Genome-wide association trans-ethnic meta-analyses identifies novel associations regulating coagulation Factor VIII and von Willebrand Factor plasma levels

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Short title: Genetic regulation of FVIII and VWF

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ABSTRACT

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 approximately 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 (MR) 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*. MR 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.

CLINICAL PERSPECTIVE

What is new?

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

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 FVIII activity levels on venous thrombosis and coronary artery disease risk, and a causal effect of plasma VWF levels on stroke risk.
- Our findings suggest that FVIII and VWF may be potential therapeutic targets to prevent thrombotic events.

INTRODUCTION

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, while 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, since severe FVIII and VWF deficiencies lead to bleeding disorders hemophilia A and von Willebrand disease, respectively. While it is currently unclear whether FVIII and VWF levels causally influence the risk of thrombotic diseases, some genetic and observational evidence point towards an effect of these proteins on thrombotic disease. Genetic variants in *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 (GWAS) 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. Last, by applying our genetic findings as instrument variables, we characterized the causal effect of plasma FVIII and VWF levels on clinical cardiovascular (CV) events using Mendelian randomization (MR) analyses.

METHODS

Due to 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. Analytic methods will be made available upon request, and summary statistics have been made publicly available through dbGaP.

Study Design and Participating Cohorts

This project was organized within the Cohorts of Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium 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 one or both of the analyses; these included participants of European (EUR), African (AFR), East Asian (ASI), and Hispanic (HIS) ancestry. Descriptions and ancestry composition of participating cohorts are found in Supplementary Table S1. 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 approximately 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 Supplementary Table S1.

Association Analyses

Plasma FVIII activity or VWF antigen levels were measured in all participants and reported in % or IU/ml*100 units. Participants with plasma FVIII or VWF levels (or activity levels) 3-standard-deviations above or below the population mean were removed, as were individuals on anticoagulation therapy. Natural-log transformed FVIII activity and VWF antigen levels (% or IU/ml*100 units) 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 males coded as 0/2.

Meta-Analysis Methods

Quality Control

Study-specific findings were uploaded centrally and a quality control (QC) pipeline of all individual studies prior to meta-analysis was conducted using the EasyQC software package[17]. Variants with beta or standard errors (SE) values > 5 or imputation values < 0.3 were excluded from the analysis. Estimated minor allele counts (eMAC) calculated for all 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 *1000 Genomes phase1 version3* reference panel and duplicated SNPs or SNPs that had inconsistencies with the reference were excluded.

Meta, Trans-Ethnic, and Multi-Phenotype Discovery Analyses

Meta-analyses were performed in METAL within each ancestry group using a fixed-effects inverse-variance weighted approach then combined in a trans-ethnic analysis using the same method[18]. The trans-ethnic 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 p-value $<2.5 \times 10^{-8}$ to correct for the low-frequency variants that were not included in the initial generation of GWAS[19] and only variants that passed QC in at least 3 cohorts were reported. Variants with MAF below 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 +/- 1Mb from the SNP with the lowest p-value, and the SNP with the lowest p-value was selected to represent the locus. Multi-phenotype methods are described in Supplementary Methods.

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 hypothesis for a biological mechanism 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 (HUVEC; Life Line Cell Technology) were plated on collagen coated 96-well plates and transfected with siRNA (Silencer Select, ThermoFisher Scientific) directed against the candidate genes using the transfection reagent oligofectamine (ThermoFisher Scientific). Cells were cultured for 4 days after transfection, and media was then replaced with control media or media containing 10 μM of histamine for 30 minutes, to stimulate an inflammatory response. The FVIII and VWF in the media was measured by an ELISA using antibodies from Fitzgerald Industries and had detection ranges of 0.003-0.21 IU/ml for the FVIII assay and 0.5-120 ng/ul for the VWF assay. Every experiment was repeated 4 times and results are expressed as the mean ± standard deviation (SD) of 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 GCTA for conditional and joint analysis using meta-analysis summary

statistics[20]. We used best-guess imputation for variants with imputation quality >0.3 in 8,481 European-ancestry individuals from the Framingham Heart Study (FHS) as the reference panel. A description of the FHS is given in the Supplementary Methods. In order 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, since different genetic variants showed the strongest association in the trans-ethnic 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 CV 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 International Network on Venous Thrombosis (INVENT) Consortium[11]. We conducted 2-sample Mendelian Randomization (MR) analyses to detect any potential causal effects of plasma FVIII and VWF levels on each CV outcome, separately. We used summary statistics to generate 1 causal estimate per significant locus as the ratio of the variant's association with disease to the variant's association 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 (IVW) estimate[24]. Additional methods to avoid bias due to heterogeneity, and the final variants composing the instrumental variables are further described in Supplementary Methods and in Supplementary Tables S2, S3, and S4. Since FVIII plasma levels are largely determined by VWF plasma levels owing to VWF's carrier role 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 CVD events, we applied a multivariable MR (MVMR) approach where we adjusted for VWF variants effects in the estimate of causal association between FVIII and CVD outcomes[25].

RESULTS

FVIII, VWF, and Multi-phenotype Meta-Analyses

Agnostic Discovery

Data used for FVIII meta-analysis was available from 25,897 European (EA), 4,500 African (AA), 773 East or Indian Asian (EAA, IAA), and 1,440 Hispanic (HA) participants. Trans-ethnic meta-analysis for FVIII resulted 13,887,196 variants passing all filters, and identified 1,431 variants that reached genome-wide statistical significance at 11 loci. Data used for VWF was available from 42,379 EA, 3,700 AA, and 275 HA participants. Meta-analysis for VWF resulted in 10,537,485 variants passing all filters, and identified 2,453 genome-wide significant variants at 17 loci (Figures 1A-B). European-specific meta-analysis identified one significant variant at one additional locus. Analysis using combined FVIII and VWF phenotypes (see Supplementary Methods) identified 2,828 variants reaching genome-wide significance at 2 additional loci, which were not identified in single-phenotype analyses.

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*). Supplementary Figures S1a-n shows regional plots for the novel loci plotted for the 2 phenotypes. The lowest MAF for the index variant was 0.02 while 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 or 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 if 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 Supplementary Tables S5 and S6. *SCARA5, ABO, VWF* and *STAB2* were predicted to have more than one independent signal both for FVIII and VWF analyses (details in Supplementary Methods and in Supplementary Tables S5 and S6), 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 (Figures 2a-b). These include the main candidate genes identified by proximity (Table 1). Our results suggest that 10 of the 12 candidate loci had one or more genes that changed VWF levels in media under basal and/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, SNIPSNAP3A* (but not *SNIPSNAP3B*), *C2CD4B, KAT2A,* and *TAB1* increased VWF release in the media, and *RAB5C* decreased VWF secretion (Table 1; Figures 2a-b). For the experiments on the 5 genes that were only shown to be associated with FVIII levels (*LINC00583, NFIB, SOX17, RP1* and *TMLHE-F8*), we could not find detectable levels of FVIII in media from treated HUVEC cells, and therefore the experiments were inconclusive.

Mendelian Randomization Analyses and Cardiovascular Events

Figure 3 show forest plots representing the results from MR analyses. We first analyzed FVIII and VWF individually using the IVW estimates that included the sentinel variant in each locus (after exclusion of variants with pleiotropic effects, see Supplementary Tables S2, S3, and S4). Both VWF and FVIII plasma levels showed a significant causal effect on CAD, IS and VTE risk. For CAD, the ORs associated with a per SD change in natural log-transformed FVIII and VWF were (OR(CI_{95}) =1.15 (1.05, 1.27) and 1.14 (1.05, 1.23), respectively. For IS, the ORs(CI_{95}) were 1.28 (1.14, 1.43) and 1.19 (1.10, 1.29), respectively. For VTE, the ORs(CI_{95}) were 2.75 (2.14, 3.55) and 2.31 (1.89, 2.81), respectively. Sensitivity analyses using both MR-Egger regression and weighted median estimates support the IVW estimates and no significant pleiotropic effect was evident after exclusion of the pleiotropic loci (Figure 3, Supplementary Table S3, Supplementary Figures S2a-c).

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

Of note, both the *ABO* and *HLA* loci were excluded from the instrumental variables for the MR analyses due to evidence of pleiotropic effects shown in the heterogeneity tests (Supplementary

Table S3). When we estimated causal effects using *ABO* alone as an instrument, estimates of causal effects were essentially the same across phenotypes and outcomes: OR(CI_{95}) 2.57 (2.47-2.67) for FVIII and VTE; OR(CI_{95}) 2.28 (2.18-2.38) for VWF and VTE; OR(CI_{95}) 1.10 (1.06-1.14) for FVIII and IS; OR(CI_{95}) 1.09 (1.05-1.13) for VWF and IS; OR(CI_{95}) 1.10 (1.06-1.14) for FVIII and CAD; and OR(CI_{95}) 1.08 (1.04-1.12) for VWF and CAD.

DISCUSSION

In the present study, we meta-analyzed data from more than 36,000 individuals with FVIII levels and more than 46,000 with VWF and identified 13 novel loci, 7 of which 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. Further, 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 and B, ST3GAL4, C2CD4B, RAB5C, KAT2A, TAB1, SYNGR1*. Last, we provide evidence in support of a causal role of FVIII levels on VTE and CAD events and of VWF levels on IS events.

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 non-coding regions, although a substantial proportion were in strong LD (R^2>0.8) with mutations

causing missense or frameshift mutations in the nearby genes (Table 2 and Supplementary Table S7). We also explored eQTL associations using publically available data and we conducted pathway analyses for the novel loci. See Supplementary Methods and Supplementary Tables S8-S13 for this information.

For most loci, several genes were identified within the associated region, and we selected 1 or more genes for further characterization using *in vitro* cell models. Based on our initial functional characterization, 1 or more plausible culprit genes regulating VWF secretion could be isolated at most loci. Interestingly, several candidate genes that showed a clear change in VWF levels upon 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.

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, LDL cholesterol, alkaline phosphatase, increased platelet aggregation, fibrinogen, CRP, and CAD (see further details and references in Supplementary Table S7). Our functional analyses showed a substantial reduction of VWF release upon *ST3GAL4* silencing, which strengthens the evidence of this gene as a novel VWF regulator in basal conditions.

SYNGR1 (Synaptogryn-1) 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 suggest 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's 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 upon silencing of either gene. Again, a reported role of these genes in vesicular trafficking[29] suggest 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 trans-ethnic multi-phenotype 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-Palate vesicles to release VWF in endothelial cells. *RAB5C* silencing caused a significant decrease of VWF release in media of endothelial cells upon stimulation with histamine.

Our *in vitro* cell work showed a significantly increased VWF secretion upon *PDHB* silencing. *PDHB* codes for a subunit of the pyruvate dehydrogenase complex, which converts pyruvate to acetyl-CoA in the mitochondrion. 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, SLE, and RA.

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 TGF-ß, WNT-1 and interleukin-1 which suggest it might have a role mediating VWF release upon certain cellular stimuli.

Similarly, silencing *C2CD4B* gene in cultured endothelial cells resulted in strong upregulation of VWF release both in basal and under stimulus conditions. Allelic variants in this gene have also been associated with fasting glucose homeostasis and type 2 diabetes. Transcripts of this gene are predominantly found 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, apoptosis, cell survival, angiogenesis, cell migration and maturation in endothelial cells, 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 upon silencing. GTPases of immunity-associated proteins (GIMAPs) are regulators of lymphocyte survival and homeostasis [33] although limited data have been published regarding the function of these proteins.

Finally, although it did not reach genome-wide significance in the trans-ethnic meta-analysis, we found a single locus that close to *SLC39A8* and that was genome-wide significant in our meta-analysis 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 (HDL) cholesterol levels and BMI. Our functional work also suggested a strong effect on VWF levels in media from endothelial cells *in vitro* upon *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.

Mendelian Randomization and Clinical Implications

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

Biological and genetic evidence indicate that circulating FVIII levels are mainly determined by levels of VWF[34]. In the present study, we calculated the genetic correlation between VWF and FVIII based on the genome-wide association results from European-descent individuals (see Supplementary Methods) 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 GWAS, 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 independent of VWF and pursued conditional analyses that adjusted for the effect of VWF plasma levels to test the causal effect of FVIII on CV events. For IS, we found no evidence of a causal effect of FVIII 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 independent of the VWF effect. As there were no genetic loci that independently associated with VWF levels and 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 biologic 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 while VWF may be a reasonable target for IS. These molecules are not currently pharmaceutical targets for the prevention of thrombotic events. In this paper, we report on 23 unique genetic loci associated with plasma levels of FVIII and/or VWF, of which 13 are newly reported associations. These discoveries offer new potential targets for the development of pharmaceutical agonistics 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 MAF of the variants in the newly associated loci where relatively common, with just 1 variant having an MAF of less than 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, as 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 and not the regulation of VWF clearance by macrophages[35]. Nor were we able to test other mechanisms that regulates synthesis in megakaryocytes but not endothelial cells. Further, 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 only be seen 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 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 co-regulation of both FVIII and VWF levels allowed us to conduct multi-phenotypes analyses and 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 CV events. With only 3 loci associated with FVIII alone, the power of the VWF-adjusted MR analyses for FVIII and CV events was limited and we could not investigate the association of VWF on CV events independent of FVIII. There is a degree of overlap between our sample and the sample from consortia providing CV events GWAS data, which might create some bias in MR analyses[36]; this is a limitation of our work.

CONCLUSIONS

We found 13 novel genetic loci with modest contributions to plasma levels of FVIII and/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 biologic pathways upon which to intervene to reduce the burden of arterial and venous outcomes.

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Table 1: Main association results for FVIII and VWF trans-ethnic GWAS meta-analysis.

Footnotes: "*Freq"* refers to the allele frequency of the effect allele. "B" = baseline; "S" = stimulated; "ns" = not significant; *p-value<0.05; **p-value<0.01; ***p-value<0.001.
Beta and Frequency refer always to the Effect_allele, and they are expressed as natural-log transformed values from the original units (reported in % or IU/ml*100 units).
†SLC39A8 was found in vWF meta-analysis of EA only (N=42,145).
‡ Although not in LD with this variant, a low-frequency variant 665Kb upstream rs9271597 was found significantly associated to vWF levels (rs80082277; p=1x10-8) 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 (PMID: 27584569).
The highest associated SNP in this locus for FVIII is rs137631 (p=9.5x10-9), located close to **RPL3 gene**, 112Kb downstream TAB1/SYNGR1 locus and in low LD with rs5750823 (R^2=0.14)
¶ Chromosome X variant for VWF only available for EU samples (N=28.685).

Table 2: Characteristics of all associated independent variants after conditional analyses.

Footnotes: †LD with top variant in the region, calculated using FHS data. ‡Primary SNP was not well imputed in FHS and no other SNPs in the region achieved genome-wide significance in conditional analyses. "slct pJ" = joint p-value from GCTA "slct". "Original p-value" = p-value from discovery meta-analysis. The putative functional column indicates the best candidate variant in high linkage disequilibrium with the associated variant (R^2>0.8) that has been identified *in silico* as the best candidate variant to have an impact on the adjacent gene/s. No functional work was performed in known genes, and these are symbolized by "-" in the last column.

Table 1													
rsID	**Effect Allele**	**Other Allele**	**Freq FVIII**	**N FVIII**	**Beta FVIII**	**P FVIII**	**Freq VWF**	**N VWF**	**Beta VWF**	**P VWF**	**Closest Gene(s)**	**Association**	**vWF Release at Baseline (B) and upon Stimulation (S) for Candidate Loci**
rs55954186	a	g	0.36	35,513	0.011	2.2E-05	0.37	46,229	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	35,513	-0.003	0.672	0.92	46,068	-0.034	**8.68E-10**	*SLC39A8*†	New	***SLC39A8 (B=↑**)***
rs548630	a	c	0.49	36,286	-0.016	**2.1E-10**	0.47	46,137	-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	36,286	-0.019	**2.2E-15**	0.46	46,212	-0.033	**5.6E-42**	*STXBP5*	Known	-
rs9271597 ‡	a	t	0.41	28,203	-0.015	**1.4E-08**	0.41	31,364	-0.012	2.1E-04	*HLA region*	New	***HLA-C (B=↑**;s=↑***)***
rs7788962	a	g	0.62	33,773	-0.010	2.3E-04	0.61	46,231	-0.014	**7.3E-09**	*GIMAP7, GIMAP4*	New	***GIMAP7*** **(B=↑***; S=ns),** *GIMAP4* (B=ns; S=ns)
rs4276643	t	c	0.66	36,286	-0.023	**1.3E-19**	0.67	44,168	-0.029	**8.8E-28**	*SCARA5*	Known	-
rs10102164	a	g	0.19	36,286	0.019	**2.4E-09**	0.20	46,230	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	28,535	-0.021	1.1E-03	0.73	24,987	-0.056	**1.5E-08**	*OR13C5, NIPSNAP*§	New	***NIPSNAP3A*** **(B=↑*; S=↑**),** ***NIPSNAP3B*** **(B=↑*; S=ns)**
rs10985344	a	g	0.25	36,286	0.011	7.5E-05	0.25	46,178	0.017	**3.5E-09**	*DAB2IP*	New	*DAB2IP (B=ns; S=ns)*
rs687289	a	g	0.36	36,286	0.145	**1.9E-770**	0.33	46,231	0.197	**5.0E-1443**	*ABO*	Known	-
9:13930481:ID	d	i	0.85	22,480	-0.032	**2.7E-10**	0.85	29,409	-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	44,020	0.060	**3.0E-12**	*ST3GAL4*	Known ‖	***ST3GAL4*** **(B=↓**; S=ns)**
rs4981022	a	g	0.69	36,286	0.025	**3.0E-20**	0.69	46,232	0.035	**6.6E-41**	*STAB2*	Known	-
rs4759787	a	c	0.40	36,286	0.011	1.1E-05	0.37	46,180	0.023	**7.7E-20**	*STX2*	Known	-
rs2238109	a	t	0.39	36,286	0.026	**3.5E-24**	0.38	46,232	0.050	**1.8E-89**	*VWF*	Known	-
rs4904820	a	g	0.49	36,286	0.014	**1.8E-08**	0.47	46,232	0.022	**6.0E-19**	*TCN2*	Known	-
rs6494314	t	c	0.18	36,286	-0.007	2.4E-02	0.17	46,232	-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	33,097	-0.010	4.4E-04	0.30	45,566	-0.022	**6.5E-16**	*CLEC4M*	Known	-
rs5750823 #	t	c	0.70	36,286	-0.013	1.5E-06	0.72	46,230	-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	20,537	0.017	**3.3E-09**	0.65	28,685	-0.005	7.0E-02	*TMLHE, F8*	Known	Too little FVIII released by endothelial cells to be detected *in vitro*

Table 2					EUR-only	TRANS-ethnic	Top Meta variant	LD† (r2)			
MarkerName	rsID	Closest Gene(s)	variant position	putative functional	Original P-value	Conditional P-value	Original P-value	Conditional P-value	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	p<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 cons	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 cons	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**	**1.3Kb 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**	-	-
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**	**9.1Kb 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	2.5Kb 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
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**	**700bp 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	5.6Kb 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**

Figure Legends

Figure 1A: Manhattan plot for the trans-ethnic analyses FVIII (named by closest gene)

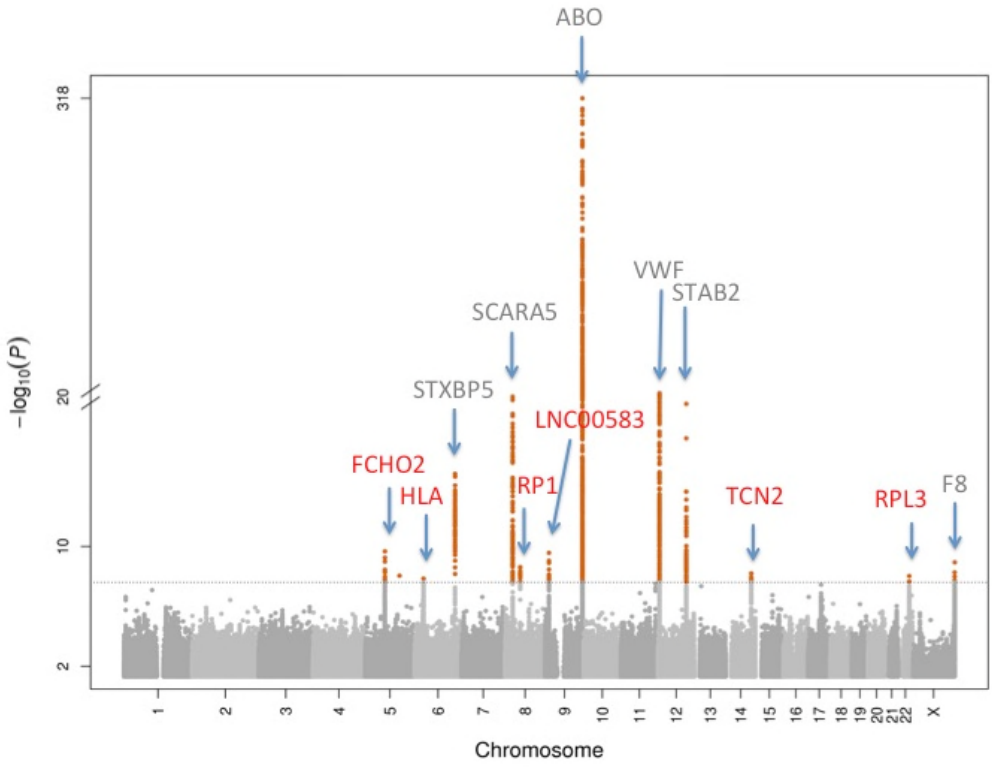


Figure 1B: Manhattan plot for the trans-ethnic analyses VWF (named by closest gene).

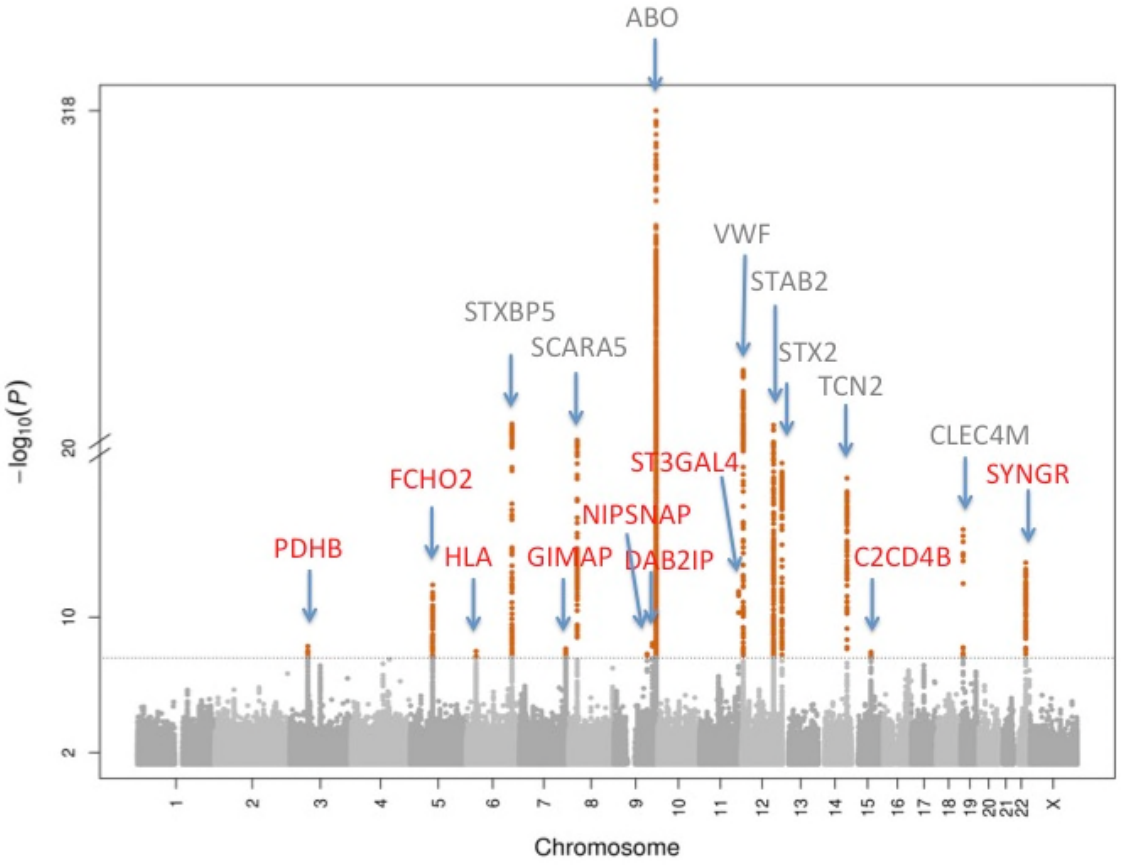


Figure 2A. Silencing candidate genes changes basal release of VWF.

HUVEC cells were transfected with siRNA against selected genes for 4 days, the media was changed, cells were cultured for 30 min, and VWF was measured in the supernatant via ELISA. n = 4 ± S.D. *p<0.05, **p<0.01, ***p<0.001. All results are relative to VWF release after transfection with a scrambled control siRNA, which is set as reference (100%).

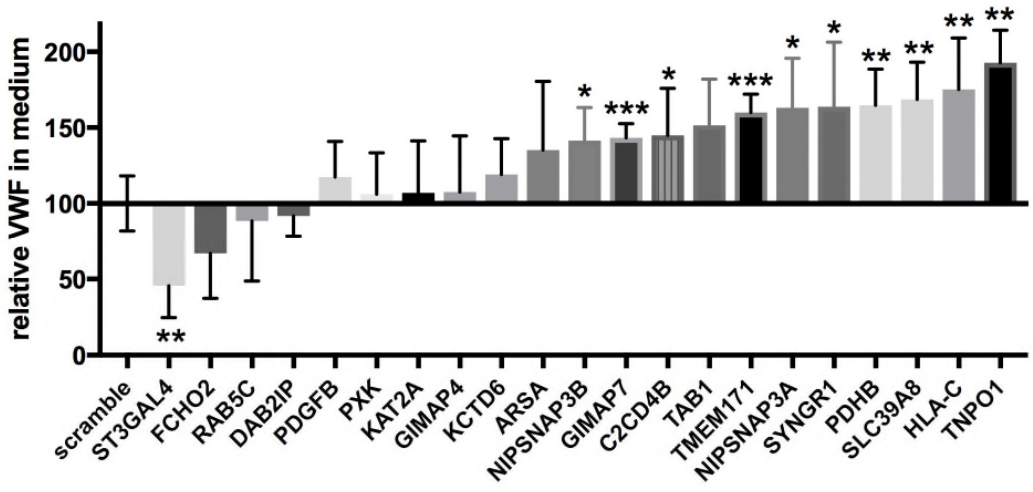


Figure 2B. Silencing candidate genes changes stimulated release of VWF.

HUVEC cells were transfected with siRNA against selected genes for 4 days, the media was changed, cells were stimulated with histamine 10 µM for 30 min, and VWF was measured in the supernatant by an ELISA. n = 4 ± S.D. *p<0.05, **p<0.01, ***p<0.001. All results are relative to VWF release after transfection with a scrambled control siRNA, which is set as reference (100%).

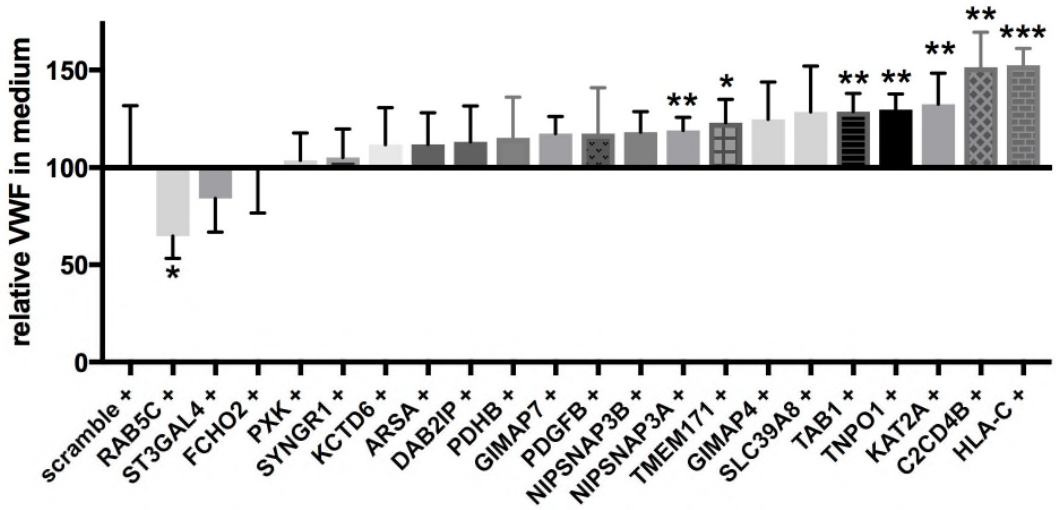
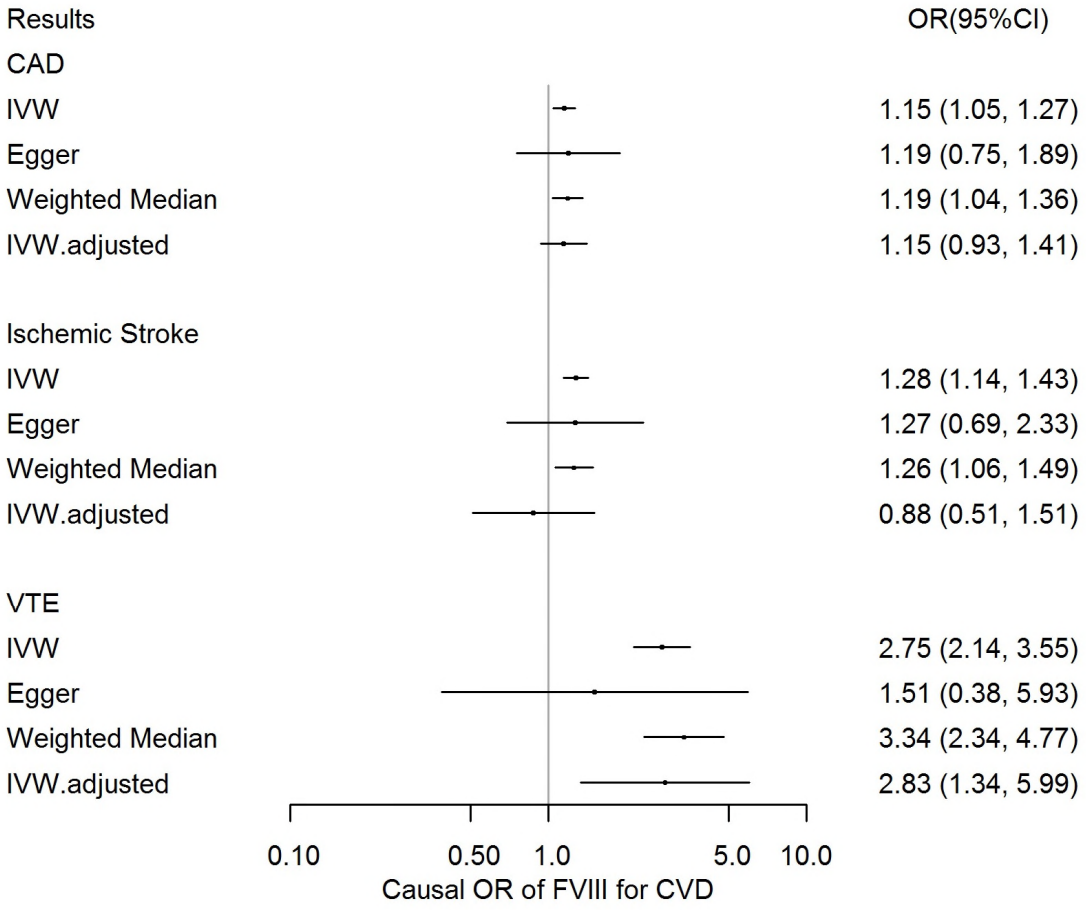

relative VWF in medium
150
100
50
0
*
**
*
**
**
**
***
scramble +
RAB5C +
ST3GAL4 +
FCHO2 +
PXK +
SYNGR1 +
KCTD6 +
ARSA +
DAB2IP +
PDHB +
GIMAP7 +
PDGFB +
NIPSNAP3B +
NIPSNAP3A +
TMEM171 +
GIMAP4 +
SLC39A8 +
TAB1 +
TNPO1 +
KAT2A +
C2CD4B +
HLA-C +

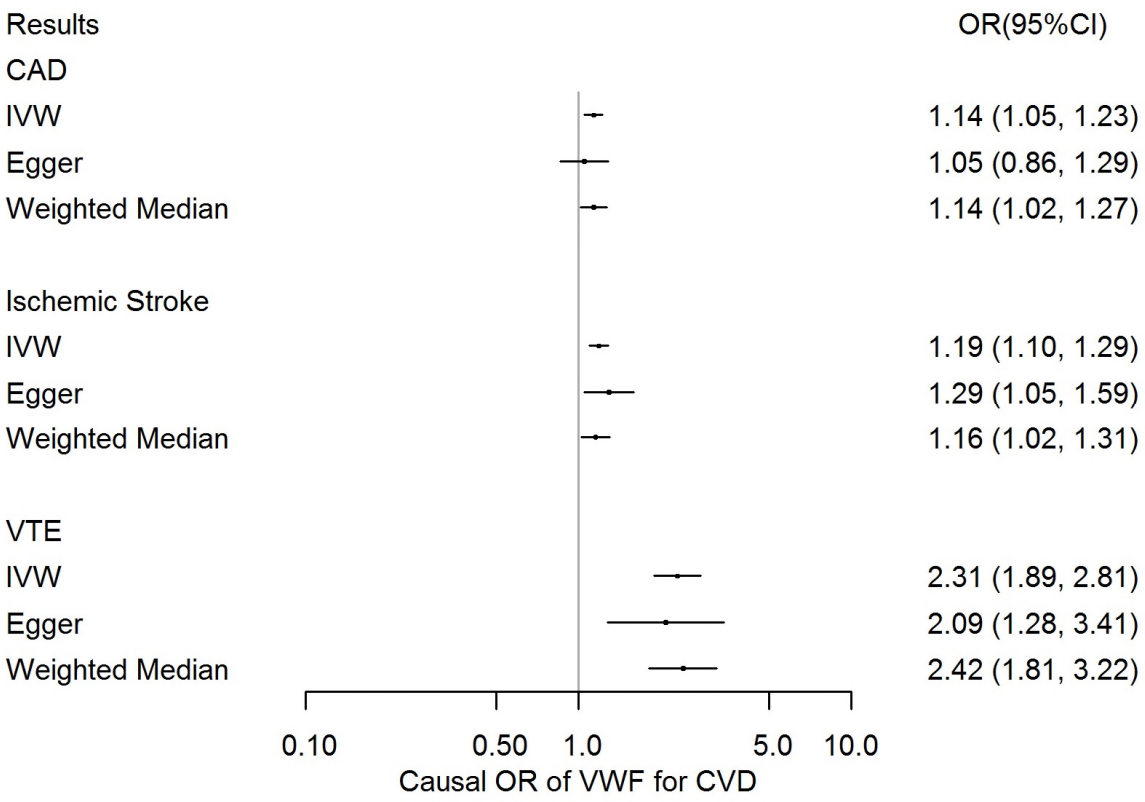
Figure 3

Mendelian Randomization results. Results show OR (95% confidential interval) per every higher standard deviation change in FVIII and VWF. CAD (Coronary Artery Disease), IVW(inverse-variance weighted method), IVW.adjusted (IVW FVIII adjusted for the effects of VWF).

a) FVIII



b) VWF


Results
OR(95%CI)
CAD
IVW
1.14 (1.05, 1.23)
Egger
1.05 (0.86, 1.29)
Weighted Median
1.14 (1.02, 1.27)
Ischemic Stroke
IVW
1.19 (1.10, 1.29)
Egger
1.29 (1.05, 1.59)
Weighted Median
1.16 (1.02, 1.31)
VTE
IVW
2.31 (1.89, 2.81)
Egger
2.09 (1.28, 3.41)
Weighted Median
2.42 (1.81, 3.22)
0.10
0.50
1.0
5.0
10.0
Causal OR of VWF for CVD

Supplementary material

Supplementary Table S1: Descriptions of participating cohorts and resources

Supplementary Table S2: Final SNPs composing instrumental variables

Supplementary Table S3: MR results for the instrumental variables individually

Supplementary Table S4: global results for the Egger Regression to evaluate heterogeneity, before and after exclusions

Supplementary Table S5: Results from conditional analyses from European-only data

Supplementary Table S6: Results from conditional analyses from transethnic data

Supplementary Table S7: Haploreg/SNIPA/GWAS catalogue for all transethnic top-SNPs

Supplementary Table S8: eQTL in other tissues

Supplementary Table S9: eQTL in whole blood

Supplementary Table S10: Factor VIII enrichment networks

Supplementary Table S11: Factor VIII prioritized genes in networks

Supplementary Table S12: von Willebrand factor enrichment networks

Supplementary Table S13: von Willebrand factor genes in networks

Supplementary Figures S1a-n: Regional plots for the novel associations

Supplementary Figures S2a-c: Scatter plots for the genetic associations between FVIII and cardiovascular events

Supplementary Figure S3: Figure shows the hypothesized effect of the genes found in the present study in relation to the possible regulatory points in VWF synthesis and secretion from endothelial cells. The specific regulatory point suggestion is based on previous literature evidence. Of note, VWF clearance or regulation in platelets were not studied in our in vitro first-pass analyses.