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Gene-centric approach identifies new and known loci for FVIII activity and VWF antigen levels in European Americans and African Americans

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Abstract

Coagulation factor VIII and von Willebrand factor (VWF) are key proteins in procoagulant activation. Higher FVIII coagulant activity (FVIII:C) and VWF antigen (VWF:Ag) are risk factors

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for cardiovascular disease and venous thromboembolism. Beyond associations with *ABO* blood group, genetic determinants of FVIII and VWF are not well understood, especially in non European-American populations. We performed a genetic association study of FVIII:C and VWF:Ag that assessed 50,000 gene-centric single nucleotide polymorphisms (SNPs) in 18,556 European Americans (EAs) and 5,047 African Americans (AAs) from five population-based cohorts. Previously unreported associations for FVIII:C were identified in both AAs and EAs with *KNG1* (most significantly associated SNP rs710446, Ile581Thr, P=5.10 × 10^{-7} in EAs and P=3.88 × 10^{-3} in AAs) and *VWF* rs7962217 (Gly2705Arg, P=6.30 × 10^{-9} in EAs and P=2.98 × 10^{-2} in AAs). Significant associations for FVIII:C were also observed with *F8/TMLHE* region SNP rs12557310 in EAs (P=8.02 × 10^{-10}), with *VWF* rs1800380 in AAs (P=5.62 × 10^{-11}), and with *MAT1A* rs2236568 in AAs (P=1.69 × 10^{-6}). We replicated previously reported associations of FVIII:C and VWF:Ag with the *ABO* blood group, *VWF* rs1063856 (Thr789Ala), rs216321 (Ala852Gln), and *VWF* rs2229446 (Arg2185Gln). Findings from this study expand our understanding of genetic influences for FVIII:C and VWF:Ag in both EAs and AAs.

Introduction

Coagulation factor VIII (FVIII) and von Willebrand factor (VWF) provide critical functions in hemostasis. In the circulation, 95% of FVIII is bound to VWF as an inactive complex that stabilizes FVIII [1,2]. Activated FVIII, after its release from VWF, acts as a cofactor for factor IXa-mediated activation of factor X and the subsequent conversion of prothrombin to its active form, thrombin [1]. VWF, an adhesive glycoprotein, also promotes platelet adhesion and aggregation [3]. Levels of FVIII coagulant activity (FVIII:C) and VWF antigen (VWF:Ag) in the top 25% of the population distribution are risk factors for venous thromboembolism and arterial vascular events [3,4].

In family studies, a significant genetic contribution has been documented for variation in FVIII:C and VWF:Ag, with estimated heritability ranging between 0.31 and 0.75 [5–7]. Shared genetic effects of FVIII:C and VWF:Ag on their risk to thrombosis have also been documented [8]. There are few reports of candidate gene studies focused on the *FVIII*, *VWF*, and *ABO* structural genes [9–14] and two genome-wide association studies (GWAS) [15,16] for FVIII:C or VWF:Ag traits. The *ABO* locus is a major contributor to their levels [10,15,16] and *ABO* alleles are also associated with the risk of venous thrombosis [17–19]. The first GWAS study, which included both FVIII:C and VWF:Ag in individuals of European ancestry (EA), reported associations at additional loci including *STXBP5*, *SCARA5*, *VWF*, *STAB2*, *STX2*, *TC2N,* and *CLEC4M.* These loci, including *ABO*, explained only 10.0-12.8% of the variation in FVIII:C and VWF:Ag [15]. The second GWAS, for VWF:Ag based on two young and healthy cohorts of EA ancestry and mixed ancestry, respectively, confirmed the associations at *ABO* and *VWF* and identified a new linkage region at chromosome 2q12–2p13 [16]. Data from other ethnic populations, such as African Americans (AAs), are limited to one report that sequenced only coding regions of the *VWF* gene [14].

In this study, we investigated the associations of circulating levels of FVIII:C and VWF:Ag with single nucleotide polymorphisms (SNPs) on the gene-centric 50K SNP ITMAT-Broad-

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CARe (IBC) genotyping array. The CARe IBC chip was designed to include a greater SNP marker density, including more non-synonymous variants, for more than 2,000 cardiovascular candidate regions, compared with the genome-wide arrays used in the published GWAS study [20]. The analyses were based upon 18,556 European Americans (EAs) and 5,047 AAs from five population-based cohorts in the Candidate Gene Association Resource (CARe) Consortium [21].

Material and Methods

Study population

This analysis included five participating cohorts in the CARe Consortium [21] that had assayed FVIII:C and/or VWF:Ag: the Atherosclerosis Risk in Communities (ARIC) study [22], the Coronary Artery Risk Development in Young Adults (CARDIA) study [23], the Cardiovascular Health Study (CHS) [24,25], the Framingham Heart Study (FHS) [26], and the Multi-Ethnic Study of Atherosclerosis (MESA) [27] (Table I). All participating institutions gave institutional review board approval for this study and all participants gave written informed consent. Details on the participating cohorts are provided in Supporting Information Methods.

Phenotype measurement

Plasma FVIII:C and/or VWF:Ag was measured in the entire cohort at baseline for ARIC (both FVIII:C and VWF:Ag) [28,29], CHS (FVIIIc) [30], and MESA (FVIIIc) [31]. In CARDIA, participants attending two field centers were measured at the years 5 and 7 exams for plasma FVIII:C and at the years 2, 5, and 7 exams for VWF:Ag [32]. Additionally, plasma VWF:Ag was measured in the FHS Offspring cohort during the fifth examination cycle [33] and in a random sample of 1,000 participants in MESA [31]. In each cohort, FVIII:C was measured using standard clinical clotting time-based assays and VWF:Ag was measured by ELISA or immunoturbidometric assays. Details on the laboratory assays are provided in Supporting Information Table SI. Information on clinical, demographic, lifestyle, and anthropometric characteristics were obtained in each cohort at the same visit as the measurements for FVIII:C and VWF:Ag.

Genotyping, imputation, and quality control

All samples were genotyped at the Broad Institute using the IBC Illumina iSELECT array [20]. The 50,000 SNPs on the IBC array are distributed across ~2,100 genes selected to cover a range of cardiovascular, metabolic, lung, blood, sleep, and inflammatory pathways [20]. The SNP selection, designed to capture maximal genetic information specific to European and African ancestries excluding rare variants, was informed by the HapMap data and supplemented by the SeattleSNPs and Environmental Genome Project resequencing data [20]. All non-synonymous variants of minor allele frequency (MAF) > 0.01 were included to supplement tagging SNPs. Imputation to the phase 2 HapMap data at gene regions for un-genotyped SNPs was conducted using MACH 1.0.16 [34], with the CEU founders of the HapMap2 as the reference panel for the CARe EA samples and the combined CEU+YRI samples as the reference panel for the CARe AA samples. SNPs with imputation quality score < 0.6 were excluded. Details on genotyping, imputation, and

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quality control (QC) in these five participating cohorts are provided in Supporting Information Methods.

Statistical analysis

Participants who were taking anticoagulants or had FVIII:C or VWF:Ag values more than 6 standard deviations from the mean were excluded. Untransformed measurements for FVIII:C and VWF:Ag were regressed on age, sex, and study site (where appropriate) using linear regression models stratified by cohort and ethnic group to create cohort and ethnic-specific residuals. These residuals were inverse normal transformed and used in genetic association analysis, performed separately for EAs and AAs within each study. In CARDIA, the residuals based on measurements of each exam year were averaged across years and the averaged values were used in the inverse normal transformation. The genetic analysis used an allele dosage for each SNP, assuming an additive genetic effect, and adjusted for the first 10 principal components derived from EIGENSTRAT [35] to account for potential population stratification. For cohorts of unrelated individuals, a linear regression model was used with PLINK V 1.0.7 [36]; for the FHS family data, a linear mixed effects model was used to account for correlation between individuals due to family structure [37]. Ethnic-specific results across studies were combined using a fixed effects, inverse variance-weighted meta-analysis as implemented in METAL [38]. Genomic control correction was applied during the meta-analysis. SNPs with MAF-weighted sample size (MAF × N) < 10 or imputed SNPs with MAF < 1% were excluded from individual cohorts. The significance threshold was $P < 2.0 \times 10^{-6}$, to account for the effective number of independent tests [39]. Heterogeneity was assessed using the I^2 inconsistency metric. To obtain clinically meaningful effect size estimates, association analyses were repeated for the most significantly associated SNPs with untransformed FVIII:C and VWF:Ag measurements. The proportion of variance explained by SNPs was based on the ARIC data, the largest study in the consortium, and calculated by subtraction of variance explained by non-SNP covariates from the total variance explained by all covariates in linear regression models.

Analysis of X chromosome SNPs

The analytical approach for X chromosome SNPs was similar to that described above for autosomal SNPs except as follows: for males, genotypes for SNPs on the X chromosome were coded as 0 or 2 [40]. In PLINK linear regression, sex was automatically adjusted for as a covariate for the analysis of X chromosome SNPs. The results from the PLINK analysis were highly consistent with those from an alternative approach in which males and females were analyzed separately in linear regression in SAS and meta-analyzed across cohort and gender groups. The percentage of variance explained by X chromosome SNPs (i.e., *F8* SNPs) was calculated by GCTA (genome-wide complex trait analysis) with full dosage compensation [41].

When multiple statistically significant SNPs clustered at a region, sequential conditional analyses were performed to adjust for the most significant SNP from each adjustment step until no other SNP attained significance. Since the association of *ABO* blood group with VWF:Ag and FVIII:C was known and the *ABO* locus was significant in this study, we used the following SNPs to tag the O, B, A2, and O2 groups in conditional analyses: rs529565

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(tag for O in EA, r^2=0.67), rs8176693 (for O in AA, r^2=0.55), rs8176749 (one of the functional variants for B group), rs8176704 (for A2, r^2=1 in both EA and AA), and rs512770 (the functional variant for O2) [42]. Linkage disequilibrium (LD) between SNPs, represented by r^2, was used to evaluate the independence of associations from a region. The r^2 statistics from the CEU sample of the HapMap phase 2 were used as a reference for the CARe EA data, while those from the combined African and African American samples of the HapMap phases 2 and 3 were used for the CARe AA data.

Results

The distributions of demographic variables, FVIII:C, and VWF:Ag in 18,556 EA and 5,047 AA participants are shown in Table I. The race-specific quantile-quantile (Q-Q) plots for observed *vs*. expected $-\log_{10} P$ values for both traits are shown in Supporting Information Figs. S1-S4. There was little influence of population stratification on the data, as evidenced by inflation factors for FVIII:C and VWF:Ag of 1.06 and 1.01, respectively, in EAs, and 0.97 and 1.00, respectively, in AAs. Table II presents the most significantly associated SNPs with FVIII:C and VWF:Ag in EAs and AAs.

Genetic associations in EAs

In EAs, 119 SNPs for FVIII:C and 140 SNPs for VWF:Ag exceeded the pre-specified significance threshold of $P < 2 \times 10^{-6}$. The significant SNP associations for FVIII:C clustered at 4 loci: *KNG1* (kininogen 1) on chromosome 3q27; *ABO* on chromosome 9q34.1-q34.2; *VWF* on chromosome 12p13.3; and *F8/TMLHE* (trimethyllysine hydroxylase, epsilon) on chromosome Xq28. The significant SNP associations for VWF:Ag clustered at 2 loci, *ABO* and *VWF*.

At the *KNG1* locus, 3 SNPs that are in high LD (r^2=0.84–1.00) were associated with FVIII:C – rs698078 (P=4.26 × 10^{-7}, Table II), rs710446 (coding, nonsynonymous, P=5.10 × 10^{-7}, Table II), and rs5030062 (P=1.81 × 10^{-6}) (Fig. 1). The P values for the other two SNPs did not attain statistical significance after adjustment for rs710446 ($P > 0.05$). At the *ABO* locus, the strongest association for both FVIII:C and VWF:Ag was with rs529565 ($P < 1.0 \times 10^{-199}$ for both FVIII:C and VWF:Ag, Table II). rs529565 is intronic to the ABO gene and tags the O blood group (r^2=0.67 with the O group variant). Simultaneous adjustment for the SNPs that tag the *ABO* O, B, A2, and O1v/O2 groups abolished all the other *ABO* SNP associations for both traits.

At the *VWF* locus, three sets of independent SNP associations, spanning 24 SNPs, were identified for FVIII:C (Table II, Supporting Information Table SII). The three sets were led by rs1063856 (coding-nonsynonymous, Thr789Ala, P=5.84 × 10^{-12}), rs7962217 (coding-nonsynonymous, Gly2705Arg, P=6.30 × 10^{-9}; P=4.03 × 10^{-8} after adjustment for rs1063856), and rs216321 (coding-nonsynonymous, Ala852Gln, P=6.30 × 10^{-9}; P=4.03 × 10^{-8} after adjustment for rs1063856 and rs7962217), respectively. The top variants rs1063856, rs7962217, and rs216321 are in low LD (r^2=0.008–0.035) and are consistent with three independent sites of genetic contribution within a single gene to variation in FVIII:C. For VWF:Ag at the *VWF* locus, two sets of independent associations of 42 SNPs emerged (Table II, Supporting Information Table SIII), led by rs1063856 (P=1.06 × 10^{-19})

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and rs216321 (P=1.71 × 10^{-17}; P=6.36 × 10^{-12} after adjusting for rs1063856), respectively. Association plots are presented for FVIII:C (Fig. 2) and VWF:Ag (Supporting Information Fig. S5) in the *VWF* locus.

Interestingly, the second set of independent SNPs in *VWF* for FVIII:C, led by rs7962217, was not associated with VWF:Ag in either the meta-analysis of all cohorts (P=0.38 for rs7962217) or individual cohorts (e.g., in ARIC, P=0.75 for rs7962217).

On the X chromosome, one *TMLHE* SNP (rs12557310, P=8.02 × 10^{-10}) and 4 SNPs from *F8* (most significant SNP, rs2096362, P=1.88 × 10^{-9}) were significantly associated with FVIII:C (Supporting Information Tables SIV, Supporting Information Fig. S6). rs12557310 is the only SNP from the *TMLHE* gene that was genotyped. Of the 4 *F8* SNPs, one is coding-nonsynonymous (rs1800291, P=9.74 × 10^{-7}) and the remaining 3 SNPs (including the most significant, rs2096362) are intronic. There was weak to moderate LD between the *TMLHE* SNP rs12557310 and the four *F8* SNPs (r^2=0.08–0.31), with stronger LD among the four *F8* SNPs (r^2=0.20–1.00), as expected. Adjustment for rs1800291 only modestly attenuated the association at *TMLHE* (P=3.12 × 10^{-5} for rs12557310, Supporting Information Fig. S6). In contrast, adjusting for rs12557310 in *TMLHE* removed the majority of the association for the *F8* SNPs (P=0.08–0.0028).

Of the associations identified above in EAs, those with the *KNG1* locus, *VWF* (rs7962217 and rs216321), and *TMLHE* (rs12557310) for FVIII:C have not been previously reported. The total variance explained by the significant and independent associated SNPs was 14.5% for FVIII:C (12.6% for autosomal SNPs and 1.9% for X chromosome SNPs) and 15.6% for VWF:Ag.

Genetic associations in AAs

In African Americans, 85 SNPs were significantly associated with FVIII:C and 76 SNPs were associated with VWF:Ag. The SNPs were located in three loci for FVIII:C – *ABO*, *MAT1A* (methionine adenosyltransferase I, alpha, chromosome 10q22), and *VWF*. In contrast to FVIII:C, the SNP associations for VWF:Ag were located in *ABO* and *VWF*.

In the *ABO* locus, 68 SNPs were significantly associated with FVIII:C and/or VWF:Ag. The most significantly associated SNP for both traits was rs8176693 (P=2.51 × 10^{-114} for FVIII:C and P=1.66 × 10^{-89} for VWF:Ag; Table II). rs8176693 is intronic in *ABO* and a tag for the O blood group (r^2=0.55). Adjustment for SNPs that tag the O, B, A2, and O1v/O2 groups did not abolish all the other *ABO* associations for either trait, with a few intergenic and intronic SNPs remaining significant (data not shown). These remaining SNP associations could be attributable to incomplete adjustment, as a result of relatively poor imputation quality for the O proxy SNP rs8176693 (0.65–0.78), as well as modest LD between rs8176693 and the O functional variant in African Americans (r^2=0.55). The remaining SNP associations at the *ABO* locus were not further investigated.

At the *MAT1A* locus, one intronic variant (rs2236568) was significantly associated with FVIII:C (P=1.69 × 10^{-6}) while other SNPs showed similar, but weaker, association with FVIII:C (Supporting Information Figures).

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At the *VWF* locus, 16 and 8 SNPs exceeded the $P < 2 \times 10^{-6}$ threshold in their associations with FVIII:C and VWF:Ag (Supporting Information Tables SV and SVI), respectively. The significant SNPs for FVIII:C included three independent sets, led by rs2229446 (coding-nonsynonymous, Arg2185Gln, P=1.95 × 10^{-20}), rs1800380 (coding-synonymous, P=5.62 × 10^{-11}; P=7.60 × 10^{-10} after conditioning on rs2229446), and rs4764482 (intronic, P=8.12 × 10^{-8}; P=9.57 × 10^{-8} after 2nd conditional analysis). The significant SNPs for VWF:Ag at *VWF* included two independent sets and were led by rs2229446 (P=1.13 × 10^{-16}) and rs1063856 (P=1.72 × 10^{-10}; P=5.14 × 10^{-12} after conditioning on rs2229446). There is low LD among the leading variants rs2229446, rs1800380, and rs1063856 (r^2=0.004–0.02). rs1063856, the second top independent SNP for VWF:Ag in AAs, is tagged by the 3rd independent set for FVIII:C (r^2=0.54–0.16, Supporting Information Results). Association plots for FVIII:C and VWF:Ag at the *VWF* region in AAs are presented in Supporting Information Figures.

Of the associations in AAs, those with the *MAT1A* locus and *VWF* rs1800380 for FVIII:C have not been previously reported. The total variance explained by the significant and independent SNPs was 11.0% for FVIII:C and 13.2% for VWF:Ag.

Details on the conditional analysis results can be found in Supporting Information Results, and association plots for other loci in Supporting Information Figures. There was no evidence of significant heterogeneity across studies for most of the associated SNPs, with the exception of one between *ABO* rs529565 with VWF:Ag in EAs (P=1.92 × 10^{-8}); however, the effect sizes were not substantially different across studies (data not shown).

Cross-ethnic comparison

The most associated SNPs for FVIII:C and VWF:Ag among EAs and AAs were compared in Table III. Most of the significantly associated SNPs identified in either EAs or AAs were replicated in the other ethnic group, with similar direction of association and nominal $p < 0.05$. An exception was noted for FVIII:C with the *TMLHE/F8* SNPs in AAs and the *MAT1A* SNP association in EAs. The strongest SNP association for FVIII:C at *F8* in AAs was different—rs5945270 (intronic, MAF=0.006, P=0.03). In both African/African American and European samples of the HapMap Project, there is low LD between the non-overlapping *VWF* SNPs identified in our EA (rs7962217 and rs216321) and AA (rs2229446 and rs1800380) populations (Supporting Information Tables SVIIa and SVIIb).

Discussion

We investigated genetic determinants of FVIII:C and VWF:Ag levels based on 50,000 SNPs from a cardiovascular gene-centric chip (supplemented by imputation data) in 18,556 EAs and 5,047 AAs from five population-based cohort studies in the USA. We identified novel loci, *KNG1* and *TMLHE* in EAs, and novel associations in *VWF* (rs7962217 in EAs and rs1800380 in AAs) that have not been reported previously for FVIII:C and VWF:Ag. The newly identified associations with *KNG1* and *VWF* SNPs were replicated across our ethnic populations. Of note, the locus represented by *VWF* rs7962217 was specific to FVIII:C, with no significant association for VWF:Ag. We also extended the association of *VWF* rs216321 with VWF:Ag previously reported in EAs [43,44] to FVIII:C in both EA and AA

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populations. Finally we confirmed previously reported associations of FVIII:C and VWF:Ag levels with *VWF* rs1063856 from EA and AA populations [13–15,43–46], *VWF* rs2229446 from an AA population [14], and the *ABO* locus [10] from a European population.

At the *KNG1* locus, the most associated SNP, rs710446, is in exon 10 of kininogen isoform 1 (high molecular weight kininogen, HMWK) and codes for an Ile to Thr substitution at amino acid 581, predicted to be involved in splicing regulation [47]. *KNG1* has not been previously associated with FVIII:C. HMWK is a cofactor for activation of kallikrein and factors XI and XII. Defects in *KNG1* are the cause of HMWK deficiency (MIM #228960), an autosomal recessive disorder. rs710446 has been associated with the activated partial thromboplastin time (aPTT), FXI, and VTE risk in EA populations [48–52]. The aPTT is highly correlated with FVIII:C and is a global coagulation test that reflects the interacting effects of factors in the classical intrinsic (FXII, FXI, FIX and FVIII) and common coagulation cascades as well as contact activation [53]. There are two possible interpretations of the identified association of *KNG1* with FVIII:C. The first is that the association was observed by virtue of the aPTT reflecting HMWK levels (in part) and that FVIII:C is measured using an aPTT-based assay; the second is that this association reflects an unknown functional link between HWMK and FVIII. Further investigations, including functional assays for HMWK levels, are needed to understand the underlying mechanisms for this association.

Because VWF serves as a carrier for FVIII, we expected overlapping genetic associations for these two phenotypes at *ABO* and *VWF*. However, we observed discrepant results for *VWF* rs7962217; this SNP was only associated with FVIII:C, indicating that the locus tagged by *VWF* rs7962217 influences FVIII:C through other pathways than VWF:Ag levels. *VWF* rs7962217 is located in exon 50 of the *VWF* gene and codes for Gly to Arg substitution at amino acid 2705 (G2705R), which is predicted to be deleterious, affecting a splicing site [47,54]. Exon 50 is within the carboxy-terminus (CK) domain of the VWF protein. The CK domain is crucial for the dimerization of the VWF subunit and mutations in this domain have been identified in type 2A von Willebrand Disease (VWD2A) [55], thought to be associated with synthesis or proteolysis of VWF multimers. Future studies are needed to investigate the mechanisms by which this *VWF* variant influences FVIII:C level, including the possibility of mediation through VWF dimerization on the interaction of VWF with FVIII.

We detected significant associations at the *F8/TMLHE* region for FVIII:C in EAs, in contrast to the published GWAS study [15] that observed no significant associations in the *F8* locus. In that GWAS, imputation data for the X chromosome were not available in two of the four studies. In our study, the *F8* SNPs on the IBC chips were selected as tag SNPs to provide a greater LD coverage than genome-wide arrays, enabling a more comprehensive investigation of *F8* effects on phenotypes. Of the four *F8* SNPs that were significantly associated with FVIII:C in our study, rs1800291 (D1241E) has been previously associated with FVIII:C in two studies of European populations [9,11]. In our study, the effect size of rs1800291 was weaker compared to the other three *F8* SNPs and *TMLHE* rs12557310. Adjustment for rs1800291 only modestly reduced the significance of association for the other SNPs, in contrast to more substantial reduction in significance after adjusting for

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rs12557310. A previously published report of the ARIC data identified eight *F8* intronic SNPs associated with FVIII:C or FVIII:C/VWF:Ag in EAs [56]. There is weak to modest LD between rs12557310 and the eight reported SNPs (r^2=0.11–0.36), in contrast to stronger LD between rs1800291 and the eight SNPs (r^2=0.14–0.67). Therefore, it is possible that the associations we observed at the *TMLHE/F8* locus are attributable to a different underlying *F8* variant than those reflected by rs1800291 or the eight *F8* SNPs. *TMLHE* encodes trimethyllysine dioxygenase, whose relevance to coagulation pathways is currently unknown. It was noted that the associations at the *TMLHE/F8* region were not observed in AAs of our study. This discrepancy could be due to different LD between the two populations as well as interactions by other ethnicity-related factors.

The association between FVIII:C and *MAT1A* SNPs has not been previously reported in any ethnic populations. *MAT1A* encodes hepatic methionine adenosyltransferase I/III, which is responsible for the synthesis of S-adenosylmethionine (SAM). SAM donates methyl groups for most biological methylations including the generation of homocysteine [57], and hyperhomocysteinemia is a modest risk factor for VTE [57]. The *MAT1A* SNP associations detected with FVIII:C should be confirmed in independent populations, given the lack of replication for this association in the EAs in this study.

Recently, Johnsen et al. [14] reported associations of FVIII:C and VWF:Ag with nonsynonymous variants in *VWF* in four AA populations, discovered by exome sequencing in the NHLBI Exome Sequencing Project; three of the cohorts overlapped with those in this study (ARIC, CARDIA and MESA). In that study [14], six *VWF* SNPs were independently associated with VWF:Ag and/or FVIII:C levels: rs1063856, rs57950734, rs11063988, rs149424724, rs76342212 (i.e., rs2229446), and rs61750625. Our study confirmed the findings for rs1063856 and extended the association for rs2229446 to EA populations. Of the other three *VWF* SNPs identified in our study (rs7962217, rs216321, and rs1800380), Johnsen et al. [14] analyzed only rs7962217 but did not find significant association (P=0.25–0.55). rs7962217 was primarily identified in EAs of our study (P=6.30 × 10^{-9}) with nominal significance in AAs (P=0.03).

While AA populations in the two studies largely overlapped, all participants in our study were genotyped for rs7962217, in contrast to 595 participants in the Johnsen et al. [14] study being sequenced and the rest imputed for all *VWF* variants. The difference in genotyping coverage might explain the discrepant significance for this SNP in AAs. Between our *VWF* SNPs (rs216321 and rs1800380) and the other four SNPs of that study, LD information in HapMap is only available for rs61750625, which was not in LD with our variants (r^2=0). In the pooled ARIC IBC data with the *VWF* sequencing and imputation data from Johnsen et al. [14], LD between our SNPs (rs216321 and rs1800380) and the other three of that study [14] (rs57950734, rs11063988, rs149424724) approached zero for most pairs ($r^2 < 0.05$), with the highest between rs1800380 and rs11063988 (r^2=0.11).

Strengths and limitations

The CARe IBC custom genotyping array provides a great advantage for this study because a majority of the genes relevant to the coagulation pathway were included and the array has better LD coverage, including more non-synonymous variants, than in the previously

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published GWAS [15]. While the EA populations in this study partially overlapped with those in the published GWAS [15], we included new populations (CARDIA and MESA) which, in combination with the advantages of the CARe IBC array and sequential conditional analysis, provided additional information beyond the published GWAS study. The increased percentage of variance explained by the most associated, independent SNPs for both traits compared to the previously published GWAS [15] demonstrates the value of the gene-centric genotyping array and contribution of this study to the understanding of genetic basis of both traits. Also, the analysis of AA data in our study represents the largest of this kind and provided an opportunity to compare associations across the two ethnic groups. However, there are limitations to our study. The assays for FVIII:C and VWF:Ag were conducted in each individual study, which may have added variation in the levels of these two measurements across studies. This variation should have a minimal influence on the genetic contribution to phenotypes, as the genetic association analysis was conducted within each study to correlate the rank of the phenotype measurements with the rank of genotype allele dosage, and the results were meta-analyzed across studies. Also, our study investigated common and low-frequency SNPs included on the pre-defined genotyping arrays and might miss rare and new SNPs that were unknown at the time of array design, or novel loci that could be detected with a denser genome-wide array and imputation.

In conclusion, using a gene-centric approach, we identified and replicated new associations for FVIII:C at *KNG1* as well as with SNPs in *VWF* that have not been reported previously. With the availability of both EA and AA samples, we were able to delineate the similarities and differences in the genetic associations between the two ethnic groups. Findings from this study expand our understanding of genetic determinants for FVIII:C and VWF:Ag and will guide future efforts to examine the contributions of the newly identified variants to the risk of thrombosis in EA and AA populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ARIC: N01-HC-5015, N01-HC-5016, N01-HC-5021, N01-HC-5019, N01-HC-5020, N01-HC-5017, and N01-HC-5018;

CARDIA: HHSN268201300025C, HHSN268201300026C, HHSN268201300027C, HHSN268201300028C, HHSN268201300029C, HHSN268200900041C, and AG032136;

CHS: N01-HC-5239, N01-HC-5079 through N01-HC-5086, N01-HC-5129, N01 HC-15103, N01 HC-55222, N01-HC-5150, N01-HC-5133, HL080295, AG-023269, AG-15928, AG-20098, AG-027058, HL-075366, and P30-AG-4827.

FHS: N01-HC-5195;

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MESA: N01-HC-5159, N01-HC-5160, N01-HC-5161, N01-HC-5162, N01-HC-5163, N01-HC-5164, N01-HC-5165, N01-HC-5166, N01-HC-5167, N01-HC-5168, and N01-HC-5169;

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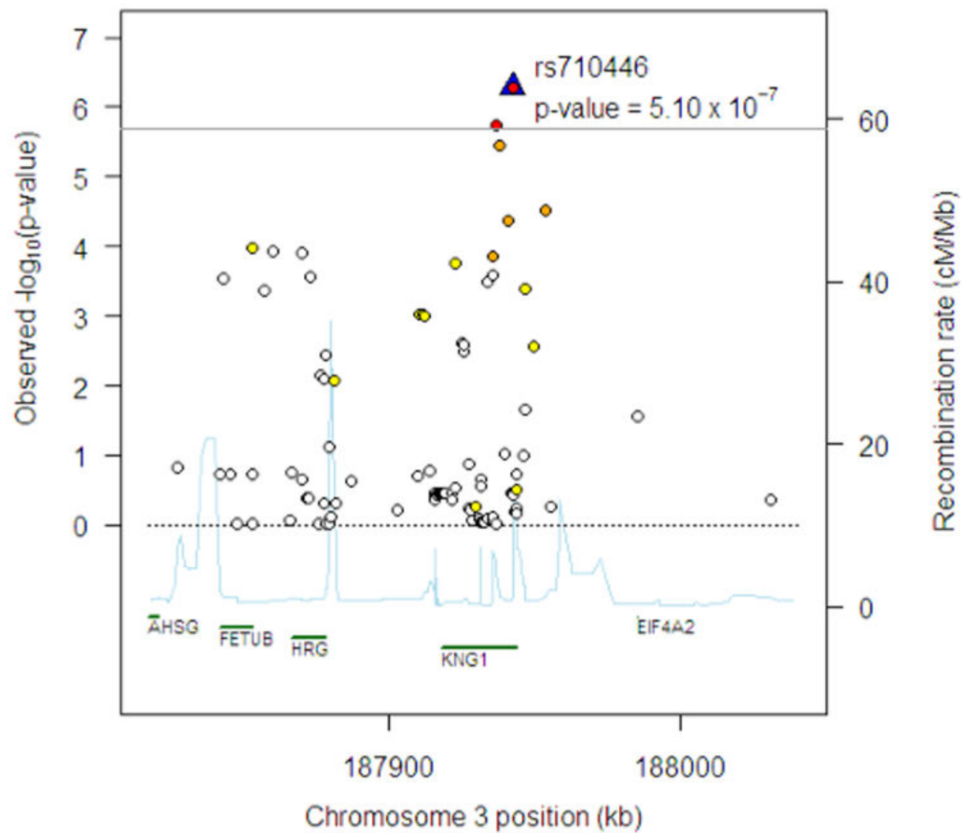

Figure 1.

Regional association plot at chromosome 3q27 region for FVIII:C in EAs. The horizontal line indicates the pre-specified significance threshold of $P = 2.0 \times 10^{-6}$. The top SNP is shown by the blue triangle and labeled. The colors of the remaining SNPs reflect the r^2 with the top SNP based on the HapMap CEU data with the following color scheme: r^2 0.8—red, 0.5 $r^2 < 0.8$—orange, 0.2 $r^2 < 0.5$—yellow, $r^2 < 0.2$—white. The light blue line represents the recombination rate (the *y* axis at right side) based on the data from the HapMap CEU, YRI and JPT1CHB populations. Gene annotations are shown along the *x* axis.

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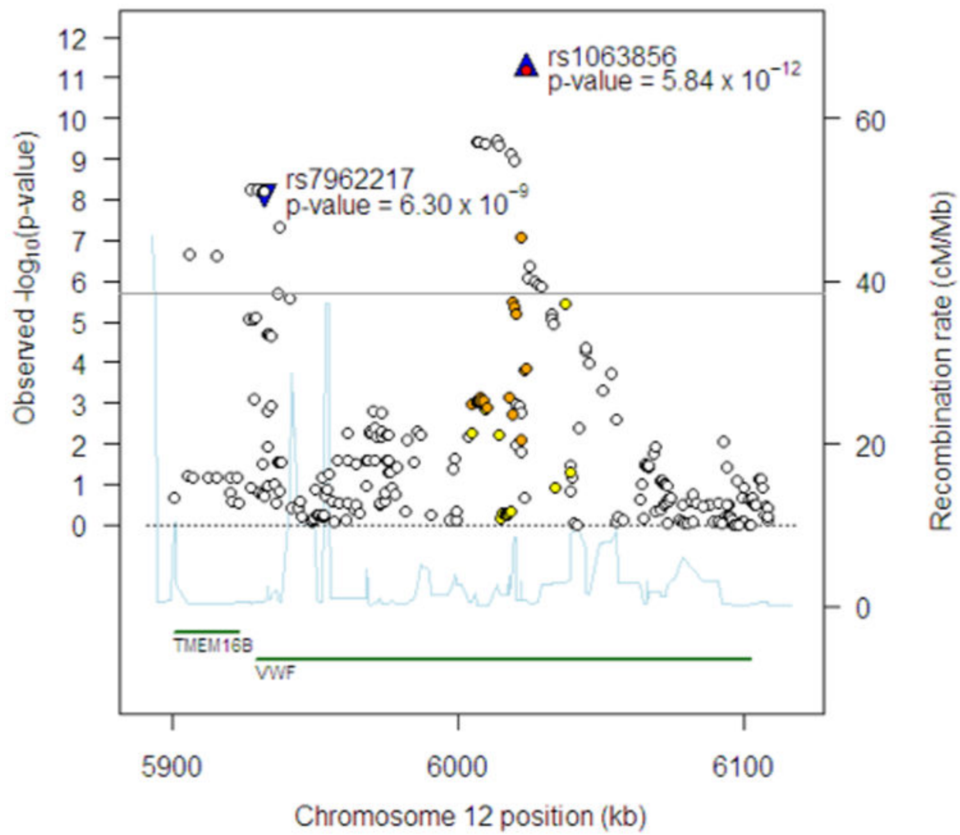

Figure 2.

Regional association plot at chromosome 12p13.3 region for FVIII:C in EAs. The 1st and 2nd top independent SNPs are shown by the blue triangle and labeled.

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Table I

Description of Basic Characteristics in the CARe Cohorts: Mean ± Standard Deviation or Percentage Unless Otherwise Mentioned

Phenotypes	ARIC EA	ARIC AA	CARDIA EA	CARDIA AA	CHS EA	CHS AA	MESA EA	MESA AA	FHS
Total *N*[a]	9,050	2,800	801	484	3,830	175	2,270	1,588	2,605
Age, years	54.1 ± 5.7	53.2 ± 5.8	25.8 ± 3.2[b]	24.4 ± 4.0[b]	72.8 ± 5.6	72.7 ± 5.6	62.7 ± 10.3	62.2 ± 10.1	54.4 ± 9.7
Age range	44–66	44–66	18–32[b]	17–34[b]	65–100	65–88	44–84	45–84	26–82
Female (%)	54.9	63.4	52.4	59.5	56.4	64.0	52.3	54.2	54.6
FVIII:C (%) (N)	125 ± 34 (9,041)	146 ± 46 (2,799)	93 ± 28 (780)	106 ± 38 (458)	121 ± 37 (3,830)	140 ± 44 (175)	95 ± 36 (2,270)	108 ± 43 (1,588)	NA
Median FVIII: C, % (IQR)	121 (101–143)	140 (114–170)	90 (74–109)	101 (78–129)	116 (95–141)	134 (106–166)	90 (70–114)	101 (77–132)	NA
VWF: Ag, % (N)	111 ± 42 (9,045)	133 ± 55 (2,799)	97 ± 32 (799)	111 ± 44 (483)	NA	NA	134 ± 54 (414)	157 ± 63 (178)	126 ± 45 (2,605)
Median VWF: Ag, % (IQR)	104 (81–133)	124 (92–164)	94 (74–115)	102 (80–134)	NA	NA	123 (95–166)	147 (115–199)	120 (91-156)

EA = European American, AA = African American, NA = phenotype not available; IQR = interquartle range.

[a] overall *N* available for the analysis of FVIII:C or VWF:Ag.

[b] Age at baseline.

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Table II

Top Significant SNP Associations for FVIII:C and VWF:Ag at *P* value <2.0 × 10^{-6} in EAs and AAs of CARe (Ordered by Chromosome)

Trait	Region	Top SNP	Position	A1/A2	AFA1	Gene (var)	Beta/SE, %	*P* value	Var%	Imput	Note
EA											
FVIII	3q27	rs698078[a]	187941921	A/G	0.59	*KNG1* (intr)	1.99/0.38	4.26×10^{-7}	0.33	0.95–1.00	New
FVIII	3q27	rs710446[a]	187942621	T/C	0.59	*KNG1* (cns)	1.97/0.39	5.10×10^{-7}	0.33	–	New
FVIII	9q34.1-.2	rs529565	135139321	T/C	0.65	*ABO* (intr)	17.03/0.37	$<1.0 \times 10^{-199}$	11.57	0.98–1.05	Repl
FVIII	12p13.3	rs1063856	6023795	T/C	0.64	*VWF* (cns)	2.68/0.40	5.84×10^{-12}	0.32	–	Repl
FVIII	12p13.3	rs7962217	5931820	C/T	0.94	*VWF* (cns)	4.84/0.83	6.30×10^{-9}	0.21	–	New
FVIII	12p13.3	rs216321	6014245	C/T	0.91	*VWF* (cns)	−3.99/0.67	4.70×10^{-10}	0.19	–	New
FVIII	Xq28	rs12557310[b]	154388892	C/T	0.72	*TMLHE* (intr)	−2.94/0.48	8.02×10^{-10}	1.86	–	New
FVIII	Xq28	rs2096362[b]	153885468	G/A	0.74	*F8* (intr)	−2.82/0.49	1.88×10^{-9}	0.13	–	Repl
VWF	9q34.1-.2	rs529565	135139321	T/C	0.66	*ABO* (intr)	22.18/0.50	$<1.0 \times 10^{-199}$	13.93	0.98–1.00	Repl
VWF	12p13.3	rs1063856	6023795	T/C	0.64	*VWF* (cns)	4.83/0.54	1.06×10^{-19}	0.88	–	Repl
VWF	12p13.3	rs216321	6014245	C/T	0.91	*VWF* (cns)	−7.24/0.92	1.71×10^{-17}	0.48	–	Repl
AA											
FVIII	9q34.1-.2	rs8176693	135127478	C/T	0.90	*ABO* (intr)	37.24/1.65	2.51×10^{-114}	8.62	0.66–0.78	Repl
FVIII	10q22	rs2236568	82025903	A/C	0.76	*MAT1A* (intr)	5.28/1.06	1.69×10^{-6}	0.69	–	New
FVIII	12p13.3	rs2229446	5973333	C/T	0.81	*VWF* (cns)	−9.47/1.13	1.95×10^{-20}	1.16	–	Repl
FVIII	12p13.3	rs1800380	6008856	C/T	0.70	*VWF* (cs)	5.72/0.95	5.62×10^{-11}	0.35	0.92–1.00	New
FVIII	12p13.3	rs4764482[c]	6039994	T/C	0.80	*VWF* (intr)	−5.74/1.10	8.12×10^{-8}	0.74	–	Repl
VWF	9q34.1-.2	rs8176693	135127478	C/T	0.90	*ABO* (intr)	48.62/2.37	1.66×10^{-89}	10.18	0.65–0.74	Repl
VWF	12p13.3	rs2229446	5973333	C/T	0.81	*VWF* (cns)	−12.67/1.68	1.13×10^{-16}	1.75	–	Repl
VWF	12p13.3	rs1063856[c]	6023795	C/T	0.59	*VWF* (cns)	−8.69/1.32	1.72×10^{-10}	1.21	–	Repl

All data presented are independent associations except those labeled with a, b, and c, A1 = allele 1 (major allele), A2 = allele 2 (minor allele), AFA1 = average allele frequency for A1 allele in the meta-analysis, var = variant class (intr = intron, cns = coding-nonsynonymous or missense, cs = coding-synonymous), Beta = change in trait level per 1 allele increase in the minor allele based on untransformed measurements, SE = standard error, Var% = variance % explained by the SNP based on the ARIC data; imput = ratio of observed to expected variance as a measure of imputation quality (range presented, "- " for genotyped SNPs), Repl = replication.

[a] r^2 = 1.0 in the HapMap CEU sample.

[b] r^2 = 0.08 in the HapMap CEU sample.

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[c] $r^2 = 0.16$ in the HapMap African and African American samples.

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Table III

Top Significant SNP Associations in EAs and AAs (in Bold) and the Corresponding Results in the Other Ethnic Population

				EA				AA			
Trait	**SNP**	**Gene**	**A1/A2**	**AFA1**	**N**	**Beta/SE, %**	**P value**	**AFA1**	**N**	**Beta/SE, %**	**P value**
FVIII	rs698078	*KNG1*	A/G	**0.59**	**15,921**	**1.99/0.38**	$\mathbf{4.26 \times 10^{-07}}$	0.49	5,020	2.08/0.88	3.38×10^{-3}
	rs710446	*KNG1*	T/C	**0.59**	**15,915**	**1.97/0.39**	$\mathbf{5.10 \times 10^{-07}}$	0.49	5,005	1.99/0.88	3.88×10^{-3}
	rs529565	*ABO*	T/C	**0.65**	**15,921**	**17.03/0.37**	$\mathbf{<1.0 \times 10^{-199}}$	0.62	5,020	17.40/0.88	1.81×10^{-92}
	rs8176693	*ABO*	C/T	0.93	15,921	14.90/0.70	5.65×10^{-105}	**0.90**	**5,020**	**37.24/1.65**	$\mathbf{2.51 \times 10^{-114}}$
	rs2236568	*MAT1A*	A/C	0.45	15,917	−0.15/0.38	0.75	**0.76**	**5,004**	**5.28/1.06**	$\mathbf{1.69 \times 10^{-6}}$
	rs1063856	*VWF*	T/C	**0.64**	**15,913**	**2.68/0.40**	$\mathbf{5.84 \times 10^{-12}}$	0.42	5,005	4.61/0.89	3.92×10^{-06}
	rs7962217	*VWF*	C/T	**0.94**	**15,914**	**4.84/0.83**	$\mathbf{6.30 \times 10^{-9}}$	0.98	4,372	6.96/3.40	2.98×10^{-2}
	rs216321	*VWF*	C/T	**0.91**	**15,917**	**−3.99/0.67**	$\mathbf{4.7 \times 10^{-10}}$	0.95	5,005	−6.64/1.97	1.13×10^{-3}
	rs2229446	*VWF*	C/T	0.99	9,039	2.85/4.78	0.89	**0.81**	**4,992**	**−9.47/1.13**	$\mathbf{1.95 \times 10^{-20}}$
	rs1800380	*VWF*	C/T	0.75	15,921	1.47/0.43	8.70×10^{-4}	**0.70**	**5,020**	**5.72/0.95**	$\mathbf{5.62 \times 10^{-11}}$
	rs4764482	*VWF*	T/C	0.48	15,916	−0.75/0.38	0.07	**0.80**	**5,005**	**−5.74/1.10**	$\mathbf{8.12 \times 10^{-8}}$
	rs12557310	*TMLHE*	C/T	**0.72**	**15,909**	**−2.94/0.48**	$\mathbf{8.02 \times 10^{-10}}$	0.15	5,005	−1.19/1.39	0.34
	rs2096362	*F8*	G/A	**0.74**	**15,814**	**−2.82/0.49**	$\mathbf{1.88 \times 10^{-9}}$	0.47	4,981	0.13/0.97	0.84
VWF	rs529565	*ABO*	T/C	**0.66**	**12,863**	**22.18/0.50**	$\mathbf{<1.0 \times 10^{-199}}$	0.62	3,460	23.90/1.30	1.23×10^{-76}
	rs8176693	*ABO*	C/T	0.93	12,863	20.50/0.96	1.16×10^{-101}	**0.90**	**3,460**	**48.62/2.37**	$\mathbf{1.66 \times 10^{-89}}$
	rs1063856	*VWF*	T/C	**0.64**	**12,855**	**4.83/0.54**	$\mathbf{1.06 \times 10^{-19}}$	0.41	3,446	8.69/1.32	1.72×10^{-10}
	rs216321	*VWF*	C/T	**0.91**	**12,858**	**−7.24/0.92**	$\mathbf{1.71 \times 10^{-17}}$	0.95	3,267	−9.86/2.96	2.50×10^{-3}
	rs2229446	*VWF*	C/T	0.99	9,043	−16.72/6.14	0.05	**0.81**	**3,435**	**−12.67/1.68**	$\mathbf{1.13 \times 10^{-16}}$
	rs1063856	*VWF*	C/T	0.36	12,855	−4.83/0.54	1.06×10^{-19}	**0.59**	**3,446**	**−8.69/1.32**	$\mathbf{1.72 \times 10^{-10}}$

A1 = allele 1 (major allele in the ethnic population in which the associations were the middle significant ones, i.e. in bold), A2 = allele 2 (minor allele in the ethnic population in which the associations were the middle significant ones), AFA1 = average allele frequency for A1 allele in the corresponding ethnic population, Beta = change in trait level per 1 allele increase in the A2 allele based on untransformed measurements, SE = standard error.