproducing the results or replicating the procedure. Analytical methods will be made available on request, and summary statistics have been made publicly available through the U.S. National Library of Medicine's National Center for Biotechnology Information database of Genotypes and Phenotypes (dbGaP).

Study Design and Participating Cohorts

This project was organized within the CHARGE Consortium (Cohorts of Heart and Aging Research in Genomic Epidemiology) Hemostasis Working Group.[15] We meta-analyzed phenotype-genotype associations of low-frequency and common (minor allele frequency [MAF] >0.01) variants in 32 610 individuals from 9 studies with FVIII levels, and in 46 354 individuals from 18 studies with VWF levels. A total of 20 studies contributed to 1 or both of the analyses; these included participants of European, African, East Asian, and Hispanic ancestry. Descriptions and ancestry composition of participating cohorts are found in Table I in the online-only Data Supplement. All studies were approved by appropriate institutional review committees, and all participants gave written informed consent for themselves and their minor children for the use of their DNA.

Study-Level Methods

Genotype Calling and Quality Control

All participating cohorts performed genotyping using commercial genotyping platforms available from Illumina or Affymetrix. Each study performed genotyping quality control checks and imputed the ≈35 million polymorphic autosomal and X-chromosome variants described in the 1000 Genomes population phase 1 version 3 for each participant using available imputation methods.[16] Variant calling and quality control procedures for each cohort are described in Table I in the online-only Data Supplement.

Statistical Analyses

Association Analyses

Plasma FVIII activity or VWF antigen levels were measured in all participants (reported in percent or international units per

milliliter x 100). Participants with plasma FVIII or VWF levels (or activity levels) 3 standard deviations (SDs) above or below the population mean were removed, as were individuals on anticoagulation therapy. Natural log–transformed FVIII activity and VWF antigen levels (percent or international units per milliliter x 100) were analyzed separately within each study. Study-specific regression analyses using an additive model of inheritance were performed for imputed variant dosages and phenotype levels, adjusting for sex, age, study design variables, and population substructure using principal components. All analyses were stratified by ancestry and then meta-analyzed. X-chromosome variants were additionally stratified by sex, with dosage values for male subjects coded as 0/2.

Quality Control

Study-specific findings were uploaded centrally, and a quality-control pipeline of all individual studies before meta-analysis was conducted with the EasyQC software package.[17] Variants with β or standard error values >5 or imputation values <0.3 were excluded from the analysis. Estimated minor allele counts calculated for all single nucleotide polymorphisms (SNPs) were a function of allele frequency, total number of samples per cohort, and imputation quality; values <10 were excluded from analysis. Alleles were harmonized according to the 1000 Genomes phase 1 version 3 reference panel, and duplicated SNPs or SNPs that had inconsistencies with the reference were excluded.

Meta, Transethnic, and Multiphenotype Discovery Analyses

Meta-analyses were performed in METAL within each ancestry group using a fixed-effects inverse-variance–weighted approach and then combined in a transethnic analysis using the same method.[18] The transethnic analyses are presented as discovery results, and we used the ancestry-specific analyses to inform and interpret these signals. An association was considered genome-wide statistically significant at a value of $P < 2.5 \times 10^{-8}$ to correct for the low-frequency variants that were not included in the initial generation of GWASs,[19] and only variants that passed quality control in at least 3 cohorts were reported. Variants with MAF <1% were filtered out after the meta-analyses. A genomic control coefficient was computed for each discovery cohort and was used to correct for cryptic relatedness. Finally, a locus was defined as ±1 Mb from the SNP with the lowest P value, and the SNP with the lowest P value was selected to represent the locus. Multiphenotype methods are described in the Methods in the online-only Data Supplement.

Functional Characterization of Candidate Loci Through Gene Silencing

In the absence of replication cohorts, we conducted first-pass functional characterization of the candidate loci to provide additional evidence of association. For each genome-wide significant locus, we selected candidate genes that could be responsible for the observed associations. Selection was based on proximity to the most associated SNPs in each region, information from public databases on putative effect of the SNPs in terms of regulation of expression and function of nearby genes, and hypothesized biological mechanisms to regulate VWF/FVIII. This selection process identified 1 to 3 candidate genes for each associated locus. To screen

for functionality, human umbilical vein endothelial cells (Life Line Cell Technology) were plated on collagen-coated 96-well plates and transfected with small interfering RNA (siRNA) siRNA (Silencer Select, Thermo Fisher Scientific) directed against the candidate genes using the transfection reagent oligofectamine (Thermo Fisher Scientific). Cells were cultured for 4 days after transfection, and media was then replaced with control media or media containing 10 µmol/L histamine for 30 minutes to stimulate an inflammatory response. The FVIII and VWF in the media were measured by an ELISA using antibodies from Fitzgerald Industries and had detection ranges of 0.003 to 0.21 IU/mL for the FVIII assay and 0.5 to 120 ng/µL for the VWF assay. Every experiment was repeated 4 times, and results are expressed as the mean±SD of the relative expression compared with a negative control (transfected with scramble siRNA).

Follow-Up Genetic Analyses

Conditional Analyses

To identify additional independent genetic signals at the associated loci, we used an approximate method implemented in Genome-Wide Complex Trait Analysis (GCTA tool) for conditional and joint analysis using meta-analysis summary statistics.[20] We used best-guess imputation for variants with imputation quality >0.3 in 8481 European-ancestry individuals from the FHS (Framingham Heart Study) as the reference panel. A description of the FHS is given in the Methods in the online-only Data Supplement. To prevent spurious conditional associations arising from a discrepancy between linkage disequilibrium in our GWAS and the reference panel, we also performed the conditional analysis on the results of the European-ancestry meta-analysis as a sensitivity analysis, because different genetic variants showed the strongest association in the transethnic analysis compared with the European-only analysis.

Mendelian Randomization

For the sentinel variant in each locus in FVIII and VWF analyses, we conducted in silico lookups for the associations of each individual variant with 3 major cardiovascular events: coronary artery disease (CAD) in the CARDIOGRAMplusC4D Consortium,[21,22] ischemic stroke (IS) in the MEGASTROKE analysis within the International Stroke Genetics Consortium,[23] and venous thromboembolism (VTE) in the INVENT Consortium (International Network on Venous Thrombosis).[11] We conducted 2-sample MR analyses to detect any potential causal effects of plasma FVIII and VWF levels on each cardiovascular outcome separately. We used summary statistics to generate 1 causal estimate per significant locus as the ratio of the association of the variant with disease to the association of the variant with the exposure, and estimates were then meta-analyzed using an inverse-variance–weighted approach as our primary MR estimate, known as the inverse-variance–weighted estimate.[24] Additional methods to avoid bias resulting from heterogeneity and the final variants composing the instrumental variables are described further in the Methods and Tables II through IV in the online-only Data Supplement. Because FVIII plasma levels are determined largely by VWF plasma levels owing to the carrier role of VWF for FVIII in plasma, essentially all genetic predictors of plasma VWF levels are also predictors of FVIII plasma levels. The inverse, however,

is not true, and a small subset of variants predict FVIII plasma levels without predicting VWF levels. To investigate the independent causal role of FVIII plasma levels from that of VWF plasma levels on cardiovascular disease events, we applied a multivariable MR approach in which we adjusted for VWF variant effects in the estimate of causal association between FVIII and cardiovascular disease outcomes.[25]

RESULTS

FVIII, VWF, and Multiphenotype Meta-Analyses

Agnostic Discovery

Data used for FVIII meta-analysis was available from 25 897 European ancestry, 4500 African ancestry, 773 East or Indian Asian ancestry, and 1440 Hispanic participants. Transethnic meta-analysis for FVIII resulted in 13 887 196 variants passing all filters and identified 1431 variants that reached genome-wide statistical significance at 11 loci. Data used for VWF were available from 42 379 European ancestry, 3700 African ancestry, and 275 Hispanic participants. Meta-analysis for VWF resulted in 10 537 485 variants passing all filters and identified 2453 genome-wide significant variants at 17 loci (Figure 1A and 1B). European-specific meta-analysis identified 1 significant variant at 1 additional locus. Analysis using combined FVIII and VWF phenotypes (Methods in the online-only Data Supplement) identified 2828 variants reaching genome-wide significance at 2 additional loci, which were not identified in single-phenotype analyses.
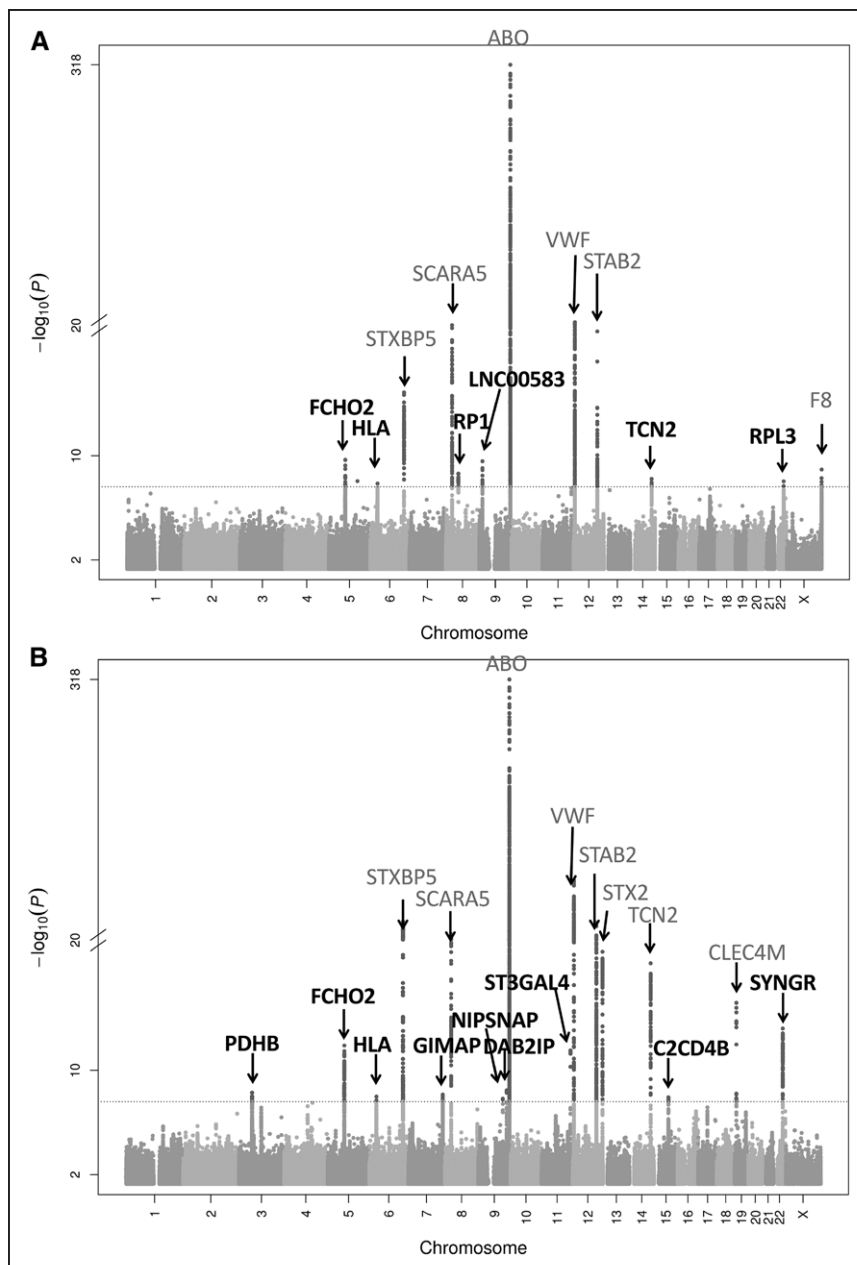
Figure 1. Manhattan plot for the transethnic analyses factor VIII (FVIII) and von Willebrand factor (VWF).
Representation of genome-wide results for FVIII (**A**) and VWF (**B**). Loci named by closest gene. Black indicates novel associations.

Table 1 shows the genetic discovery results for the FVIII, VWF, and combined FVIII-VWF phenotypes. Overall, 23 unique loci were identified. Among these, 13 were new associations not previously reported. Among the newly identified loci, 7 were associated with FVIII levels (*FCHO2/TMEM171/TNPO1*, *HLA*, *SOX17/RP1*, *LINC00583/NFIB*, *RAB5C/KAT2A*, *RPL3/TAB1/SYNGR1/PDGB*, and *ARSA*), and 11 were associated with VWF levels (*PDHB/PXK/KCTD6*, *SLC39A8*, *FCHO2/TMEM171/TNPO1*, *HLA*, *GIMAP7/GIMAP4*, *OR13C5/NIPSNAP*, *DAB2IP*, *C2CD4B*, *RAB5C/KAT2A*, *RPL3/TAB1/SYNGR1/PDGB*, and *ARSA*). Figure IA through IN in the online-only Data Supplement shows regional plots for the novel loci plotted for the 2 phenotypes. The lowest MAF for the index variant was 0.02, whereas the effect size per allele ranged from 0.015 to 0.032 (in log-transformed units) for FVIII levels and from 0.014 to 0.060 for VWF levels.

Among the 23 genome-wide significant findings, 10 loci were previously reported to be associated with plasma levels of FVIII, VWF, or both: *STXBP5*, *SCARA5*, *ABO*, *ST3GAL4*, *STAB2*, *STX2*, *VWF*, *TCN2*, *CLEC4M*, and *TMLHE-F8* region.

Conditional Analyses and Variant Characterization

In follow-up analyses, we conditioned on sentinel variants to determine whether secondary independent genome-wide significant signals were present. Results and additional independent variants are summarized in Table 2, along with findings from in silico investigations of the putative functional variant, and in Tables V and VI in the online-only Data Supplement. *SCARA5*, *ABO*, *VWF*, and *STAB2* were predicted to have >1 independent signal both for FVIII and VWF analyses (details in Methods and Tables V and VI in the online-only Data Supplement), some of which are in agreement with previous publications.[6] Among the independently associated variants within the *ABO* locus, SNPs rs10901252 and rs687621 perfectly discriminate B and O blood groups from A, and rs8176685 can reasonably capture information to tag A1/A2 (r2 0.59/D' 0.99 with the tag SNP), confirming that ABO blood groups are essential determinants of VWF and FVIII plasma levels.

Variance Explained

Overall, the top variants for these loci (including the strongest independent associated variants in each locus that reached genome-wide significance after conditional analyses) explain 17% of the phenotypic variance for FVIII and 21.3% of the variance for VWF. *ABO* locus was by far the strongest determinant, alone explaining 13.6% and 16.2% of these variances, respectively.

Functional Analyses

We silenced 21 genes across 12 loci to assess the in vitro impact on FVIII and VWF secretion (Figure 2A and 2B). These include the main candidate genes identified by proximity (Table 1). Our results suggest that 10 of the 12 candidate loci had ≥1 genes that changed VWF levels in media under basal or histamine-stimulated conditions. Specifically, silencing *PDHB*, *SLC39A8*, *TMEM171*, *TNPO1*, *HLA-C*, *GIMAP7*, *NIPSNAP3A*, *NIPSNAP3B*, *C2CD4B*, and *SYNGR1* increased VWF release into media under basal conditions, whereas *ST3GAL4* silencing decreased VWF secretion. When cells were stimulated with histamine, silencing *TMEM171*, *TNPO1*, *HLA-C*, *SNIP-SNAP3A* (but not *SNIPSNAP3B*), *C2CD4B*, *KAT2A*, and *TAB1* increased VWF release in the media, and *RAB5C* decreased VWF secretion (Table 1 and Figure 2A and 2B). For the experiments on the 5 genes that were shown to be associated only with FVIII levels (*LINC00583*, *NFIB*, *SOX17*, *RP1*, and *TMLHE-F8*), we could not find detectable levels of FVIII in media from treated human umbilical vein endothelial cells, and therefore, the experiments were inconclusive.

MR Analyses and Cardiovascular Events

Figure 3 shows forest plots representing the results from MR analyses. We first analyzed FVIII and VWF individually using the inverse-variance–weighted estimates that included the sentinel variant in each locus (after exclusion of variants with pleiotropic effects; Tables II through IV in the online-only Data Supplement). Both VWF and FVIII plasma levels showed a significant causal effect on CAD, IS, and VTE risk. For CAD, the odds ratios (ORs) associated with a per 1-SD change in natural log–transformed FVIII and VWF were 1.15 (95% CI, 1.05–1.27) and 1.14 (95% CI, 1.05–1.23), respectively. For IS, the ORs were 1.28 (95% CI, 1.14–1.43) and 1.19 (95% CI, 1.10–1.29), respectively. For VTE, the ORs were 2.75 (95% CI, 2.14–3.55) and 2.31 (95% CI, 1.89–2.81), respectively. Sensitivity analyses using both MR-Egger regression and weighted median estimates support the inverse-variance–weighted estimates, and no significant pleiotropic effect was evident after exclusion of the pleiotropic loci (Figure 3 and Table III and Figure IIA through IIC in the online-only Data Supplement).

We then performed multivariable MR analyses of the FVIII phenotype to identify causal effects of FVIII activity levels that were independent of VWF levels. For VTE and CAD outcomes, after adjustment of FVIII results by the effect of VWF, the ORs were modestly attenuated (20% and 16%, respectively) compared with the unadjusted estimates and CIs widened. For IS, however, adjustment of FVIII results by the effect of VWF resulted in an 86% attenuation of the OR to 0.88 (95% CI, 0.51–1.51). We could not demonstrate a causal association of VWF levels with VTE and CAD that was independent of FVIII levels.

Of note, both the *ABO* and *HLA* loci were excluded from the instrumental variables for the MR analyses

Table 1. Main Association Results for FVIII and VWF Transethnic GWAS Meta-Analysis

rsID	Effect Allele	Other Allele	Freq FVIII	N FVIII	β FVIII	*P* Value FVIII	Freq VWF	No. VWF	β VWF	*P* Value VWF	Closest Gene(s)	Association	VWF Release at B and on S for Candidate Loci
rs55954186	a	g	0.36	35513	0.011	2.2E−05	0.37	46229	0.015	5.2E−09	*PDHB, PXK, KCTD6*	New	*PDHB* (B=↑**; S=NS), *PXK* (B=NS; S=NS), *KCTD6* (B=NS; S=NS)
rs6855246	a	g	0.93	35513	−0.003	0.672	0.92	46068	−0.034	8.68E−10	*SLC39A8*†	New	*SLC39A8* (B=↑**)
rs548630	a	c	0.49	36286	−0.016	2.1E−10	0.47	46137	−0.018	1.2E−12	*FCHO2, TMEM171, TNPO1*	New	*FCHO* (B=NS; S=NS), *TMEM171* (B=↑***; S=↑*), *TNPO1* (B=↑***; S=↑**)
rs9390460	t	c	0.47	36286	−0.019	2.2E−15	0.46	46212	−0.033	5.6E−42	*STXBP5*	Known	…
rs9271597‡	a	t	0.41	28203	−0.015	1.4E−08	0.41	31364	−0.012	2.1E−04	*HLA* region	New	*HLA-C* (B=↑**;s=↑***)
rs7788962	a	g	0.62	33773	−0.010	2.3E−04	0.61	46231	−0.014	7.3E−09	*GIMAP7, GIMAP4*	New	*GIMAP7* (B=↑***; S=NS), *GIMAP4* (B=NS; S=NS)
rs4276643	t	c	0.66	36286	−0.023	1.3E−19	0.67	44168	−0.029	8.8E−28	*SCARA5*	Known	…
rs10102164	a	g	0.19	36286	0.019	2.4E−09	0.20	46230	0.009	2.9E−03	*SOX17, RP1*	New	Too little FVIII released by endothelial cells to be detected in vitro
rs6479259	t	c	0.73	28535	−0.021	1.1E−03	0.73	24987	−0.056	1.5E−08	*OR13C5, NIPSNAP*§	New	*NIPSNAP3A* (B=↑*; S=↑**), *NIPSNAP3B* (B=↑*; S=NS)
rs10985344	a	g	0.25	36286	0.011	7.5E−05	0.25	46178	0.017	3.5E−09	*DAB2IP*	New	*DAB2IP* (B=NS; S=NS)
rs687289	a	g	0.36	36286	0.145	1.9E−770	0.33	46231	0.197	5.0E−1443	*ABO*	Known	…
9:13930481:ID	d	i	0.85	22480	−0.032	2.7E−10	0.85	29409	−0.003	4.4E−01	*LINC00583, NFIB*	New	Too little FVIII released by endothelial cells to be detected in vitro
rs35458154	a	g	0.03	33,871	0.048	3.1E−08	0.03	44020	0.060	3.0E−12	*ST3GAL4*	Known‖	*ST3GAL4* (B=↓**; S=NS)
rs4981022	a	g	0.69	36286	0.025	3.0E−20	0.69	46232	0.035	6.6E−41	*STAB2*	Known	…
rs4759787	a	c	0.40	36286	0.011	1.1E−05	0.37	46180	0.023	7.7E−20	*STX2*	Known	…
rs2238109	a	t	0.39	36286	0.026	3.5E−24	0.38	46232	0.050	1.8E−89	*VWF*	Known	…
rs4904820	a	g	0.49	36286	0.014	1.8E−08	0.47	46232	0.022	6.0E−19	*TCN2*	Known	…
rs6494314	t	c	0.18	36286	−0.007	2.4E−02	0.17	46232	−0.018	1.1E−08	*C2CD4B*	New	*C2CD4B* (B=↑*; S=↑**)
rs1869365					Significant in combined multiphenotype analysis				<5.0E−8	*RAB5C, KAT2A*	New	*RAB5C* (B=NS; S=↓*), *KAT2A* (B=NS; S=↑**)
rs2277998	a	g	0.30	33097	−0.010	4.4E−04	0.30	45566	−0.022	6.5E−16	*CLEC4M*	Known	…
rs5750823¶	t	c	0.70	36286	−0.013	1.5E−06	0.72	46230	−0.020	6.0E−14	*RPL3, TAB1, SYNGR1, PDGB*	New	*TAB1* (B=NS; S=↑**), *SYNGR1* (B=↑*; S=NS), *PDGFB* (B=NS; S=NS)
rs9616897					Significant in combined multiphenotype analysis				<5.0E−8	*ARSA*	New	*ARSA* (B=NS; S=NS)
rs150926226#	c	g	0.62	20537	0.017	3.3E−09	0.65	28685	−0.005	7.0E−02	*TMLHE, F8*	Known	Too little FVIII released by endothelial cells to be detected in vitro

B indicates baseline; FVIII, factor VIII; Freq, allele frequency of the effect allele; NS, not significant; rsID, rs identify of unique variant; S, stimulated; and VWF, von Willebrand factor. β and frequency refer always to the effect allele, and they are expressed as natural log–transformed values from the original units (reported in percent or international units per milliliter times 100 U). No functional work was performed in known genes, and these are indicated by "…" in the last column.

*$P<0.05$; **$P<0.01$; ***$P<0.001$.

†SLC39A8 was found in VWF meta-analysis of European ancestry only (n=42 145).

‡Although not in linkage disequilibrium with this variant, a low-frequency variant 665 kb upstream of rs9271597 was found significantly associated to VWF levels (rs80082277; $P=1×10^{-8}$), and we consider it within the HLA region; thus, we pursued this gene for further functional validation.

§Olfactory receptor family was not considered for further functional validation for its low expression in the relevant tissues (mainly artery and whole blood).

‖The *ST3GAL4* locus was new at the time of analyses, although reported in a recent candidate gene study lacking replication.[26]

¶The highest associated single nucleotide polymorphism in this locus for FVIII is rs137631 ($P=9.5×10^{-9}$), located close to *RPL3* gene, 112 kb downstream of the TAB1/SYNGR1 locus, and in low linkage disequilibrium with rs5750823 ($R^2=0.14$)

#Chromosome X variant for VWF available only for European-ancestry samples (n=28 685).

Table 2. Characteristics of All Associated Independent Variants After Conditional Analyses

Marker Name	rsID	Closest Gene(s)	Variant Position	Putative Functional	EUR-only Original P Value	EUR-only Conditional P Value	TRANS-ethnic Original P Value	TRANS-ethnic Conditional P Value	Top Meta Variant	LD* (R²)	LD* (D′)
FVIII (n=29 573 EUR; n=36 286 TRANS)											
5:72406659	rs548630	TNPO1; FCHO2; TMEM171	9.5 kb 5′ TMEM171	…	5.14E−10	3.96E−10	2.10E−10	2.06E−10	5:72406659	Top variant	Top variant
6:147701217	rs9390461	STXBP5	Intronic	rs1039084, missense	2.74E−13	4.06E−13	1.73E−14	…	6:147703299	0.98	1.00
6:147703299	rs9399599	STXBP5	Intronic	rs1039084, missense	3.73E−13	…	1.41E−15	4.70E−16	6:147703299	Top variant	Top variant
8:27778148	rs55829013	SCARA5	Intronic	…	3.39E−10	8.54E−10	5.81E−10	…	8:27805815	0.51	0.77
8:27823832	rs11780263	SCARA5	Intronic	…	3.41E−17	6.96E−17	1.39E−19	…	8:27805815	0.10	0.98
8:27805815	rs7816579	SCARA5	Intronic	SiPhy conserved	5.32E−16	…	6.52E−21	4.07E−21	8:27805815	Top variant	Top variant
8:55421614	rs10102164	SOX17; RP1	Intergenic	…	1.66E−07	…	2.38E−09	2.44E−09	8:55421614	Top variant	Top variant
9:13930481:ID	rs35468074	LINC00583	Intronic	…	1.74E−10	1.24E−10	2.66E−10	3.10E−10	9:13930481:ID	Top variant	Top variant
9:136116662	rs11793768	ABO	14 kb 3′ ABO	…	3.74E−13	1.29E−62	2.16E−13	…	9:136132908:ID	0.08	0.72
9:136137065	rs687621	ABO	Intronic	…	1.18E−647	<1E−320	6.85E−778	…	9:136132908:ID	0.89	0.98
9:135976698	rs35108384	ABO	Intronic	…	4.58E−12	…	2.24E−12	6.53E−12	9:136132908:ID	0.00	0.14
9:136114000	rs78490142	ABO	17 kb 3′ ABO	…	1.12E−03	…	6.53E−05	1.48E−13	9:136132908:ID	0.01	1.00
9:136132908:ID	rs8176719	ABO	Intronic	SiPhy conserved	1.62E−307	…	3.52E−403	P<1E−320	9:136132908:ID	Top variant	Top variant
9:136178821	rs574237	ABO	Intergenic	…	2.89E−29	…	6.94E−38	5.38E−26	9:136132908:ID	0.01	0.18
9:136181539	rs551924	ABO	Intergenic	…	2.81E−48	…	9.21E−75	2.20E−63	9:136132908:ID	0.00	0.14
9:136207218	rs607900	ABO	Intergenic	rs116779216, missense SURF1	6.86E−03	…	3.21E−15	1.85E−13	9:136132908:ID	0.00	0.19
9:136255149	rs62575992	ABO	Intergenic	…	3.46E−42	…	8.45E−45	1.83E−26	9:136132908:ID	0.02	0.80
9:136344853	rs3124758	ABO	Intergenic	…	2.71E−21	…	1.74E−18	5.88E−15	9:136132908:ID	0.00	0.14
12:6062894	rs57040304	VWF	Intronic	rs7962217, missense, SiPhy conserved	1.51E−15	1.22E−14	7.71E−12	…	12:6160614	0.00	0.09
12:6160614	rs7135039	VWF	Intronic	rs1063856, missense	3.00E−19	7.02E−18	2.32E−24	7.21E−20	12:6160614	Top variant	Top variant
12:6070845	rs12423482	VWF	Intronic	rs7962217, missense, SiPhy conserved	2.49E−15	…	2.92E−18	1.57E−17	12:6160614	0.00	0.15
12:6160146	rs11064010	VWF	Intronic	…	8.36E−07	…	2.41E−11	2.51E−09	12:6160614	0.01	0.33
12:104000319	rs1070073	STAB2	Intronic	…	1.07E−13	5.04E−13	2.25E−14	…	12:104149874	0.00	0.16
12:104147207	rs3751198	STAB2	Intronic	…	2.16E−17	5.91E−17	5.88E−18	…	12:104149874	0.70	0.98
12:104000470	rs2723889	STAB2	Intronic	…	1.08E−13	…	2.14E−14	1.21E−14	12:104149874	0.00	0.16
12:104149874	rs4981022	STAB2	Intronic	…	1.16E−17	…	2.95E−20	4.47E−21	12:104149874	Top variant	Top variant
14:92268531	rs10498631	TC2N	Intronic	…	6.69E−09	8.63E−09	2.30E−08	…	14:92302972	0.54	0.82
14:92302972	rs58204830	TC2N	Intronic	…	2.57E−08	…	6.28E−09	7.03E−09	14:92302972	Top variant	Top variant
22:39717706	rs137631	RPL3	1.3 kb 5′ of RPL3	…	2.35E−07	…	9.48E−09	1.34E−08	22:39717706	Top variant	Top variant
X:154721357	rs150926226	TMLHE,F8	…	…	…	†	3.25E−09	†	X:154721357	…	…

(Continued)

Table 2. Continued

Marker Name	rsID	Closest Gene(s)	Variant Position	Putative Functional	EUR-only		TRANS-ethnic		Top Meta Variant	LD* (R²)	LD* (D')
					Original *P* Value	Conditional *P* Value	Original *P* Value	Conditional *P* Value			
VWF (n=42 256 EUR; n=46 232 TRANS)											
3:58383174	rs55692656	*PXK*; *KCTD6*; *PDHB*	Intronic *PXK*	rs56384862, missense PXK; rs34579268 missense PXK; rs200687616 frameshift PDHB	6.48E−09	1.01E−08	3.33E−08	...	3:58436476	0.94	1.00
3:58436476	rs55954186	*PXK*; *KCTD6*; *PDHB*	17 kb 3' of *PXK*	rs56384862, missense PXK; rs34579268 missense PXK; rs200687616 frameshift PDHB	8.72E−09	...	5.20E−09	8.06E−09	3:58436476	Top variant	Top variant
5:72403453	rs7733340	*TNPO1*; *FCHO2*; *TMEM171*	Intergenic	...	5.80E−13	1.52E−12	3.40E−12	...	5:72406659	0.93	0.99
5:72406659	rs548630	*TNPO1*; *FCHO2*; *TMEM171*	9.5 kb 5' *TMEM171*	...	1.33E−12	...	1.22E−12	6.19E−13	5:72406659	Top variant	Top variant
6:31192766	rs9263993	*HLA* region	Intergenic	...	8.54E−09	3.50E−09	1.69E−07	...	6:31925848	0.00	0.63
6:31941629	rs116420479	*HLA* region	Intronic *STK19*	...	3.10E−08	1.49E−08	1.02E−08	...	6:31925848	0.94	0.98
6:31158633	rs9263861	*HLA* region	Intergenic	...	3.08E−08	...	3.25E−08	8.64E−09	6:31925848	0.00	1.00
6:31906828	rs78593564	*HLA* region	Intronic *C2*	...	3.15E−08	...	1.03E−08	2.76E−09	6:31925848	1.00	1.00
6:147694334	rs9390460	*STXBP5*	Intronic	rs1039084, missense	6.32E−38	1.67E−38	5.58E−42	1.27E−42	6:147694334	Top variant	Top variant
7:150296496	rs13230842	*GIMAP7*; *GIMAP4*	Intergenic	...	2.06E−08	1.96E−08	2.95E−08	...	7:150227227	0.18	0.97
7:150227227	rs7788962	*GIMAP7*; *GIMAP4*	9.1 kb 3' of *GIMAP7*	...	6.29E−08	...	7.30E−09	1.36E−08	7:150227227	Top variant	Top variant
8:27803599	rs4276643	*SCARA5*	Intronic	...	5.77E−27	1.19E−29	8.77E−28	7.69E−31	8:27803599	Top variant	Top variant
8:27815481	rs62496810	*SCARA5*	Intronic	...	3.74E−08	4.38E−11	4.98E−08	6.04E−11	8:27803599	0.02	1
9:124416940	rs10985344	*DAB2IP*	Intronic	...	4.11E−09	3.76E−09	3.47E−09	...	9:124416940	Top variant	Top variant
9:124421965	rs4837886	*DAB2IP*	Intronic	...	3.84E−09	...	4.43E−09	3.94E−09	9:124416940	0.79	0.97
9:135919501	rs138796740	*ABO*	Intergenic	...	2.61E−08	7.06E−11	3.59E−08	...	9:136132908:ID	0.00	0.15
9:136128546	rs7857390	*ABO*	2.5 kb 3' of *ABO*	...	*P*<1E−320	7.81E−294	2.15E−393	...	9:136132908:ID	0.36	1
9:136145414:ID	rs202001822	*ABO*	Intronic	...	5.90E−78	1.46E−39	2.26E−80	...	9:136132908:ID	0.10	1
9:136147553	rs660340	*ABO*	Intronic	...	*P*<1E−320	*P*<1E−320	1.11E−492	...	9:136132908:ID	0.33	0.87
9:136177394	rs656105	*ABO*	Intergenic	...	1.20E−66	2.62E−129	4.23E−73	...	9:136132908:ID	0.02	0.19
9:136128000	rs10901252	*ABO*	3.1 kb 3' of *ABO*	rs8176747, missense; rs8176746, missense; rs8176743 missense	1.78E−291	...	2.61E−351	*P*<1E−320	9:136132908:ID	0.16	0.99
9:136130677	rs62641786	*ABO*	3' UTR	...	2.79E−96	...	2.59E−106	8.85E−30	9:136132908:ID	0.03	0.91
9:136138765:ID	rs8176685	*ABO*	Intronic	...	*P*<1E−320	...	5.95E−507	*P*<1E−320	9:136132908:ID	0.31	1
9:136316367	rs28680325	*ABO*	Intronic *ADAMTS13*	...	1.04E−09	...	2.88E−10	1.12E−09	9:136132908:ID	0.01	0.25

(Continued)

Table 2. Continued

Marker Name	rsID	Closest Gene(s)	Variant Position	Putative Functional	EUR-only		TRANS-ethnic		Top Meta Variant	LD* (R²)	LD* (D')
					Original P Value	Conditional P Value	Original P Value	Conditional P Value			
11:126296825	rs35458154	*ST3GAL4*	Intronic	…	6.35E−12	8.31E−12	2.96E−12	2.42E−12	11:126296825	Top variant	Top variant
12:6157394	rs2283335	*VWF*	Intronic	rs1063856, missense	6.48E−83	5.55E−79	2.08E−89	…	12:6153967	1	1
12:6160146	rs11064010	*VWF*	Intronic	…	1.60E−27	8.75E−22	7.90E−27	2.40E−21	12:6153967	0.01	0.30
12:6225931	rs112814955	*VWF*	Intronic	…	1.43E−42	3.14E−49	9.02E−43	4.00E−50	12:6153967	0.01	0.34
12:6153967	rs2238109	*VWF*	Intronic	rs1063856, missense	1.37E−82	…	1.77E−89	3.41E−87	12:6153967	Top variant	Top variant
12:104000319	rs1070073	*STAB2*	Intronic	…	2.28E−36	8.23E−31	2.03E−36	6.72E−32	12:104149874	0.00	0.16
12:104007418	rs11111679	*STAB2*	Intronic		2.31E−14	4.79E−10	1.22E−14	1.60E−10	12:104149874	0.00	0.02
12:104127353	rs73192004	*STAB2*	Intronic	rs17034433, missense	2.29E−08	2.33E−08	5.09E−09	6.57E−09	12:104149874	0.01	0.53
12:104149874	rs4981022	*STAB2*	Intronic	…	5.44E−37	4.69E−41	6.57E−41	8.65E−45	12:104149874	Top variant	Top variant
12:131287011	rs6486599	*STX2*	Intronic	rs17564, missense	4.23E−18	1.92E−18	1.14E−18	…	12:131290180	1.00	1
12:131290180	rs4759787	*STX2*	Intronic	rs17564, missense	4.45E−18	…	7.73E−20	3.84E−20	12:131290180	Top variant	Top variant
14:92290744	rs10498632	*TCN2*	Intronic	…	2.58E−18	4.74E−18	5.00E−18	-	14:92318935	0.53	0.99
14:92318935	rs4904820	*TCN2*	Intronic	…	8.30E−18	…	6.04E−19	2.40E−19	14:92318935	Top variant	Top variant
15:62455019	rs6494314	*C2CD4B*	700 bp 3' of gene	rs8040712, missense	9.63E−08	…	1.14E−08	1.30E−08	15:62455019	Top variant	Top variant
19:7831628	rs2277998	*CLEC4M*	Missense	rs2277998, missense	2.02E−15	7.30E−16	6.47E−16	1.74E−15	19:7831628	Top variant	Top variant
22:39790191	rs2413590	*PFGFB; SYNGR1; TAB1*	5.6 kb of *TAB1*	…	3.96E−12	4.44E−12	2.02E−12	…	22:39829973	0.75	0.94
22:39829973	rs5750823	*PFGFB; SYNGR1; TAB1*	Intronic *TAB1*	…	2.98E−11	…	5.95E−14	7.58E−14	22:39829973	Top variant	Top variant

Original *P* value is the *P* value from discovery meta-analysis. The putative functional column indicates the best candidate variant in high LD with the associated variant (*R²*>0.8) that has been identified in silico as the best candidate variant to have an impact on the adjacent gene/genes. EUR indicates European; FVIII, factor VIII; LD, linkage disequilibrium; TRANS, transethnic; UTR, untranslated region; and VWF, von Willebrand factor.

*LD with top variant in the region, calculated with Framingham Heart Study data.

†Primary single nucleotide polymorphism was not well imputed in Framingham Heart Study, and no other single nucleotide polymorphisms in the region achieved genome-wide significance in conditional analyses. Ellipses indicate that conditional analyses were not performed.

because of evidence of pleiotropic effects shown in the heterogeneity tests (Table III in the online-only Data Supplement). When we estimated causal effects using *ABO* alone as an instrument, estimates of causal effects were essentially the same across phenotypes and outcomes: OR of 2.57 (95% CI, 2.47–2.67) for FVIII and VTE, 2.28 (95% CI, 2.18–2.38) for VWF and VTE, 1.10 (95% CI, 1.06–1.14) for FVIII and IS, 1.09 (95% CI, 1.05–1.13) for VWF and IS, 1.10 (95% CI, 1.06–1.14) for FVIII and CAD, and 1.08 (95% CI, 1.04–1.12) for VWF and CAD.

DISCUSSION

In the present study, we meta-analyzed data from >36 000 individuals with FVIII levels and >46 000

with VWF and identified 13 novel loci, of which 7 associated with FVIII plasma levels and 11 associated with VWF levels. Overall, new discoveries yielded an additional 6.2% and 8.1% proportion of variance explained for FVIII and VWF, respectively, from previous estimations,[8] and suggest that a great proportion of the genetic variance is explained by common variation. Furthermore, we presented experimental evidence of biological function on VWF regulation for 14 of these genes from gene silencing in vitro: *PDHB*, *SLC39A8*, *TMEM171*, *TNPO1*, *HLA-C*, *GIMAP7*, *NIPSNAP3A*, *NIPSNAP3B*, *ST3GAL4*, *C2CD4B*, *RAB5C*, *KAT2A*, *TAB1*, and *SYNGR1*. Finally, we provide evidence in support of a causal role of FVIII levels on VTE and CAD events and of VWF levels on IS events.
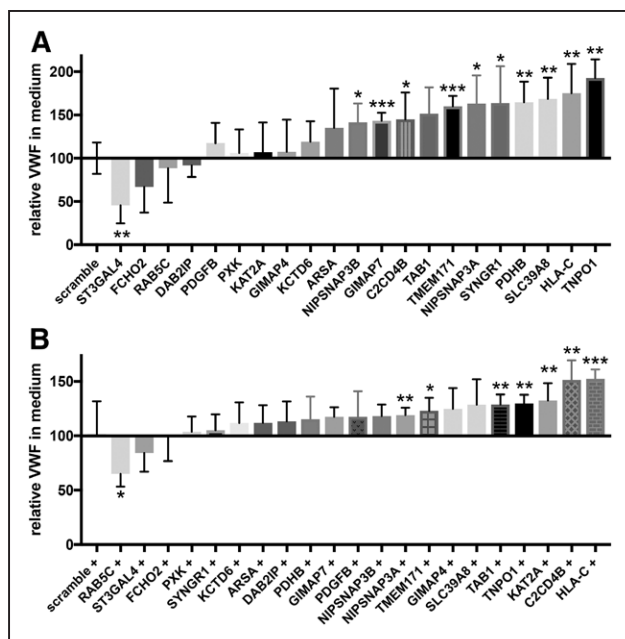
Figure 2. Silencing candidate genes changes basal release and stimulated release of von Willebrand factor (VWF).
Human umbilical vein endothelial cells were transfected with short interfering RNA (siRNA) against selected genes for 4 days; the media was changed; and the cells were cultured for 30 minutes for basal release (**A**) or were stimulated with histamine 10 μmol/L for 30 minutes (**B**). VWF was then measured in the supernatant by an ELISA. n=4±SD. All results are relative to VWF release after transfection with a scrambled control siRNA, which is set as reference (100%). *$P<0.05$. **$P<0.01$. ***$P<0.001$.

Characterization of the Novel Loci Regulating FVIII and VWF

As expected for traits with strong genetic correlation, most of the newly associated loci regulate both FVIII and VWF levels in blood. Our results show that most of the highest signal-independent variants associated with these traits were located in introns or noncoding regions, although a substantial proportion were in strong linkage disequilibrium ($R^2>0.8$) with mutations causing missense or frameshift mutations in the nearby genes (Table 2 and Table VII in the online-only Data Supplement). We also explored expression quantitative trait loci associations using publicly available data, and we conducted pathway analyses for the novel loci. See the Methods and Tables VIII through XIII in the online-only Data Supplement for this information.

For most loci, several genes were identified within the associated region, and we selected ≥1 genes for further characterization using in vitro cell models. On the basis of our initial functional characterization, ≥1 plausible culprit genes regulating VWF secretion could be isolated at most loci. Several candidate genes that showed a clear change in VWF levels on silencing have been shown to participate in vesicle trafficking and exocytosis, as well as intracellular signaling and inflammatory response. The most relevant functional genes

are described below and summarized in Figure III in the online-only Data Supplement.

VWF Biogenesis, Vesicle Trafficking, and Secretion

ST3GAL4 is a Golgi transferase that catalyzes transfer of sialic acids in VWF glycan branches that are essential to its biogenesis, adhesive activity, and clearance.[26] It also has a role in clearance of desialylated platelets with effects on platelet homeostasis. Genetic variants in *ST3GAL4* locus have been associated with total cholesterol, low-density lipoprotein cholesterol, alkaline phosphatase, increased platelet aggregation, fibrinogen, C-reactive protein, and CAD (see further details and references in Table VII in the online-only Data Supplement). Our functional analyses showed a substantial reduction of VWF release on *ST3GAL4* silencing, which strengthens the evidence of this gene as a novel VWF regulator in basal conditions.

SYNGR1 encodes an integral membrane protein associated with presynaptic vesicles in neuronal cells. Several commonalities have been described between the exocytic machinery that drives vesicle trafficking and membrane fusion in endothelial cells and synaptic machinery found in neurons,[27,28] which suggests that SYNGR1 could have a role in vesicle trafficking and exocytosis of VWF from the Weibel-Palade bodies. Genetic variation in this locus has also been associated with IgG glycosylation, rheumatoid arthritis, and inflammatory bowel disease/Crohn disease, the last 2 consistent with an effect of deregulation of interleukin and inflammatory signaling pathways.

NIPSNAP3A and *NIPSNAP3B* were selected as the main biologically plausible genes for locus on chromosome 9, and results from the functional study show evidence of significant upregulated levels of VWF on silencing of either gene. Again, a reported role of these genes in vesicular trafficking[29] suggests that these genes could be important in Weibel-Palade formation and exocytosis of VWF, both in basal conditions and under inflammatory stimuli.

Among the 2 new loci found in the transethnic multiphenotype analysis, *RAB5C* is particularly interesting. It is a member of the Rab protein family, thought to ensure fidelity in the process of docking and fusion of vesicles with their correct acceptor compartment,[30] which may be relevant to the process of fusion of Weibel-Palade vesicles to release VWF in endothelial cells. *RAB5C* silencing caused a significant decrease of VWF release in media of endothelial cells on stimulation with histamine.

Our in vitro cell work showed a significantly increased VWF secretion on *PDHB* silencing. *PDHB* codes for a subunit of the pyruvate dehydrogenase complex, which converts pyruvate to acetyl-CoA in the mitochondrion.
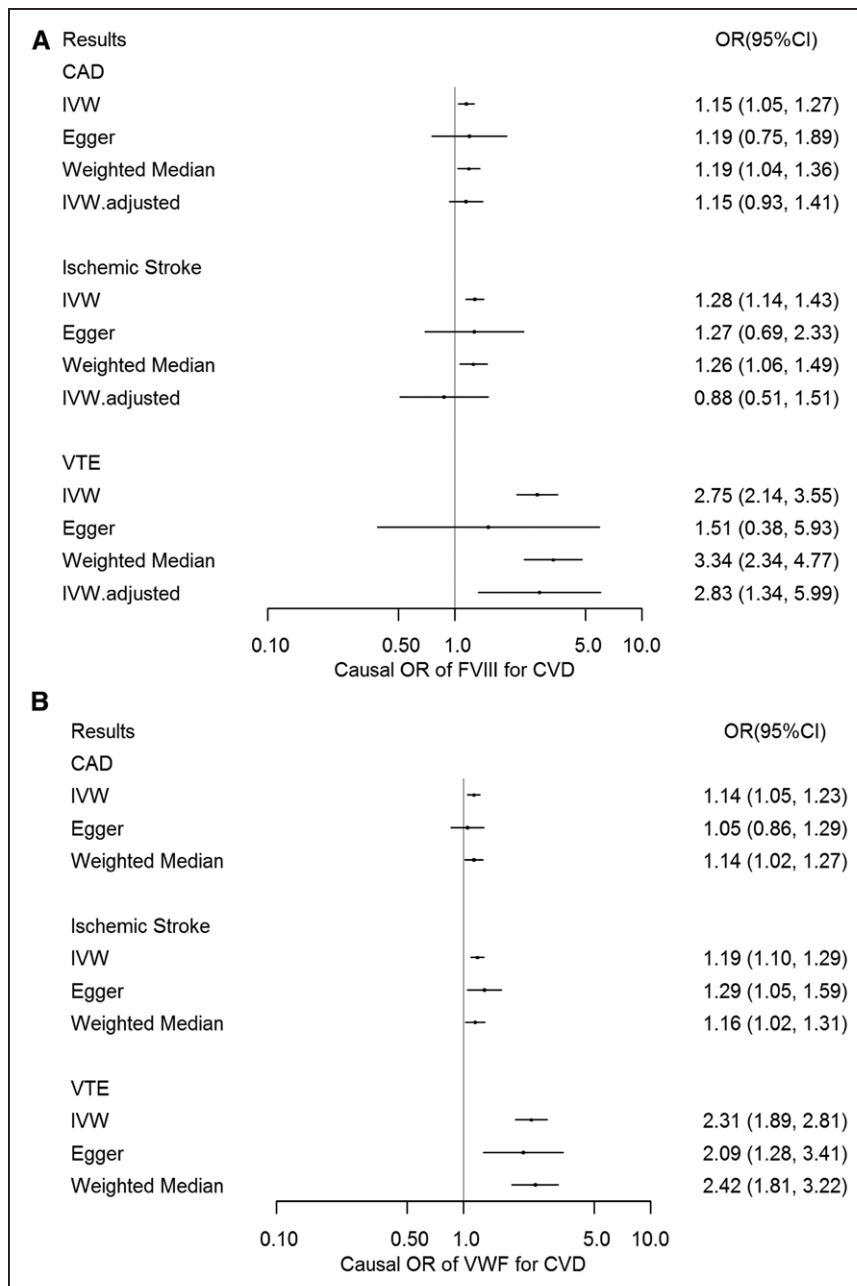
Figure 3. Mendelian randomization results. Results show odds ratio (OR) (95% CI) per every higher SD change in FVIII (**A**) and von Willebrand factor (VWF; **B**). CAD indicates coronary artery disease; CVD, cardiovascular disease; FVIII, factor VIII; IVW, inverse-variance–weighted method; IVW.adjusted, IVW factor VIII adjusted for the effects of VWF; and VTE, venous thromboembolism.

We speculate that it is possible that the metabolism of endothelial cells regulates vesicle trafficking and exocytosis of VWF, meaning that the exocytosis process is dependent on the energetic status of the endothelial cell. Genetic variation in this locus has also been associated with total cholesterol, systemic lupus erythematosus, and rheumatoid arthritis.

Intracellular Signaling and Inflammatory Response

TAB1 silencing increased VWF released in media in our in vitro functional analyses, whereas no effect could be seen for *PDGFB*, a gene that has been implicated in CAD and VTE risk. TAB1 is a regulatory protein that

acts as a mediator of several intracellular signaling pathways, especially those mediated by transforming growth factor-ß, WNT-1, and interleukin-1, which suggests that it might have a role mediating VWF release on certain cellular stimuli.

Similarly, silencing the *C2CD4B* gene in cultured endothelial cells resulted in strong upregulation of VWF release both in basal conditions and under stimulus conditions. Allelic variants in this gene have also been associated with fasting glucose homeostasis and type 2 diabetes mellitus. Transcripts of this gene are found predominantly in the nuclear compartment of endothelial cells, and a possible role in regulation of transcription that might increase vascular permeability in acute inflammation has been suggested.[31] Similarly, *TNPO1*

codes for a nuclear receptor (Transportin-1[32]), which mediates nuclear import of several proteins, which could also suggest a role in regulation of transcription under certain circumstances.

DAB2IP is involved in several relevant cell-signaling pathways in response to inflammation, innate immune response, and cell growth inhibition, and genetic variation in this gene has been associated with abdominal aortic aneurysm and heart rate. Despite the strong genetic signal in our data, functional confirmation could not be achieved for *DAB2IP* in our secretion experiment, so additional investigative work is needed.

GIMAP7 showed a significant increase of VWF release on silencing. GTPases of immunity-associated proteins are regulators of lymphocyte survival and homeostasis,[33] although limited data have been published on the function of these proteins.

Finally, although it did not reach genome-wide significance in the transethnic meta-analysis, we found a single locus close to *SLC39A8* that was genome-wide significant in our meta-analysis of VWF associations in European-ancestry samples. This gene, which encodes a zinc transporter that functions in the cellular import of zinc at the onset of inflammation, has also been associated with blood pressure, high-density lipoprotein cholesterol levels, and body mass index. Our functional work also suggested a strong effect on VWF levels in media from endothelial cells in vitro on *SLC39A8* silencing.

Although further functional characterization of these genes is needed to fully characterize the role of all the investigated genes in VWF regulation, our results demonstrate that these studies are a valid tool to elucidate functional genes coming from genetic associations and to shed light into the most relevant biological pathways implicated in the regulation of the phenotype under study.

MR and Clinical Implications

Our results provide insights into the causal role of FVIII and VWF in 3 cardiovascular events, which are the leading causes of deaths globally.

Biological and genetic evidence indicates that circulating FVIII levels are determined mainly by levels of VWF.[34] In the present study, we calculated the genetic correlation between VWF and FVIII on the basis of the genome-wide association results from European-descent individuals (Methods in the online-only Data Supplement) and found that the proportion of shared heritability of between these 2 phenotypes is 83.5%. This result is strengthened by the overlapping findings found in the individual GWASs and suggests that, with some exceptions, the genetic pathways that regulate VWF levels indirectly regulate FVIII levels. Given the role of VWF regulating FVIII, we used 3 loci that were uniquely associated with FVIII independently of VWF and

pursued conditional analyses that adjusted for the effect of VWF plasma levels to test the causal effect of FVIII on cardiovascular events. For IS, we found no evidence of a causal effect of FVIII that was independent of the VWF effect, which suggests that VWF biology may causally contribute to IS risk. For VTE and CAD, however, we found evidence supporting a causal effect of FVIII independently of the VWF effect. Because no genetic loci were independently associated with VWF levels but not FVIII levels, we could not adjust the VWF analyses for FVIII. Nonetheless, given the similarities in the magnitude of the VWF-adjusted FVIII causal ORs with the VWF causal ORs for VTE and CAD, our data suggest that the VWF causal association for VTE and CAD may be driven primarily by the biological effect of FVIII, although this hypothesis could not be tested.

The results of the MR analyses suggest that both FVIII and VWF may be reasonable targets for the prevention or intervention of CAD and VTE, whereas VWF may be a reasonable target for IS. Indeed, over the past decade, this line of thinking and research has been pursued, and these molecules are currently under investigation as pharmaceutical targets for the prevention of thrombotic events.[35–38] Here, we report 23 unique genetic loci associated with plasma levels of FVIII or VWF, of which 13 are newly reported associations. These discoveries may offer new targets in the development of pharmaceutical agonists or antagonists that may modulate thrombotic risk.

Strengths and Limitations

A major strength of the study was the relatively large sample size and the use of a denser imputation panel than was used in past discovery efforts. With this approach, we had hoped to identify uncommon associated variants, but the MAFs of the variants in the newly associated loci were relatively common, with just 1 variant having an MAF of <0.10. Our study design did not identify new associations marked by rare variation. Increasing the number of study participants to increase statistical power or improving the quality of the imputation from genotyping arrays may help to identify uncommon or rare variants associated with the outcomes. Some of the novel findings may be false-positives because we did not have access to independent populations to replicate our discoveries. Replication is required to validate genetic associations, especially for those close to the threshold for statistical significance. To offset this limitation, we conducted functional validation by silencing candidate genes and measuring VWF release; we view this functional work as a strength of the study. We were able to test only the regulation of VWF expression, not the regulation of VWF clearance by macrophages.[39] Nor were we able to test other mechanisms that regulate syn-

thesis in megakaryocytes but not endothelial cells. Furthermore, the need for a particular cellular stimulus that cannot be mimicked by histamine stimulation for the effect to be produced would be missed by our approach. Finally, it could be that the effect of some genetic associations can be seen only through overexpression rather than silencing of the gene. We attempted to also measure FVIII release, but levels were too low, so new models are required to validate the impact of the candidate genes on FVIII levels; this is a limitation of our approach. All functional work was done in vitro, which carries limitations relative to in vivo investigations. The strong genetic coregulation of both FVIII and VWF levels allowed us to conduct multiphenotype analyses and to increase statistical power for discovery. Our MR approach using improved instrumental variants allows to establish for the first time a causal relationship between VWF and FVIII and several cardiovascular events. With only 3 loci associated with FVIII alone, the power of the VWF-adjusted MR analyses for FVIII and cardiovascular events was limited, and we could not investigate the association of VWF with cardiovascular events independently of FVIII. There is a degree of overlap between our sample and the sample from consortia providing cardiovascular events GWAS data, which might create some bias in MR analyses;[40] this is a limitation of our work.

CONCLUSIONS

We found 13 novel genetic loci with modest contributions to plasma levels of FVIII or VWF. Our discovery approach including first-pass functional validation has provided relevant information on the best candidate gene at the novel loci. Finally, MR analyses provided some evidence implicating FVIII plasma levels in the risk of CAD and VTE and VWF plasma levels in the risk of IS. In summary, our work has identified novel loci regulating proteins essential for hemostasis and coagulation. These findings may provide genetic tools for therapeutic and preventive strategies and may be useful to identify new biological pathways on which to intervene to reduce the burden of arterial and venous outcomes.

ARTICLE INFORMATION

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Disclosures

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