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D-dimer in African Americans: Whole Genome Sequence Analysis and Relationship to CVD Risk in the Jackson Heart Study

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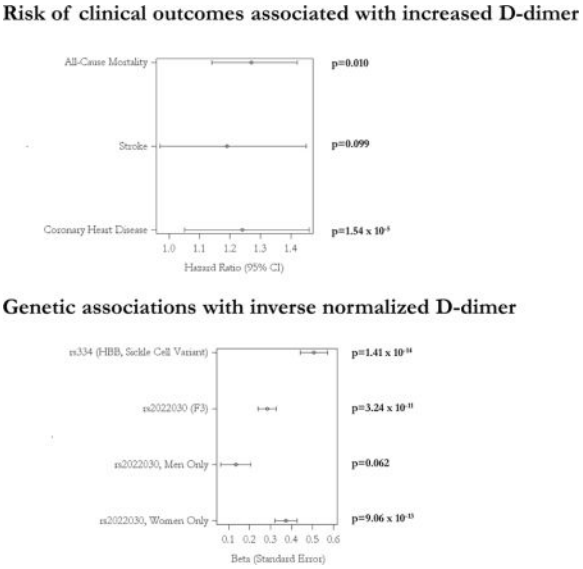
Abstract

Objective—Plasma levels of the fibrinogen degradation product D-dimer are higher among African Americans (AAs) compared to those of European ancestry and higher among women compared to men. Among AAs, little is known of the genetic architecture of D-dimer or the relationship of D-dimer to incident CVD.

Approach and Results—We measured baseline D-dimer in 4,163 AAs aged 21–93 years from the prospective Jackson Heart Study (JHS) cohort and assessed association with incident CVD events. In participants with whole genome sequencing data (n=2,980), we evaluated common and rare genetic variants for association with D-dimer. Each standard deviation higher baseline D-dimer was associated with a 20–30% increased hazard for incident coronary heart disease, stroke, and all-cause mortality. Genetic variation near *F3* was associated with higher D-dimer (rs2022030, $\beta = 0.284$, $p = 3.24 \times 10^{-11}$). The rs2022030 effect size was nearly three times larger among women ($\beta = 0.373$, $p = 9.06 \times 10^{-13}$) than men ($\beta = 0.135$, $p = 0.06$, p-interaction= 0.009). The sex by rs2022030 interaction was replicated in an independent sample of 10,808 multi-ethnic men and women (p-interaction=0.001). Finally, the African ancestral sickle cell variant (*HBB* rs334) was significantly associated with higher D-dimer in JHS ($\beta = 0.507$, $p = 1.41 \times 10^{-14}$), and this association was successfully replicated in 1,933 AAs ($p = 2.3 \times 10^{-5}$).

Conclusion—These results highlight D-dimer as an important predictor of CVD risk in AAs and suggest that sex-specific and African ancestral genetic effects of the *F3* and *HBB* loci contribute to the higher levels of D-dimer among women and AAs.

Graphical abstract



Keywords

genetic epidemiology; cardiovascular disease; coagulation

Subject Codes

Genetic; Association Studies; Cardiovascular Disease; Epidemiology

INTRODUCTION

Plasma D-dimer, a fibrin degradation product, is a coagulation related biomarker. Clinically, extremely elevated levels of D-dimer are a hallmark of disseminated intravascular coagulation, while D-dimer levels in the normal range are used to exclude a diagnosis of deep venous thrombosis and pulmonary embolism.[1] In healthy people, higher D-dimer predicts future risk of cardiovascular disease (CVD) events,[2, 3] particularly venous thrombosis,[4, 5] stroke,[6, 7] and all-cause mortality[8, 9] independently of conventional CVD risk factors. More modest associations have generally been observed for incident coronary heart disease (CHD).[6, 7, 10–12] D-dimer is also associated with CVD events and mortality in patients with peripheral arterial disease[13] and mortality in patients with cancer.[14]

African Americans (AAs) have higher mean levels of D-dimer than Europeans, independent of other CVD risk factors.[15–17] However, the relationship of D-dimer to CVD events specifically in AAs has only been addressed in one recent study.[7] D-dimer levels are also higher in women than men, for unknown reasons.[6, 8, 9] Family-based heritability studies and genome-wide association studies (GWAS) including mainly individuals of European descent have shown significant heritability for D-dimer, and variants in three coagulation factor-related loci (*F3*, *F5*, and *FGA/FGG*) have been associated with D-dimer.[18] Despite their higher D-dimer levels, little is known about the genetic architecture or environmental

correlates of D-dimer in AAs, or factors that account for higher D-dimer in women compared to men.

We measured baseline D-dimer in 4,163 AAs from the Jackson Heart Study (JHS), of whom 2,980 had available whole genome sequencing (WGS) data through the National Heart, Lung, and Blood Institute (NHLBI) Trans-Omics for Precision Medicine (TOPMed) project. We assessed the environmental and genetic correlates of D-dimer, as well as the association of baseline D-dimer with incident CVD events and all-cause mortality in JHS. We then performed WGS association analysis, including coding and non-coding genetic variants, which encompass African population-specific variants that may not be captured by conventional GWAS genotyping platforms. For any genome-wide significant loci, we tested for sex by genotype interaction. Finally, we sought replication and follow-up of sex- or ancestry-specific genetic association findings using independent samples from the Multi-Ethnic Study of Atherosclerosis (MESA), Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), and REasons for Geographic and Racial Differences in Stroke (REGARDS) studies.

MATERIALS AND METHODS

Materials and Methods are available in the online-only Data Supplement.

RESULTS

Genetic and environmental correlates of D-dimer levels in JHS AAs

Older age, female sex, and higher BMI were each strongly and independently associated with higher D-dimer, using a Bonferroni correction for multiple comparisons ($p<0.004$) (Table 1, Figure S1). Adjusting for age and sex, heritability (h^2) of D-dimer was estimated as 0.284 (standard error (SE) = 0.057, $p= 3.75 \times 10^{-9}$). In age- and sex-adjusted models or multivariate adjusted models (Table 1, Figure S1), higher CRP and higher fibrinogen were each associated with higher D-dimer. Greater estimated African ancestry percentage was nominally associated with higher D-dimer levels in a model adjusted for CVD risk factors.

Association of D-dimer levels with incident CVD events and mortality in JHS

In minimally and fully-adjusted models, higher D-dimer was nominally associated with increased hazard for CHD, stroke, and all-cause mortality (all $p\leq 0.039$) (Table 2). One standard deviation (SD) difference in log-transformed D-dimer was associated with a 20–30% greater hazard. Adjustment for CRP attenuated the associations with stroke (hazard ratio (HR) 1.19 (95% CI 0.97, 1.45), p=0.099), while the association with CHD (HR 1.24 (95% CI 1.05, 1.46), p=0.010) and mortality (HR 1.27 (95% CI 1.14, 1.42), $p= 1.54 \times 10^{-5}$) remained similar.

Whole genome sequence association analysis of D-dimer in JHS AAs

We performed both (1) single-variant association tests and (2) gene-based association tests of aggregated variants with a minor allele frequency <5%. Manhattan plots of the single-variant whole genome association results and gene-based rare variant results are displayed in

Figure S2. No significant systematic inflation was observed on QQ-plots (Figure S3); the calculated genomic inflation factor (λ) was 1.006 for single-variant analysis and 1.06 for gene-based analysis. Two loci, on chromosomes 1 (near *F3*) and 11 (*HBB*), reached significance in single variant testing (Figure S2a). *HBB* also was significantly associated with D-dimer levels in gene-based testing ($p=2.35 \times 10^{-10}$) (Figure S2b). These are described further in detail below.

Of other single variants previously associated with D-dimer, we observed significance and the same direction of association (β= 0.112, p= 0.01, effect allele frequency (EAF) 10.1%) for the coagulation factor V (*F5)* locus variant (rs6687813) associated with D-dimer in Europeans.[18] We also observed a concordant direction of association for rs6025 (FV Leiden) (β= 0.277, p= 0.093, EAF 0.6%),[16] a well-studied variant at this locus associated with VTE risk.[19] The fibrinogen alpha/fibrinogen gamma chain (*FGA/FGG)* locus variant (rs13109457) did not replicate (β= 0.010, p= 0.755, EAF 23.7%),[18] nor did a coding variant in *FGA* (rs6050) from analysis of a candidate gene array in AAs (β= −0.003; p= 0.912, EAF 36.8%).[16] An insertion variant at nucleotide 545 in *FGL1* previously associated with D-dimer in Finns was not observed[20]; no exonic or splicing variants in this gene were associated with D-dimer (p>0.05).

Chromosome 1 locus near F3—The chromosome 1 signal was an intergenic locus near tissue factor (*F3*, top variant rs2022030, β= 0.284, SE= 0.043, $p=3.24 \times 10^{-11}$, effect allele frequency (EAF) = 10.3%) explained 1.47% of the phenotypic variance). A LocusZoom plot[21] is displayed in Figure S4. A total of 9 variants at the chr 1 locus were significant at the 1×10^{-9} level, including two index SNPs previously associated with D-dimer in European GWAS (rs12029080[18], rs2022309[22]). However, none of these variants remained significant after conditioning on the lead variant rs2022030 (Figure S5a). Similarly, adjustment for lead European meta-analysis variant rs12029080 greatly attenuated the association signal at rs2022030 (β= 0.323, SE=0.136, p= 0.017) (Figure S5b) in JHS. These two variants were in strong linkage disequilibrium (LD) in JHS (r^2=0.90).

As the *F3* variant associated with D-dimer is common among all major continental ancestries, we attempted to further explore the *F3* rs2022030 genotype association with *F3* RNA expression and monocyte tissue factor expression using RNA sequencing and flow cytometry data available in the multi-ethnic MESA cohort. The minor allele of the index SNP *F3* rs2022030 associated with higher D-dimer was nominally associated with increased *F3* expression in monocytes (β= 0.182, SE= 0.073, p= 0.013). Index SNP rs2022030 was also associated with an increased percentage of TF+ monocytes in the LPS stimulated expression assay (β = 0.119, SE= 0.038, p= 0.002) but was not associated with percentage of TF+ monocytes in the unstimulated assay (β = −0.023, SE= 0.061, p= 0.705).

We also assessed associations of rs2022030 with other coagulation-related biomarkers that were previously measured in MESA and CHS. The *F3* lead variant rs2022030 was associated with higher levels of PAP (β=0.025, SE=0.007, p=0.00043). There were no associations between rs2022030 and either thrombin generation measured *ex vivo* or selected coagulation/fibrinolysis biomarkers related to TF or extrinsic pathway activation, including circulating levels of soluble TF, factor VIIa, or TFPI (Table S1).

Chromosome 11 HBB locus—The second genome-wide significant locus for D-dimer was on chromosome 11. The lead variant, *HBB* rs334 (p.E6V, EAF =4.2%), which encodes the sickle cell mutation, was associated with higher D-dimer levels (β= 0.507, SE=0.065, $p=1.41 \times 10^{-14}$) and explained 1.97% of the phenotypic variance (Figure S6a). Excluding two homozygotes for the minor allele of rs334 did not substantively change this result (β= 0.485, $p=3.77 \times 10^{-13}$). An additional chromosome 11 variant, intergenic indel rs149481026 near *OR51V1*, was also significant (β= 0.490, SE=0.079, $p= 6.19 \times 10^{-10}$, EAF=2.9%). Conditioning on rs334 attenuated the rs149481026 signal (β= −0.003, SE= 0.1339, p= 0.983, Figure S6b). These two variants were in moderate LD in JHS (r^2=0.67). The gene-based association signal at *HBB* was driven by only two variants, rs334 and non-significant variant rs33930165 (β= −0.003, SE=0.119, p=0.981, EAF=1.3%).

As rs334 is an African ancestry specific variant, we confined our replication analysis for this variant to an independent, single-ethnicity sample of N=1,933 AA participants from MESA and REGARDS. We found a similar association of *HBB* rs334 with higher D-dimer levels (β= 0.286, SE= 0.068, $p= 2.31 \times 10^{-5}$). To investigate the possible mechanism or influence on other coagulation-related parameters, we analyzed the relationship of *HBB* rs334 genotype with additional coagulation measures available in MESA and/or REGARDS. We observed no association of *HBB* rs334 with fibrinogen, PAP, factor VIII, factor IX, factor XI, or protein C levels (Table S2).

Gene-sex interaction of *F3* variant on D-dimer levels

Since D-dimer is higher in women than men, we performed an exploratory WGS association analysis stratified by sex in JHS AA. Manhattan and QQ-plots are shown in Figure S7. No variants were genome-wide significant in men. In women, both the chromosome 1 *F3* rs2022030 variant (β= 0.373, SE= 0.052, $p= 9.06 \times 10^{-13}$) and the chromosome 11 *HBB* rs334 variant (β= 0.523, SE= 0.085, $p= 9.91 \times 10^{-10}$) were genome-wide significant. The lead variant at the *F3* locus in women was rs4609438 (β= 0.356, SE= 0.048, $p= 2.72 \times 10^{-13}$, EAF 12.8%), which is in strong LD (r^2=0.8) with rs2022030.

Though the chromosome 11 *HBB* rs334 variant did not reach genome-wide significance in men, it had a similar estimated effect in men (β= 0.468, SE= 0.100, $p= 3.12 \times 10^{-6}$, p-interaction=0.923) compared to women. In contrast, *F3* rs2022030, the lead variant in the sex combined analyses, had greatly reduced estimated effect size in JHS men (β= 0.135, SE= 0.072, p= 0.062) compared to JHS women. The rs2022030–sex interaction in JHS was significant (p interaction= 0.009) (Table 3).

We next tested for additional evidence of *F3* rs2022030–sex interaction in an independent sample of 10,808 multi-ethnic men and women from MESA, FHS, and CHS (Table 3, Figure S8). In the combined analysis for these three cohorts, the effect size of rs2022030 was higher (β= 0.073, SE= 0.023) among women than men, and this sex difference was also highly significant (p interaction=0.001).

D-dimer associated genetic variants and risk of incident events in JHS

Finally, we assessed the relationship between D-dimer associated genetic variants and risk of clinical events in JHS. No significant associations with all-cause mortality, coronary heart disease, or stroke were observed for *HBB* rs334 (Table 4). For *F3* variant rs2022030, nominal associations were observed with increased risk of CHD (HR 1.43 (95% CI 1.01, 2.03), p=0.045) and stroke (HR 1.66 (95% CI 1.11, 2.47), p=0.013) in models adjusted for age, sex, and ancestry principal components. The results remained essentially unchanged when adjusted for BMI, current smoking, alcohol use, diabetes mellitus, hypertension, systolic blood pressure, low-density lipoprotein cholesterol, and CRP. We also constructed a simple genetic risk score, summing the number of D-dimer raising alleles at rs334 and rs2022030 (Table 4). This score was significantly associated with increased stroke risk (HR 1.58 (95% CI 1.15, 2.17), p=0.004), but not with CHD (HR 1.21 (95% CI 0.88, 1.67), p=0.246) or mortality (HR 1.03 (95% CI 0.82, 1.29), p=0.825). The association between *F3* rs2022030 and risk of CHD and stroke and the association between the genetic risk score and risk of stroke were only partially attenuated by adjustment for D-dimer.

DISCUSSION

There were three main findings from this study. First, higher D-dimer was confirmed as an independent risk marker for future CVD and total mortality in AAs. Second, both acquired and genetic factors (including those that are shared with ancestral European populations such as *F3* rs2022030) contributed to D-dimer variation among AAs. Third, we identified genetic factors that may in part account for gender and ethnic differences in D-dimer. Specifically, the African ancestral sickle cell variant (*HBB* rs334), was associated with higher D-dimer. Moreover, a sex-specific association of the *F3* gene locus was seen in women but not men, which might explain, in part, the higher D-dimer levels among women compared to men.

Prior studies examining the role of D-dimer in risk prediction of stroke[6, 7], CHD[6], venous thromboembolism (VTE)[4, 5], all CVD[3], and mortality[9] included both AA and European descent participants. Only one recent study in REGARDS reported the results stratified by race/ethnicity, which suggested a stronger association for CHD than stroke, particularly in AAs.[7] Our results in JHS for the association of D-dimer with these CVD and mortality outcomes in AAs were generally consistent with these prior studies, although our precision was limited by the available sample size.[3, 6, 9] Comparison of results between studies may be complicated by differences in sample sizes and numbers of events, length of follow-up/proximity of clinical events to time of D-dimer measurement, and covariate adjustment, all of which may impact the reported risk estimates.

Among related AA individuals from JHS, we estimated the polygenic heritability of D-dimer as 0.284. Prior studies reported heritability estimates ranging from 0.11–0.65.[23–26] Global or genome-wide estimates of African ancestry have been associated with higher D-dimer levels in some populations,[27] but not others,[28] with a modest positive association observed in JHS. Apart from any differences in genetic architecture of D-dimer levels due to ancestry-specific genetic factors, it is possible that polygenic influences may differ on the basis of age and/or sex. In the context of the observed *F3* genotype-sex interaction we report

in JHS, it is interesting to note the relatively higher heritability estimate for D-dimer (65% in female European twin pairs) reported in the one prior study that only included women.[23]

To our knowledge, the current report is the first genome-wide, sequencing-based association study of D-dimer in a population of African ancestry, including analysis of coding and non-coding variants across the entire allele frequency spectrum. Two prior genetic association analyses of D-dimer in AAs were limited to variants in candidate gene regions.[16, 27] An analysis of variants from 42 coagulation genes in 327 AA participants from CHS found no significant associations.[27] In an analysis of ITMAT-Broad-CARe (IBC) array data in 2,192 AA participants, Weng et al. replicated the association of a coding variant in *FGA* (rs6050) with D-dimer previously observed in European populations.[16]

The genome-wide association of *HBB* rs334 (sickle cell trait) with D-dimer in JHS is consistent with previous work[29–32] and the higher risk of VTE with sickle cell trait.[5] While sickle cell disease is a hypercoagulable state, few prior studies have examined hemostatic measures in *HBB* rs334 heterozygotes.[5, 30, 31, 33] We did not find any association between *HBB* rs334 and other hemostasis biomarkers, though our analysis may be limited by sample size. The mechanism responsible for higher D-dimer in sickle cell mutation carriers is unknown. Relatively low partial pressure of oxygen and dehydration in certain tissues[34, 35] may lead to erythrocyte sickling and endothelial activation, which may result in tissue damage, exposure of extracellular and intracellular proteins (e.g., tissue factor), which ultimately trigger activation of the blood coagulation system. The roles of subclinical rhabdomyolysis, proteinuria, or erythrocyte sickling and resultant erythrocyte phosphatidylserine exposure, protein S levels, and thrombin generation[36] require further investigation to elucidate the mechanisms relating sickle trait to higher D-dimer and VTE risk.

Our results show that the association of the *F3* locus with D-dimer initially observed in Europeans[18] generalizes to AAs. *F3* encodes tissue factor (TF), a necessary co-factor for factor VIIa in the initiation of the extrinsic blood coagulation pathway. TF is constitutively expressed in various extravascular cells, and can be induced in endothelial cells and monocytes/macrophages by a variety of agonists. According to high-resolution chromatin conformation capture assay, our lead variant rs2022030 physically interacts with the *F3* promoter region in trophoblast and spleen tissue.[37] In analyses from the MESA cohort, our lead variant was also associated with increased monocyte *F3* expression and an increased percentage of TF+ LPS-stimulated monocytes. Another LD proxy variant rs143015276 located ~50 kb upstream of *F3* is within promoter marks for lung and cervical tissue and enhancer histone marks for mesenchymal stem cells, skin, muscle, breast, and placental tissue.[38] Taken together, these data suggest a potential regulatory role for one or more of the D-dimer-associated variants on tissue factor expression.

The observed rs2022030-sex interaction at *F3* is concordant with higher levels of D-dimer in women[6, 8, 9] (JHS mean 0.49, SD 0.65 in men, 0.62, SD 0.63 in women). Notably, TF expression can be induced by progesterone in human endometrial stromal cells and appears to play a role in reproductive and peripartum hemostasis.[39] A genome-wide significant *F3* variant (rs143015276, $p = 1.34 \times 10^{-9}$) in LD with rs2022030 is located within predicted

binding motifs for *FOXA1*, a transcription factor for the estrogen receptor.[38] A previous study did not find any association of the *F3* variant and risk of incident VTE,[40] however, further study of the *F3* variant in women and in the contexts of exogenous sex hormones (oral contraceptives, hormone therapy) is warranted.

The connection between D-dimer associated genetic variants *F3* rs2022030, *HBB* rs334, and CVD events is unclear. While we provide some additional suggestive evidence that D-dimer raising alleles are associated with increased risk of incident CHD and stroke in AA, the interpretation of results is limited by the relatively small number of CVD events in JHS. Moreover, the genetic variant – CVD associations were only partially attenuated by adjustment for D-dimer, suggesting that additional intermediates or pathways may mediate the putative genetic susceptibility. A prior meta-analysis of incident VTE and *F3* variant rs12029080 in Europeans found no significant association.[40] Further analyses, including a more formal Mendelian randomization analysis involving considerably larger sample sizes, will be required to clarify the relationship of D-dimer associated genetic variants to CVD risk, particularly in African ancestry populations.

Several additional limitations of our analysis should be noted. To date, VTE have not been adjudicated within the JHS, so we were unable to assess associations with D-dimer. Much larger sample sizes (currently accruing through WGS projects such as TOPMed) may be needed to assess the role of lower frequency variants associated with D-dimer levels. The lack of association between the *F3* or *HBB* variants and other coagulation biomarkers may be due to heterogeneity of participant characteristics or assay methods between studies.

In summary, our results extend the importance of D-dimer as a CVD biomarker and predictor in AAs. Genetic factors including sickle cell trait and common variants at the *F3* locus contribute to the higher D-dimer levels among AAs and AA women, respectively. Given the role of D-dimer in the clinical evaluation and diagnosis of VTE, and potentially additional vascular conditions, future studies should address the clinical and public health implications of these ethnic and gender-related genetic influences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AAs	African Americans
CVD	cardiovascular disease
CHS	Cardiovascular Health Study
CRP	C-reactive protein
FHS	Framingham Heart Study
GWAS	genome-wide association studies
JHS	Jackson Heart Study
LD	linkage disequilibrium
LPS	lipopolysaccharide
MAF	minor allele frequency
MESA	Multi-Ethnic Study of Atherosclerosis
NHLBI	National Heart Lung, and Blood Institute
PAP	plasmin-antiplasmin complex
PCs	principal components
REGARDS	REasons for Geographic and Racial Differences in Stroke
SD	standard deviation
SE	standard error
TF	tissue factor
TFPI	tissue factor pathway inhibitor
TOPMed	Trans-Omics for Precision Medicine
VTE	venous thromboembolism
WGS	whole genome sequencing

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HIGHLIGHTS

- Fibrin degradation product D-dimer is associated with risk of incident cardiovascular disease in African Americans (AAs) from the Jackson Heart Study (JHS).
- Analysis of whole genome sequencing data in JHS revealed associations of intergenic variants near *F3* and sickle cell trait (*HBB* locus) with D-dimer.
- The signal at the *F3* locus was driven mostly by women, and this significant sex interaction was replicated in a multi-ethnic sample.
- Sex-specific and African ancestral effects of the *F3* and *HBB* loci, respectively, may contribute to higher D-dimer among women and AAs.

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Table 1

Demographic characteristics of Jackson Heart Study participants with measured D-dimer and assessment of association with D-dimer.

Trait				Model 1		Model 2		Model 3	
	Mean ± SD or %	Median (Min, Max)	N	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value
D-dimer (μg/mL)	0.573 ± 0.645	0.4 (0.01, 11.98)	4163						
Age in Years	55 ± 12.9	55.4 (20.6, 93.1)	4163	0.017 (0.001)	$<1 \times 10^{-15}$	0.016 (0.001)	$<1 \times 10^{-15}$	0.017 (0.001)	$<1 \times 10^{-15}$
Sex (% Female)	62.1%		4163	0.333 (0.026)	$<1 \times 10^{-15}$	0.315 (0.024)	$<1 \times 10^{-15}$	0.277 (0.025)	$<1 \times 10^{-15}$
Body Mass Index (kg/m^2)	31.9 ± 7.3	30.6 (16, 91.8)	4157	0.013 (0.002)	2.73×10^{-14}	0.011 (0.002)	1.34×10^{-12}	0.012 (0.002)	1.26×10^{-13}
Current Smoker (%)	13.7%		4126	−0.040 (0.033)	0.226	0.067 (0.032)	0.037	0.091 (0.032)	0.005
Diabetes Status (%)	22.0%		4161	0.101 (0.031)	0.001	−0.020 (0.031)	0.504	−0.077 (0.031)	0.014
Hypertension (%)	59.5%		4163	0.249 (0.024)	$<1 \times 10^{-15}$	0.065 (0.025)	0.010	0.044 (0.027)	0.104
Systolic Blood Pressure (mmHg)	126.9 ± 18.5	125 (73, 237)	4151	0.005 (0.001)	5.65×10^{-10}	0.001 (0.001)	0.418	−0.0004 (0.001)	0.618
Diastolic Blood Pressure (mmHg)	78.9 ± 10.6	79 (31, 121)	4151	−0.007 (0.001)	4.42×10^{-7}	−0.002 (0.001)	0.230	−0.004 (0.002)	0.014
Fasting LDL Cholesterol (mg/dL)	126.5 ± 36.6	124 (22, 361)	3823	−0.0001 (0.0004)	0.759	−0.0003 (0.0003)	0.323	−0.0004 (0.0003)	0.228
Fibrinogen (mg/dL)	417.2 ± 91.1	406 (153, 946)	4162	0.003 (0)	$<1 \times 10^{-15}$	0.002 (0.0001)	$<1 \times 10^{-15}$	0.002 (0.0001)	$<1 \times 10^{-15}$
C-Reactive Protein (mg/dL)	0.52 ± 0.943	0.269 (0, 33.8)	4162	0.131 (0.025)	1.10×10^{-7}	0.107 (0.018)	2.68×10^{-9}	0.092 (0.015)	8.79×10^{-10}
History of Cardiovascular Disease (%)	10.5%		4163	0.139 (0.043)	0.001	0.033 (0.041)	0.431	0.017 (0.042)	0.688
Any Self-reported Alcohol Consumption in Last 12 Months (%)	47.0%		4142	−0.195 (0.025)	1.78×10^{-15}	−0.014 (0.025)	0.565	−0.017 (0.025)	0.490
Individual African ancestry (%)	82.6% (8.7%)	84.4% (22.1%, 98.5%)	3522	0.466 (0.154)	0.003	0.398 (0.143)	0.005	0.360 (0.140)	0.010

Model 1 is unadjusted. Model 2 is adjusted for age and sex. Model 3 is adjusted for age, sex, body mass index, current smoking, alcohol use in last 12 months, diabetes mellitus, hypertension, systolic blood pressure, and low-density lipoprotein cholesterol. All characteristics were assessed at visit 1. Only individuals with complete covariates for all models (n=3,754) are included. β values reported per standard deviation difference in ln transformed measure. Abbreviations: maximum (Max), minimum (Min), odds ratio (OR)

Table 2

Association of natural log transformed D-dimer with mortality and incident cardiovascular disease events in JHS.

	Coronary Heart Disease				Stroke				All-Cause Mortality			
Model	**HR (95% CI)**	**p-value**	**Events**	**N**	**HR (95% CI)**	**p-value**	**Events**	**N**	**HR (95% CI)**	**p-value**	**Events**	**N**
Model 1	1.25 (1.07, 1.47)	0.005	146	3408	1.23 (1.01, 1.51)	0.039	106	3509	1.32 (1.19, 1.46)	2.14×10^{-7}	384	3753
Model 2	1.26 (1.07, 1.48)	0.007	146	3408	1.24 (1.01, 1.52)	0.037	106	3509	1.30 (1.16, 1.45)	2.36×10^{-6}	384	3753
Model 3	1.24 (1.05, 1.46)	0.010	146	3408	1.19 (0.97, 1.45)	0.099	106	3509	1.27 (1.14, 1.42)	1.54×10^{-5}	384	3753

Model 1 is adjusted for age and sex. Model 2 is adjusted for Model 1 + body mass index, current smoking, alcohol use in last 12 months, diabetes mellitus, hypertension, systolic blood pressure, and low-density lipoprotein cholesterol. Model 3 is adjusted for Model 2 + C-reactive protein. Hazard ratios are reported per standard deviation. Only individuals with complete covariates for all models are included.

Table 3

Association between *F3* rs2022030 and natural log D-dimer, stratified by sex, in JHS discovery sample and in replication studies.

Study	Race/Ethnicity		Women				Men				
		MAF	β	SE	p-value	N	β	SE	p-value	N	P for interaction
JHS	AA	0.103	0.258	0.035	5.56×10^{-13}	1851	0.089	0.053	0.089	1129	0.009
CHS	EA	0.310	0.190	0.034	1.91×10^{-7}	854	0.077	0.050	0.126	604	0.038
FHS	EA	0.277	0.108	0.022	1.30×10^{-6}	1623	0.092	0.027	0.0006	1440	0.467
MESA	AA	0.109	0.254	0.069	2.30×10^{-4}	846	0.123	0.075	0.099	732	0.303
MESA	EA	0.318	0.200	0.034	7.84×10^{-9}	1306	0.016	0.039	0.675	1198	3.78×10^{-4}
MESA	Hispanic	0.250	0.162	0.052	0.002	739	0.195	0.049	7.94×10^{-5}	695	0.626
MESA	Chinese American	0.270	0.187	0.067	0.006	392	0.078	0.076	0.301	379	0.366

In combined analysis of CHS, FHS, and MESA, the effect size of rs2022030 was higher (β= 0.073, SE= 0.023) among women than men (p interaction=0.001). JHS and FHS analyses adjusted for age and the first 10 principal components. CHS and MESA analyses adjusted for age, site, and the first 10 principal components. Abbreviations: EA, European American.

Table 4

Association of D-dimer-associated genetic variants with mortality and incident cardiovascular disease events in Jackson Heart Study.

	Coronary Heart Disease (Events=111, N=2481)		Stroke (Events=82, N=2552)		All-Cause Mortality (Events=282, N=2698)	
Genotype	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
***HBB* rs334**						
Model 1	0.74 (0.36,1.53)	0.418	1.39 (0.71,2.69)	0.336	0.97 (0.66,1.43)	0.877
Model 2	0.78 (0.38,1.62)	0.511	1.47 (0.73,2.96)	0.275	0.99 (0.66,1.48)	0.964
Model 3	0.67 (0.32,1.39)	0.278	1.28 (0.64,2.59)	0.486	0.86 (0.58,1.29)	0.465
***F3* rs2022030**						
Model 1	1.43 (1.01,2.03)	0.045	1.66 (1.11,2.47)	0.013	1.05 (0.8,1.39)	0.720
Model 2	1.46 (1.02,2.08)	0.037	1.66 (1.09,2.51)	0.018	1.05 (0.8,1.37)	0.744
Model 3	1.37 (0.96,1.97)	0.084	1.62 (1.08,2.42)	0.019	1.02 (0.78,1.35)	0.874
Genetic risk score (rs334 + rs2022030)						
Model 1	1.21 (0.88,1.67)	0.246	1.58 (1.15,2.17)	0.004	1.03 (0.82,1.29)	0.825
Model 2	1.25 (0.9,1.73)	0.186	1.60 (1.15,2.24)	0.006	1.03 (0.82,1.29)	0.803
Model 3	1.14 (0.81,1.61)	0.444	1.53 (1.10,2.13)	0.012	0.97 (0.77,1.22)	0.775

Model 1 is adjusted for age, sex, and the first 10 ancestry principal components. Model 2 is adjusted for Model 1 + body mass index, current smoking, alcohol use in last 12 months, diabetes mellitus, hypertension, systolic blood pressure, and low-density lipoprotein cholesterol, and C-reactive protein. Model 3 is adjusted for Model 1+ natural log transformed D-dimer. Hazard ratios are reported per standard deviation. Only individuals with complete covariates for all models are included.