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Coagulation factor VIII: Relationship to Cardiovascular Disease Risk and Whole Genome Sequence and Epigenome-Wide Analysis in African Americans

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Abstract

Background: Prospective studies have suggested higher factor VIII (FVIII) levels is an independent risk factor for coronary heart disease (CHD) and stroke. However, limited information, including on genetic and epigenetic contributors to FVIII variation, is available specifically among African Americans (AAs), who have higher FVIII levels than Europeans.

Objectives: We measured FVIII levels in ~3,400 AAs from the community-based Jackson Heart Study and assessed genetic, epigenetic, and epidemiological correlates of FVIII, as well as incident cardiovascular disease (CVD) associations.

Methods: We assessed cross-sectional associations of FVIII with CVD risk factors as well as incident CHD, stroke, heart failure, and mortality associations. We additionally assessed associations with TOPMed whole genome sequencing data and an epigenome-wide methylation array.

Results: Our results confirmed associations between FVIII and risk of incident CHD events and total mortality in AAs; mortality associations were largely independent of traditional risk factors. We also demonstrate an association of FVIII with incident heart failure, independent of B-type natriuretic peptide. Two genomic regions were strongly associated with FVIII (*ABO* and *VWF*). The index variant at *VWF* is specific to individuals of African descent and is distinct from the previously reported European *VWF* association signal. Epigenome-wide association analysis showed significant FVIII associations with several CpG sites in the *ABO* region. However, after adjusting for *ABO* genetic variants, *ABO* CpG sites were not significant.

Conclusions: Larger sample sizes of AAs will be required to discover additional genetic and epigenetic contributors to FVIII phenotypic variation, which may have consequences for CVD health disparities.

Keywords

factor VIII; coagulation; thrombosis; GWAS; African American

INTRODUCTION

Coagulation factor VIII (FVIII) circulates bound to von Willebrand factor (VWF) and serves as a co-factor for factor IX-mediated activation of factor X, which ultimately generates a fibrin blood clot. Mutations of the factor VIII gene (*F8*) result in low levels of FVIII and the hereditary X-linked bleeding disorder hemophilia A [1] . Conversely, higher basal levels of FVIII are a risk factor for primary and recurrent venous thromboembolism (VTE) [2]. FVIII is an acute phase protein and levels tend to correlate with other inflammation biomarkers as well as traditional cardiovascular disease (CVD) risk factors such as age, body mass index (BMI), and diabetes [3]. Nonetheless, in some studies, higher FVIII levels were an independent risk factor for arterial thrombotic disease such as myocardial infarction (MI) or stroke [4-9], as well as overall mortality[10]. More recently, instrumental variable or Mendelian randomization analyses of FVIII have suggested FVIII levels may be causally related to both CHD and VTE risk [11].

Cardiovascular diseases, including MI, ischemic stroke, venous thromboembolic disease (VTE), and heart failure (HF) disproportionately affect African Americans (AAs) [12, 13]. FVIII levels are higher among AAs than individuals of European ancestry (EAs) [14, 15] and may be a stronger risk factor for VTE in AAs than EAs [16, 17], but the role of FVIII as a risk factor for CVD outcomes has been less well-studied among AAs [18].

Genetic factors contribute to inter-individual variation in FVIII levels, with heritability estimates in the range of 40-60% [19-21]. A major determinant is ABO blood group [19], but familial aggregation of high FVIII levels persists even after adjustment for ABO [22]. Through genome-wide association studies (GWAS) and exome studies, several additional FVIII associated loci have been discovered, though these studies were conducted primarily in individuals of European descent [11, 23-25].

Compared to traditional GWAS, whole genome sequencing (WGS) assesses genetic variants (both coding and non-coding) in the lower frequency range, as well as African population-specific variants poorly represented on genotyping arrays and current imputation reference panels [26]. Epigenetic factors can also influence complex traits such as FVIII level, but association of DNA methylation at a genome-wide scale with FVIII levels in population-based samples has not been previously examined. To further characterize the epidemiologic, genetic, and epigenetic correlates of FVIII, and the relationship of FVIII to CVD risk in AAs, we performed a series of analyses in ~3,400 AAs from the Jackson Heart Study (JHS).

METHODS

The Jackson Heart Study (JHS)

Between 2000 and 2004, JHS recruited 5,306 AA participants from the Jackson, Mississippi metropolitan area. A range of measures, including traditional and putative CVD risk factors, health behaviors, detailed demographic, socioeconomic and sociocultural factors, medication use, anthropometry, blood pressure, assessments of kidney function and diabetes, and biochemical analytes, were obtained at the baseline JHS examination and in subsequent clinic visits [23]. The current analysis is confined to 3,493 individuals who had FVIII measured as part of the JHS ancillary study “Thrombosis Genetics in African Americans” and gave consent that allows genetic research (Figure S1). Computed tomography (CT), ultrasound, and echocardiographic imaging data collection, reading, and quality control in JHS for assessment of carotid IMT, Left ventricular mass index (LVMI), LV hypertrophy (LVH), Ankle brachial index (ABI), coronary artery calcification (CAC) and abdominal aortic calcification (AAC), have been previously described [27-29]. All-cause mortality and incident coronary heart disease (CHD) and stroke events were adjudicated from the beginning of the study through 2014, while adjudication of incident HF events began in 2005 [30]. Overall CHD includes fatal CHD, myocardial infarction, coronary artery bypass surgery, or angioplasty. Hard CHD includes fatal CHD and myocardial infarction. Strokes were defined according to the World Health Organization definition and include both ischemic and hemorrhagic subtypes. Individuals with a prior history of stroke or CHD (prior to 2000) or HF (prior to 2005) or who did not consent to medical record abstraction were excluded from incident event analyses. Median follow-up for mortality is ~14 years, ~12 years for stroke and CHD, and ~10 years for HF.

Laboratory measurements

FVIII antigen level (as the percent of pooled normal plasma) was measured at the University of Vermont using EDTA plasma from the JHS baseline exam and a sandwich ELISA (Affinity Biologicals). Values above the upper limit of detection were set to 800%. High-sensitivity C-reactive protein (CRP), total cholesterol, high-density lipoprotein cholesterol (HDLc) and triglycerides (TG), serum creatinine and B-type natriuretic peptide (BNP) were measured as previously described.

Statistical Analysis of FVIII with CVD risk factors and outcomes

Cross-sectional associations of FVIII with baseline JHS participant characteristics and with measures of subclinical CVD were assessed using generalized estimating equations (GEE), to account for familial correlation, with FVIII as the independent variable and covariate adjustment for age and sex, and baseline characteristics and subclinical CVD measures treated as dependent variables. Effect estimates were reported per standard deviation (SD) change in FVIII. AAC, CAC, LVMI, cIMT, CRP, TG, and BMI (in JHS) were natural log(LN)-transformed prior to analysis. Cox proportional hazards models with sandwich variance estimator, to account for relatedness in the sample, were used to calculate hazard ratios and 95% confidence intervals (CI) for covariate-adjusted associations with all-cause mortality and incident CVD events. Associations were reported both using FVIII as a continuous trait (transformed as a z-score) and as a categorical variable divided into FVIII quartiles. All associations were assessed using SAS 9.3. Heritability of FVIII was estimated using a subset of 1,578 related JHS individuals from 433 families, adjusting for age and sex [30].

Whole genome sequencing (WGS) and FVIII association analysis

Eligible JHS participants underwent ~30X WGS at the Northwest Genomics Center at University of Washington through the NHLBI Trans-Omics for Precision Medicine (TOPMed) project. Details of the sequencing, variant calling, and quality control (QC) protocols used in TOPMed are described at https://www.nhlbiwgs.org/data-sets. Regression of inverse normalized FVIII on genotype was adjusted for age, sex, and the first ten principal components (PCs) for global ancestry. We used a linear mixed model approach to account for familial relationships, as implemented in SAIGE on the University of Michigan ENCORE server (https://encore.sph.umich.edu) [35]. We included 31,176,270 single nucleotide variants and small indels with sequence depth >10 and minor allele count >20. A significance threshold of 1×10^{-9} was used for single variant analyses [31]. To assess the number of distinct signals at a given locus, we performed step-wise conditional regression analysis.

We also performed genome-wide gene-based testing for rare variants with inverse-normalized FVIII using the mixed model SMMAT aggregate association testing method (adjusting for potential relatedness using an estimated kinship matrix and covariates) using Wu weights for each variant [32]. We aggregated variants for association tests grouping variants by gene and restricting to variants of MAF<1% that are either loss of function or missense and predicted to be pathogenic on the basis of a FATHMM-XF coding score>0.5

[33]. We use a Bonferroni correction for the number of tested genes containing more than one polymorphic variant (n= 18,750, $p< 2.67 \times 10^{-6}$).

Global and local ancestry estimation and admixture mapping

We utilized an earlier version of TOPMed WGS (freeze 5b, September 2017) to estimate global and local ancestry among JHS participants (n=2,958). Using as a reference panel 37 African, 35 European, and 20 Native American individuals with phased sequence data (for chromosomes 1-22) [34], we used RFMix version 1.5.4 [35] to infer the number of alleles inherited from each ancestral population (African, European, Native American). To estimate the overall admixture proportions for each JHS participant, we calculated the genome-wide average local ancestry. We used GENESIS [36] to perform admixture mapping using a linear mixed model, investigating each ancestral group (African, European, and Native American) separately, adjusting for age, sex and overall admixture proportions as fixed effects. To account for relatedness, we included ancestry-adjusted kinship estimates as a random effect. We used the genome-wide p-value significance threshold of 5.5×10^{-6}, as estimated using the test statistic simulation approach described in [37].

Epigenome-wide analysis

Illumina Methylation EPIC array data (containing over 850,000 CpG methylation sites) was generated using blood samples collected at the JHS baseline exam. Methylation β values (the ratio of intensities between methylated and un-methylated alleles) were normalized with respect to background color intensity using the normal-exponential out-of-band (NOOB) preprocessing method in the R package minfi[38]. Cell counts (granulocytes, monocytes, natural killer, CD4+ T lymphocytes, naïve CD8+ T lymphocytes, exhausted cytotoxic CD8+ T cells (defined as CD8 positive CD28 negative CD45R negative), and plasma blasts) were estimated according to the method of Houseman et al and Horvath et al [39, 40]. Methylation β values were adjusted for important batch covariates (sample batch, plate, and plate position) using Combat as implemented in the sva and ChAMP R packages. Epigenome-wide association analysis (EWAS) was performed using ln transformed FVIII as the dependent variable, adjusted for age, sex, the first ten ancestry PCs from Affymetrix 6.0 GWAS array data, and estimated cell counts (n=1670). EWAS was performed using linear mixed models in R. For top CpGs, we adjusted for family structure as a random effect in the R package lmer; this was done for presented results. Effect sizes were based on Pearson correlation coefficients. We performed sensitivity analyses adjusting top CpGs for potential confounders of methylation levels (BMI, smoking, and socioeconomic status (SES) (as represented by income)), in a reduced sample size of n=1430. To assess statistical significance of findings, we used a genome-wide significance threshold of $P = 3.6 \times 10^{-8}$ [41].We removed lead CpGs which overlap common SNPs in African populations from 1000 Genomes, based on suggested masking from http://zwdzwd.github.io/InfiniumAnnotation [42].

RESULTS

Association of FVIII with cardiovascular risk factors and subclinical CVD

Of the 3,493 JHS participants included in the current analysis, the mean age was 55.6 years (range 21- 93), 38% were male, 13% were current smokers, 54% were obese, 57% had hypertension, 23% had diabetes, 11% had a prior history of CVD, and 2.9% were taking anticoagulant medication. FVIII levels ranged between 16% and 800% (median 135%, mean 145%, SD 59%). FVIII levels were strongly correlated with age and were higher in women (mean 149%, SD 60%) than men (mean 139%, SD 57%) (Table 1). One male participant had circulating levels (FVIII=16%) compatible with a diagnosis of mild hemophilia A, but bleeding history in this individual is unknown.

In analyses adjusted for age and sex), higher BMI, larger waist circumference, higher TG, higher CRP, lower HDLc, higher fasting glucose, diabetes, and hypertension were each significantly associated with higher FVIII (all $P<0.01$) (Table 1). In a multivariate regression model containing terms for all CVD risk factors significantly associated with FVIII ($P<0.01$) (see Table 1), age, diabetes, triglycerides, and CRP remained strongly associated with higher FVIII levels (all $P<0.001$). As shown in Table 2, among subclinical disease outcomes available in JHS, higher FVIII was nominally associated with LVH ($P=0.01$). There was no evidence of association of FVIII with ABI, cIMT, LVMI, continuous or dichotomous AAC or CAC (all $P>0.05$).

Association of FVIII with incident clinical outcomes

In Cox models minimally adjusted for age and sex, continuous FVIII level was significantly associated with overall CHD, hard CHD, HF, and death (all $P \leq 0.05$), but not with stroke (Table 3). These associations were somewhat attenuated upon adjustment for traditional CVD risk factors, including CRP (Table 3). In the model adjusted for CVD risk factors including CRP, the p-values for association with HF and mortality remained significant (both $P < 0.05$), and the association with hard CHD was marginally significant ($P = 0.05$). The hazard ratios (HR) per standard deviation increase in FVIII were 1.15 (95% confidence interval (CI) 1.03 – 1.28), 1.16 (1.08 – 1.25), and 1.15 (1.00 – 1.33) for HF, mortality, and hard CHD, respectively. When the FVIII association with HF was additionally adjusted for BNP, the HR remained significant (1.14; 95% CI 1.02 – 1.28; $P=0.02$). When the FVIII association with mortality was additionally adjusted for BNP, white blood count (WBC), and estimated glomerular filtration rate (eGFR), this association also remained significant (HR 1.15; 95% CI 1.06 – 1.24 per SD unit; $P = 0.001$).

When analyzed according to FVIII quartiles (Table S1), there again was a significant linear increase in risk of hard CHD, HF, and death in the minimally-adjusted model ($P\leq0.05$), but only HF and mortality remained significant in the fully adjusted model. Individuals in the upper quartile of FVIII among JHS participants had an estimated 2.35-fold increased risk of HF (1.44 – 3.84) and 1.97-fold increased risk of death (1.50 – 2.59), compared to those in the bottom quartile.

Genetic association analysis of FVIII

Adjusting for age and sex, heritability (h^2) of inverse normalized natural log transformed FVIII was estimated as 0.47 (standard error (SE) = 0.06, p= 2.69×10^{-18}) in JHS. In WGS-based association analysis of 3,349 JHS TOPMed participants, two genomic regions were strongly associated with FVIII (Figure S2). On chromosome 9q34, there was a broad association peak containing 312 genome-wide significant variants ($P<1 \times 10^{-9}$) centered on the *ABO* gene (index variant chr9:133257521_T/TC or rs8176719, MAF of 0.29, associated with 0.52 ± 0.03 SD unit higher FVIII, $P=5 \times 10^{-84}$) (Figure 1A). The effect allele of the 1 base pair (bp) indel index variant corresponds to the non-O allele of the ABO blood group. Upon conditional analysis adjusting for the O/non-O rs8176719 variant, the next strongest independent signal was chr9:133255669_CG/C_rs56392308 (MAF=0.064, $P= 4.5 \times 10^{-15}$) associated with 0.46 ± 0.05 lower FVIII, another 1 bp indel which encodes the A2 allele (ABO*A2.06). After a second round of conditional analysis adjusting for both O/non-O and A2 indels, there was a residual marginally significant signal at chr9:133264269_C/T (rs41302905 or rs141515001) associated with lower FVIII (MAF=0.003; $P=5\times10^{-5}$). This variant is part of an extended haplotype which includes two missense variants, rs41302905 and rs55876802, which together comprise part of several O2 alleles (ABO*O.02).

The second FVIII-associated region located on chromosome 12p13.31 contained 33 genome-wide significant variants ($P<1 \times 10^{-9}$) centered around the *VWF* gene (Figure 1B). The peak association signal at the *VWF* locus included nine single nucleotide variants in near complete LD with one another (rs115708869, rs142033986, rs115364369, rs184911391, rs74731445, rs76100694, rs114537734, rs114018824, rs57950734; MAF of ~0.11 for each) that were associated with 0.34 ± 0.05 SD unit lower FVIII ($P=1\text{-}2 \times 10^{-18}$). Based on 1000 Genomes allele frequencies, each of these nine SNV are considerably more prevalent among African (MAF ~0.15) compared to non-African populations (MAF<0.001). Eight of the nine SNVs are intronic, whereas rs57950734 encodes the missense variant p.His817Gln, which was previously associated with lower FVIII in a *VWF* gene-based association analysis conducted in AAs [43]. Upon conditional regression analysis of FVIII in JHS adjusting for the index *VWF* variant rs115708869, the strongest remaining association was chr12:6044584_A/G or rs216294 (MAF=0.05, $P=1 \times 10^{-5}$) with the minor allele associated with higher FVIII.

In gene-based rare variant association analysis of FVIII, there were no associations that reached the genome-wide significance threshold of $< 2.7 \times 10^{-6}$ (Figure S3, Table S2).

We further compared our single variant WGS-based FVIII association results in JHS AAs to previously published FVIII GWAS or exome-based association analyses that have been performed predominantly in European ancestry individuals (Table 4). At the *ABO* locus, the O/non-O variant rs8176719 variant most strongly associated with FVIII in our WGS-based analysis in AAs is in moderate LD (r^2=0.82 in EUR, r^2=0.51 in AFR in 1000 Genomes phase 1 data) with the sentinel variant rs687289 reported in the largest existing FVIII GWAS (n~36,000 multi-ethnic individuals, of whom ~70% were European ancestry) [11]. As expected, rs687289 is also strongly associated with FVIII in JHS AAs ($P=1.8 \times 10^{-50}$). By contrast, the sentinel variant at the *VWF* locus reported in [11] (rs2238109; MAF=0.39; β=0.026; *P*=3.5E−24) was only nominally associated with FVIII in JHS AAs (MAF=0.42;

β=0.06; P=0.019). Of other *VWF* coding variants previously reported to be associated with FVIII (or VWF) levels [48], we observed nominally significant and directionally consistent associations with FVIII for rs1063856 (Thr789Ala), rs216321 (Ala852Gln), and rs2229446 (Arg2185Gln) in our JHS AA samples. Two other variants (at *STXBP5* and *ST3GAL4*) reported in [11] had p-values ~0.06 in JHS and the estimated beta coefficients were directionally consistent (Table 4). We were unable to replicate a previously reported *MAT1A* rs2236568 variant suggestively associated with FVIII in AAs [24].

Admixture analysis of FVIII

In a subset of n=2,958 JHS participants with genome-wide ancestry estimates and FVIII measurements, each percentage point higher of age- and sex-adjusted African ancestry was associated with 28%±10% higher mean FVIII (P=0.006). After additional adjustment for other FVIII correlates (BMI, triglycerides, CRP, diabetes, hypertension) and SES, the association of African ancestry proportion with FVIII was no longer significant (β = 13% ± 11%; P=0.21). We performed an admixture mapping scan in JHS using genome-wide local-ancestry estimates for African and European ancestry. None of the regions tested reached the empirically-derived significance threshold of $P<5.5 \times 10^{-6}$ (Figure S4).

Epigenome-wide association analysis of FVIII

EWAS identified 30 genome-wide significant CpGs ($P<3.6 \times 10^{-8}$) associated with FVIII. Nine of these CpG sites are located in or near *ABO* (Table 5). The most significant CpG was cg21160290 in *ABO* (Pearson's correlation coefficient = 0.19, $P=5.21 \times 10^{-22}$) located in the *ABO* 3' UTR. Following additional adjustment for potential environmental or lifestyle confounders of methylation levels (BMI, smoking, and SES), the strong signal near ABO remained significant, with the same lead CpG (cg21160290), whereas most of the other CpG associations became non-significant after these adjustments. (Table S3). Some of the CpG associations were likely attenuated due to reduced sample size (n= 1430) due to missing covariate data but others may have been confounded by BMI, smoking, or SES. Finally, when we conditioned all CpGs within 1 Mb on each side of cg21160290 on our lead *ABO* genetic association signal rs8176719 in n= 1,657 individuals overlapping the WGS and EWAS datasets; the EWAS signal in the *ABO* region was markedly attenuated (cg21160290 p=0.06); this result is unsurprising given the high correlation between *ABO* SNP genotypes and CpG methylation beta values (r=0.58 for cg21160290 and rs8176719). Non-*ABO* CpG associations were not further examined, as they were not coincident with a genetic signal, and require further replication in additional African American cohorts.

Relationship of *ABO* and *VWF* FVIII-associated variants to clinical CVD outcomes

There was no evidence of association for either *ABO* rs8176719 or *VWF* rs115708869 with incident CHD, stroke, HF, or mortality in JHS (Table S4), though these analyses have limited statistical power due to the relatively small number of incident events. We used the Cerebrovascular Disease and Cardiovascular Disease Knowledge Portals to access summary statistics from larger GWAS analyses for stroke and CVD. African specific VWF lead variant rs115708869 was not present in CAD, stroke, or heart failure GWAS summary statistics. rs8176719 was also not present in summary statistics, but moderately correlated variant rs687289 (the lead from the largest FVIII GWAS [11]; r^2=0.51 in 1000G AFR with

rs8176719) was associated with coronary artery disease ($p=4.76 \times 10^{-6}$, OR=1.04, n=184,305) in the CARDIoGRAMplusC4D analysis [44], with ischemic stroke in the MEGASTROKE analysis ($p=2.67 \times 10^{-4}$, OR=1.03, n= 521,612) [45], and with heart failure in European UK Biobank participants ($p=2.38 \times 10^{-5}$, OR=1.08, n=394,156) [46]. Further analysis is needed to clarify the association of the multiple signals at the ABO locus with cardiovascular events, and to disentangle FVIII mediated effects from pleiotropic effects of ABO genetic variation on lipid and glycemic measures.

DISCUSSION

In a large prospective community-based study of AAs, we confirmed the association of FVIII with clinical events including hard CHD and total mortality. These FVIII associations were largely independent of traditional risk factors, including other inflammation biomarkers. We also demonstrate an association of FVIII with incident HF, independent of BNP. In WGS-based association analysis, we observed two genomic regions strongly associated with FVIII in AAs, the *ABO* region on chromosome 9q34 and the *VWF* gene on chromosome 12p13.31. The association signal at the *VWF* locus includes the African ancestral coding variant p.His817Gln and is distinct from the previously reported European *VWF* association signal for FVIII. At the *ABO* locus, there were at least two conditionally-independent association signals (O/non-O allele the A2 allele) in our AA sample.

FVIII and CVD risk in AAs

The role of FVIII as a risk factor for incident CHD [4-7] and stroke [8, 9] has been suggested in several prospective studies of healthy middle-aged and older adults, though it remains less clear whether the FVIII association is independent of other CVD risk factors [47]. FVIII is strongly correlated with atherosclerosis-related risk factors such as age, BMI, diabetes and other coagulation and inflammatory biomarkers [3]. Results from our cross-sectional analyses in JHS confirm age, diabetes, triglycerides, and CRP as the major correlates of higher FVIII in AAs. In a previous race-stratified analysis from REGARDS, FVIII was found to be associated with risk of overall and hard CHD, and to a lesser extent stroke, independent of traditional CVD risk factors and CRP in both EAs and AAs [18]. Among REGARDS AAs, the HR per SD unit increase in FVIII were 1.65 (1.28 – 2.13), 1.64 (1.28 – 2.11), and 1.38 (1.15 – 1.66) for hard CHD, overall CHD, and stroke, respectively. The weaker associations with incident CVD events observed in JHS may reflect the smaller numbers of cases compared to REGARDS, FVIII assay heterogeneity, or FVIII measurement error.

The stronger associations of FVIII with mortality compared to associations with incident CVD in JHS are consistent with the findings in several other multi-ethnic studies [52,53], with an approximately two-fold increased risk of mortality comparing the bottom quartile to the upper quartile. Some of these prior studies included individuals of both European and African ancestry, but our analysis is the first to demonstrate the association of FVIII with mortality in AAs. In MESA, FVIII was also associated with cancer-specific mortality [47]. The reason for the stronger relationship of FVIII to morality compared to incident CVD is unclear. FVIII is an acute phase reactant, and thus, similar to CRP and fibrinogen, may

reflect a low-grade chronic inflammatory state characteristic of various chronic diseases including not only atherosclerosis but cancer and other age-related diseases.

The associations of FVIII with LVH and HF have not been reported. A study of British men found no association of FVIII with HF [48]. Nonetheless, hypercoagulability is a general feature of HF [49, 50], and arterial and venous thrombotic events are a common complication of patients with HF [51]. FVIII has also been associated with increased risk of incident atrial fibrillation [52], an important risk factor for HF. Several coagulation markers have been correlated with N-terminal-pro hormone BNP (NT-proBNP), suggesting increased coagulation activity may be related to neurohormonal activation and cardiac stress [53]. Given the importance of HF in CVD health disparities, further study of FVIII as a predictor of HF is warranted, particularly as the association observed in JHS was independent of adjustment for other risk factors and biomarkers such as BNP and renal function.

Genetics of FVIII in AAs

Our results show that the overall contribution of genetic factors to phenotypic variation in FVIII in AAs is similar to that previously reported in EAs (h^2~50%) [19-21]. We were also able to confirm and extend the association of *ABO* and *VWF* variants to FVIII in AAs. In particular, the 1 bp deletion variants that define O/non-O and A2 alleles were directly genotyped in our analysis through WGS, thereby allowing us to “fine-map” these two ABO groups as the strongest determinants of FVIII (as opposed to correlated non-coding proxy variants). Our results are consistent with previous studies based on targeted genotyping or haplotype imputation showing O and A2 blood group alleles associated with lower FVIII [54] and that ABO accounts for ~10% of the variability of FVIII phenotypic variance in otherwise unselected individuals [55]. The effects of ABO blood groups on FVIII appear to be mainly mediated by VWF levels; lower VWF levels in O-group subjects are due to shorter VWF survival, mainly attributable to faster clearance [56-58]. A smaller, direct or VWF-independent ABO influence on FVIII has also been reported [54].

Epigenetic association analyses may identify novel mechanisms that contribute to regulation of hemostasis and thrombosis. Changes in gene expression and methylation of *F8* and other inflammation-related genes were associated with SES [59] and neighborhood characteristics [60], suggesting that such epigenetic changes may mediate the effects of environmental or psycho-social stressors on CVD. By performing EWAS for FVIII in a subset of AAs from JHS, we identified several candidate loci, including differentially methylated CpG sites in the 3’UTR of *ABO*, which were also correlated with O vs non-O variant rs8176719. *ABO* gene transcription is dependent on differential DNA methylation of promoter and 3’ flanking regions [61, 62], and the non-O/O variant rs8176719 has been strongly associated both with *ABO* gene expression across a variety of tissues and with methylation at the same CpG sites [63]. Finally, it is important to note that our EWAS was performed using peripheral whole blood DNA. As methylation is a tissue-specific process, whole blood DNA methylation marks might not be a good proxy for methylation status at more biologically relevant cells, such as endothelial cells where FVIII is mainly expressed.

In contrast to the *ABO* locus, where the FVIII-associated variants appear to be largely consistent between AAs and EAs, we observed very little association with the previously

reported European *VWF* rs2238109 variant associated with FVIII. Instead, the main association signal at the *VWF* locus in our AA sample is highly specific to AAs. Of the nine variants that comprise this AA-specific association signal, the most likely causal variant is rs57950734 which encodes p.His817Gln. This variant has been previously associated with lower FVIII in a targeted coding sequence analysis of *VWF* among ~4500 AAs [43]. FVIII circulates bound to VWF, which protects FVIII from early degradation. The VWF p.His817Gln variant, located within the VWF D′ domain within the FVIII binding region, is preferentially associated with lower FVIII (and has little effect on plasma VWF levels) [43]. This variant has also been reported in several patients with type 2N von Willebrand disease in which patients' experience bleeding due to the inability of FVIII to appropriately bind to VWF [66]. Moreover, *in vitro*, the p.His817Gln amino acid substitution results in significantly lower FVIII binding capacity [64], suggesting it is the causal variant for lower FVIII observed in the JHS sample.

Paradoxically, the VWF p.His817Gln variant is common in AAs, but associated with *lower* FVIII. On average, FVIII levels are *higher* in AAs compared to EAs, yet we were unable to identify any African ancestral genetic factors that account for these differences through our WGS-based association analyses or through a complementary genome-wide local ancestry-based admixture scan in JHS. Genome-wide African admixture proportion was not associated with FVIII in JHS or in other studies[65], upon adjustment for other FVIII correlates including SES. We also did not observe any genome-wide significant association signal at the *F8* structural gene locus. Of 37 missense variants within *F8* identified by WGS in JHS with an allele frequency of 0.1% or greater, only rs1800297 showed suggestive evidence of association with higher/lower FVIII (P=0.06). The rs1800297 variant, which encodes a B-domain substitution D1241E, has been previously associated with FVIII levels in Europeans [68]. There was no association of FVIII with the common African rs1800291 missense variant (p.M2257V) in JHS, (P=0.71), consistent with the hypothesis that this is likely a benign African-derived variant [68,69]. Together, these observations suggest that non-genetic, epigenomic, or environmental factors may have a major contribution to the inter-ethnic FVIII phenotypic differences.

Mechanistic and causal relationships between FVIII, ABO, and CVD risk

While very large studies have established that ABO blood group, particularly O vs. non-O is associated with risk of VTE, CHD, and stroke [66-69], the mechanism is most clearly established for VTE, where both FVIII and VWF are important mediators. For arterial thrombotic diseases, the mechanistic relationships are less established, as ABO alleles are pleiotropically associated with many other potential CVD risk factors and mediators, particularly LDL cholesterol [70]. In JHS, we did not observe any association between O vs. non-O and risk of CHD, stroke, HF, or mortality, but our sample size is orders of magnitude smaller than those assessed in European studies. In a multi-ethnic analysis from REGARDS, ABO blood type did not account for the higher stroke risk among AAs [71].

The association of FVIII with VTE and the protection from CHD in individuals with genetically low FVIII (hemophilia A) [72] and hemophilia carriers [73] support a direct causal role of FVIII in arterial thrombosis. Other observational data have suggested that the

association of FVIII with CVD risk appears to be independent of ABO blood group [4] but not necessarily independent of VWF [6]. A limitation of our study is the lack of availability of measured VWF (which is highly correlated with FVIII) in JHS; therefore a direct comparison of risk assessment was not possible. Recently, instrumental variable or Mendelian randomization analyses of FVIII using results of recent FVIII GWAS in Europeans have suggested FVIII levels may be causally related to both CHD and VTE risk, as the risk estimates were only modestly attenuated upon adjustment for VWF levels, whereas VWF (but not FVIII) was causally related to ischemic stroke [11]. The disparate association of the African VWF p.His817Gln variant with lower FVIII (but not VWF) levels [43, 64, 74] makes it a potentially attractive genetic instrument to further assess the causal role of FVIII in AAs. We were unable to observe any significant association with clinical events in JHS, but our power to detect such an association was likely limited by the small number of CVD cases and length of follow-up. Another limitation of our study is that VTE events have not been adjudicated in JHS; therefore, we were unable to assess the risk of our main FVIII-associated genetic variants with VTE risk. It will be important to perform genetic discovery in even larger samples of AAs to more comprehensively characterize the distinct and shared genetic determinants of FVIII and to assess whether the potential causal relationship of FVIII with CVD, and also the novel association of FVIII with heart failure/LVH, can be extended to broader AA populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Membership in the NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium and the TOPMed Hematology & Hemostasis Working Group is listed in the supplemental material.

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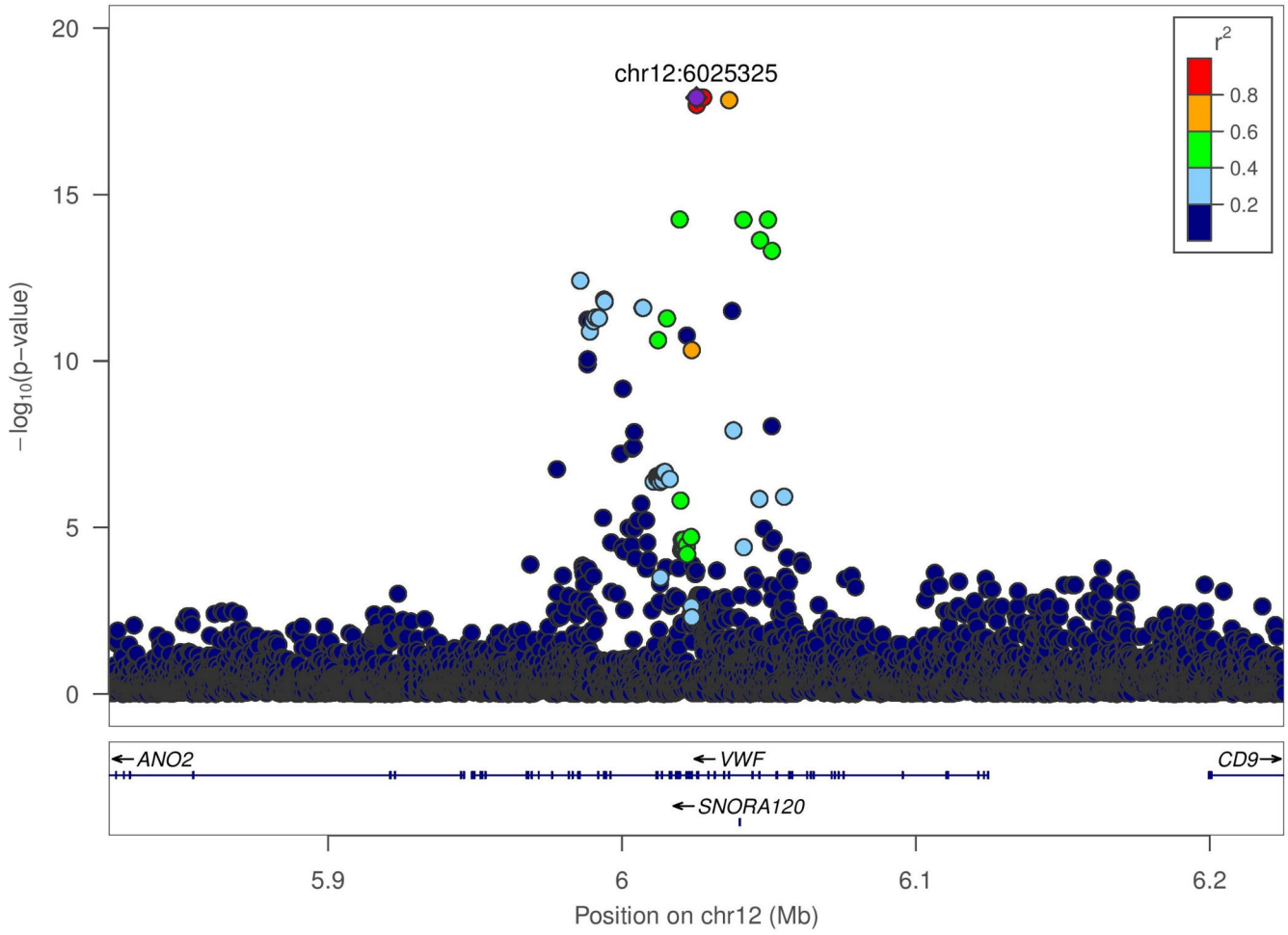
ESSENTIALS

Higher factor VIII (FVIII) levels are a known cardiovascular disease risk factor.

We here examine the epidemiological and genetic correlates of FVIII in African Americans.

FVIII was associated with incident heart failure, mortality, and coronary heart disease.

Genetic variants at *ABO* and *VWF*, as well as methylation at *ABO*, associated with FVIII.



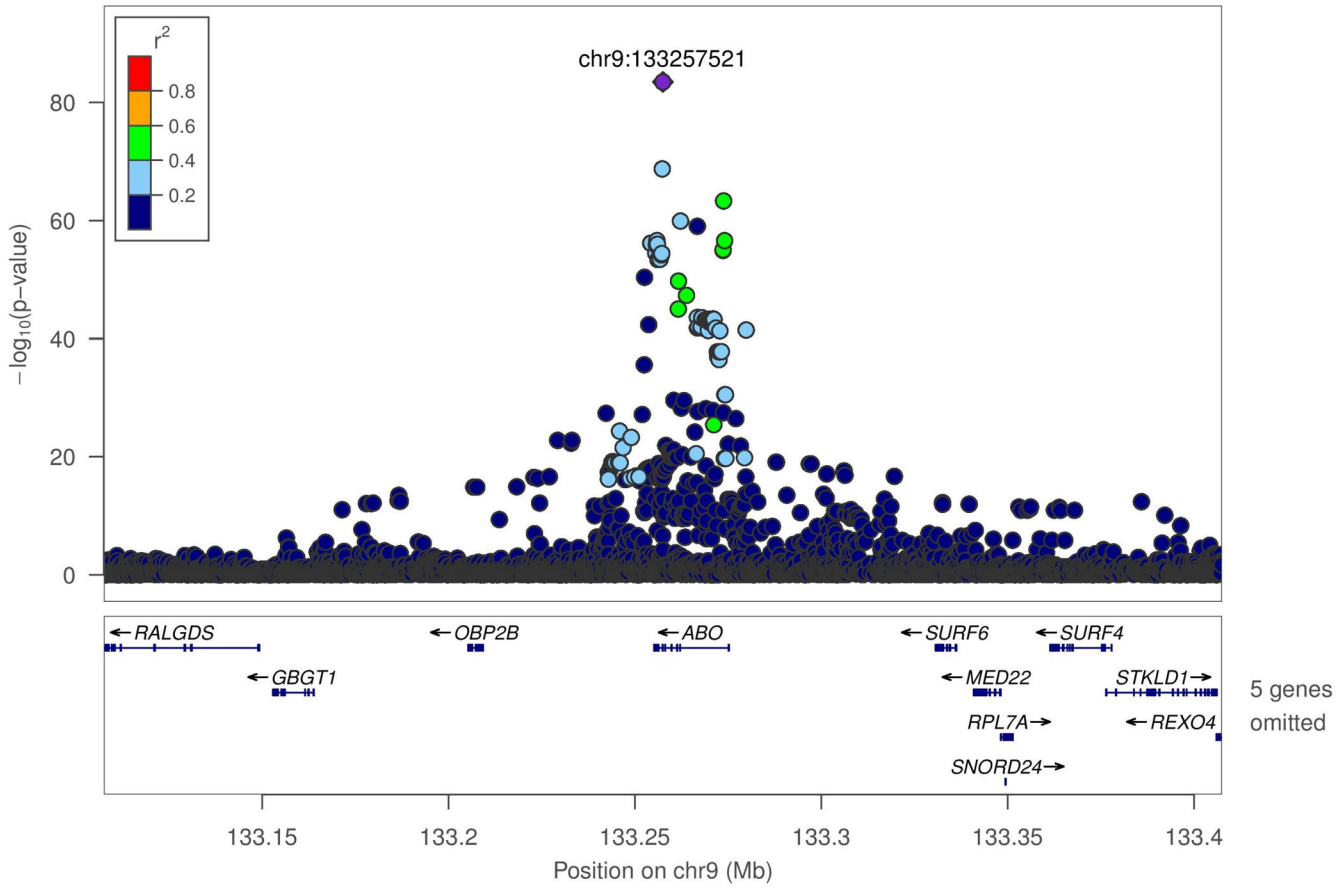


Figure 1.

LocusZoom plots for *ABO* and *VWF* loci, with linkage disequilibrium calculated in Jackson Heart Study participants with TOPMed freeze 6 data.

A. *VWF* locus (rs115708869)

b. *ABO* locus (rs8176719)

Table 1.

Associations between factor VIII and cardiovascular disease risk factors in the Jackson Heart Study (JHS), reported as the difference in the listed variable per standard deviation higher factor VIII. The mean (SD) or, for dichotomous variables, %, for each variable (untransformed) is also listed.

Trait	Mean (SD) or %	N	β	Standard Error	p-value
Age (years)	55.59 (12.80)	3493	2.91	0.25	<0.0001
Male sex	37.79%	3493	−0.17	0.04	<0.0001
Current smoker	13.31%	3463	−0.07	0.06	0.25
Ln BMI (kg/m^2)	31.89 (7.31)	3486	0.02	0.004	<0.0001
Waist (cm)	101.2 (16.27)	3486	2.04	0.29	<0.0001
Systolic BP (mmHg)	127.37 (16.61)	3487	0.32	0.29	0.26
Diastolic BP (mmHg)	75.77 (8.75)	3487	−0.18	0.14	0.20
Fasting glucose (mg/dL)	90.45 (8.86)	2591	0.47	0.17	0.01
Total Cholesterol (mg/dL)	199.21 (40.62)	3238	1.07	0.79	0.18
LDLc (mg/dL)	126.49 (36.94)	3205	0.35	0.68	0.61
HDLc (mg/dL)	51.64 (14.78)	3237	−1.03	0.29	0.0003
Triglycerides (mg/dL)	107.57 (82.18)	3238	0.07	0.01	<0.0001
C-reactive protein (mg/dL)	0.53 (0.98)	3487	0.20	0.02	<0.0001
Hypertension	57.34%	3493	0.10	0.05	0.01
Diabetes	23.15%	3491	0.28	0.05	<0.0001

Models (other than those for age and sex) are adjusted for age and sex.

Abbreviations: SD=standard deviation; HDLc=high density lipoprotein cholesterol; LDLc=low density lipoprotein cholesterol; BP=blood pressure.

BMI, C-reactive protein, and triglycerides were natural log transformed.

Fasting glucose was only tested in those without diabetes at visit 1.

Table 2.

Associations between factor VIII and subclinical disease measures in the Jackson Heart Study (JHS), reported as the difference in the listed variable per standard deviation higher FVIII. The mean (SD) or, for dichotomous variables, %, for each variable is also listed.

Trait	Mean (SD) or %	N	β	Standard Error	p-value
Carotid IMT (mm)	0.73 (0.19)	3318	0.0001	0.003	0.97
CAC (Agatston score)	167.8 (506.93)	1938	0.08	0.06	0.17
AAC (Agatston score)	895.77 (1629.07)	1937	0.02	0.07	0.75
Any CAC	48.86%	1938	0.003	0.06	0.96
Any AAC	66.39%	1937	−0.004	0.07	0.95
Ankle-brachial index	1.21 (0.17)	3100	−0.003	0.003	0.32
LVMI (g/m^2)	36.31 (9.79)	2233	0.003	0.006	0.58
Left ventricular hypertrophy	7.84%	2233	0.17	0.07	0.01

Carotid IMT, CAC, AAC, and LVMI were natural log transformed prior to analysis. Any CAC and any AAC are reported as dichotomous variables (AAC>0 vs. AAC=0). AAC and CAC measures are from visit 2, not visit 1 when FVIII was measured. Models are adjusted for age and sex.

Abbreviations: IMT=intima media thickness; CAC=Coronary artery calcium; AAC=Abdominal aortic calcium; ABI=Ankle-brachial index; LVMI=Left ventricular mass index

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Table 3:

Association between factor VIII and risk of clinical outcomes in the Jackson Heart Study

			Model 1				Model 2				Model 3			
Outcome	**N**	**Events**	**HR**	**95% CI**		**P**	**HR**	**95% CI**		**P**	**HR**	**95% CI**		**P**
Stroke	2990	110	1.09	0.92	1.30	0.31	1.06	0.87	1.28	0.57	1.02	0.84	1.24	0.87
Overall CHD	2899	147	1.13	1.00	1.28	0.05	1.06	0.93	1.22	0.37	1.05	0.92	1.21	0.46
Hard CHD	2905	101	1.22	1.08	1.39	0.001	1.16	1.01	1.35	0.04	1.15	1.00	1.33	0.05
Heart Failure	2755	190	1.21	1.10	1.32	<0.0001	1.15	1.03	1.28	0.01	1.15	1.04	1.28	0.01
Mortality	3172	559	1.22	1.14	1.30	<0.0001	1.18	1.10	1.27	<0.0001	1.16	1.08	1.25	<0.0001

Abbreviations: CHD=coronary heart disease; HR=hazard ratio; CI=confidence interval; CRP=C-reactive protein.

Hazard ratios are reported per standard deviation of factor VIII. Only individuals with complete covariates for all models are included. **Model 1:** Adjusted for age, sex; **Model 2:** Model 1 + BMI, blood pressure medications, type 2 diabetes, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, current smoking; **Model 3:** Model 2 + CRP

Table 4.

Significant loci from previous genome-wide association studies or exome-wide association studies, with results from Jackson Heart Study (JHS) (N=3,348). Effect sizes from previous studies are reported in study-specific units (often but not always for inverse normalized Factor VIII) and should only be compared for direction of effect.

rsID	Closest gene(s)	Chr	Position (Build 38)	Effect allele	Other allele	Effect allele frequency	β	*P*	Effect allele frequency	β	*P*
Sabater-Lleal M et al., *Circulation*, 2019 (N=up to 46,354; multi-ethnic; genome-wide association study, includes n= 4,500 African Americans)									**JHS (N=3,348)**		
rs548630	*FCHO2, TMEM171, TNPO1*	5	73110832	A	C	0.49	−0.016	2.1×10^{-10}	0.75	−0.016	0.56
rs9390460	*STXBP5*	6	147373198	T	C	0.47	−0.019	2.2×10^{-15}	0.51	−0.045	0.065
rs9271597	*HLA* region	6	32623514	A	T	0.41	−0.015	1.4×10^{-08}	0.33	−0.041	0.11
rs4276643	*SCARA5*	8	27946082	T	C	0.66	−0.023	1.3×10^{-19}	0.54	−0.036	0.13
rs10102164	*SOX17, RP1*	8	54509054	A	G	0.19	0.019	2.4×10^{-09}	0.18	0.033	0.30
rs687289	*ABO*	9	133261703	A	G	0.36	0.15	1.9×10^{-770}	0.41	0.37	1.8×10^{-50}
9:13930481	*LINC00583, NFIB*	9	13930481	Del	Ins	0.85	−0.032	2.7×10^{-10}	0.97	−0.009	0.90
rs35458154	*ST3GAL4*	11	126426930	A	G	0.03	0.048	3.1×10^{-08}	0.005	0.33	0.065
rs4981022	*STX2*	12	103756096	A	G	0.69	0.025	3.0×10^{-20}	0.69	0.023	0.39
rs2238109	*VWF*	12	6044801	A	T	0.39	0.026	3.5×10^{-24}	0.58	0.059	0.019
rs4904820	*TCN2*	14	91852591	A	G	0.49	0.014	1.8×10^{-08}	0.69	0.007	0.80
rs150926226	*TMLHE, F8*	X	155491696	C	G	0.62	0.017	3.3×10^{-09}	0.43	−0.001	0.98
Huffman JE et al., *Blood*, 2015 (N=28,291; multi-ethnic; Exome-chip), results displayed are from multi-ethnic analysis, including n=6 079 African Americans, *KATNB1* significant in African Americans only									**JHS (N=3,348)**		
rs7962217	*VWF*	12	5952393	T	C	0.046	5.16	2.5×10^{-13}	0.02	0.15	0.13
rs41276738	*VWF*	12	6034812	T	C	0.0040	−16.89	2.2×10^{-13}	0.001	−1.02	0.001
rs141041254	*STAB2*	12	103759154	A	G	0.00087	26.81	2.1×10^{-8}	Not in JHS results (minor allele count <20)		
rs1800291	*F8*	X	154930010	C	G	0.27	−1.73	8.20×10^{-8}	0.33	0.008	0.71
rs142508811	*KATNB1*	16	57754931	T	C	0.00027	39.36	4.80×10^{-4}	0.001	0.696	0.06
Tang W et al., Am J Hematol, 2015 (N=20,941; multi-ethnic; IBC Illumina iSELECT array, African American results from n= 5,020 displayed here)									**JHS (N=3,348)**		
rs8176693	*ABO*	9	133262254	T	C	0.1	37.24	2.51×10^{-114}	0.11	0.65	1.17×10^{-60}
rs2236568	*MAT1A*	10	80276167	C	A	0.24	5.28	1.69×10^{-6}	0.23	0.003	0.93

rsID	Closest gene(s)	Chr	Position (Build 38)	Effect allele	Other allele	Effect allele frequency	β	*P*	Effect allele frequency	β	*P*
Sabater-Lleal M et al., *Circulation*, 2019 (N=up to 46,354; multi-ethnic; genome-wide association study, includes n = 4,500 African Americans)									**JHS (N=3,348)**		
rs2229446	*VWF*	12	5993906	T	C	0.19	−9.47	1.95×10^{-20}	0.21	−0.21	1.44×10^{-12}
rs1800380	*VWF*	12	6029429	T	C	0.3	5.72	5.62×10^{-11}	0.32	0.06	0.02
rs4764482	*VWF*	12	6060567	C	T	0.2	−5.74	8.12×10^{-8}	0.19	−0.04	0.19

Table 5.

Top CpG sites associated with factor VIII levels in Jackson Heart Study (N=1,670), with a p-value < 3.6×10^{-8}. Models are adjusted for age, sex, cell counts, and 10 ancestry principal components, as well as for family as a random effect.

CpG	Chr	Position (Build 37)	Position (Build 38)	Gene	Pearson Correlation	p-value
cg21160290	9	136149941	133274525	*ABO*	0.19	5.21×10^{-22}
cg12020464	9	136131183	133255796	*ABO*	−0.20	1.94×10^{-21}
cg22535403	9	136150032	133274616	*ABO*	0.19	2.35×10^{-21}
cg24267699	9	136151359	133275943	*ABO*	0.16	1.44×10^{-15}
cg14440550	9	136131118	133255731	*ABO*	−0.17	7.54×10^{-15}
cg11879188	9	136149908	133274492	*ABO*	0.13	3.78×10^{-13}
cg13660174	9	136238392	133371516	*SURF4*	0.14	1.36×10^{-11}
cg09376613	10	102087334	100327577	*PKD2L1*	0.14	8.57×10^{-11}
cg06015525	12	57872123	57478340	*ARHGAP9*	0.13	9.29×10^{-10}
cg14209264	11	64382444	64614972	*NRXN2*	0.13	1.48×10^{-09}
cg26657675	16	85575407	85541801		0.12	1.65×10^{-09}
cg17980786	3	32933637	32892145	*TRIM71*	0.13	1.73×10^{-09}
cg02650017	17	47301614	49224252	*PHOSPHO1*	−0.13	2.91×10^{-09}
cg07793033	16	85256423	85222817		0.12	3.19×10^{-09}
cg24044988	16	30197947	30186626	*CORO1A*	0.13	4.14×10^{-09}
cg06495135	22	40336939	39940935	*GRAP2*	0.13	4.68×10^{-09}
cg06388937	10	104406651	102646894	*TRIM8*	0.13	6.63×10^{-09}
cg13506600	9	136150361	133274945	*ABO*	0.12	7.81×10^{-09}
cg03315921	22	18243630	17760864	*BID*	0.12	8.27×10^{-09}
cg03044066	8	130047260	129035014		0.12	9.01×10^{-09}
cg04883291	9	136126473	133251086		0.12	9.35×10^{-09}
cg06192883	15	52554171	52261974	*MYO5C*	0.13	9.90×10^{-09}
cg18645241	21	43022102	41601942		−0.12	1.08×10^{-08}
cg07023538	10	71719637	69959881		0.14	1.12×10^{-08}

CpG	Chr	Position (Build 37)	Position (Build 38)	Gene	Pearson Correlation	p-value
cg26098679	1	23681008	23354515		0.13	1.47×10^{-08}
cg06646796	14	24104741	23635532	*DHRS2*	0.13	1.99×10^{-08}
cg16248756	7	127795594	128155542		0.13	9.60×10^{-09}
cg07817261	20	47566479	48949942	*ARFGEF2*	0.13	3.50×10^{-08}
cg00964361	20	30459158	31871355	*DUSP15*	0.12	3.53×10^{-08}
cg19748455	17	76274856	78278775	*LOC100996291*	−0.13	3.53×10^{-08}