Hemostatic Factors, *APOL1* Risk Variants, and the Risk of ESRD in the Atherosclerosis Risk in Communities Study

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Abstract

Background and objectives Hemostatic factors have been associated with kidney function decline, and evidence suggests stronger effects among African Americans. The presence of *APOL1* renal risk variants, common in African Americans, might partly underlie this risk difference.

Design, setting, participants, & measurements A total of 13,337 participants in the Atherosclerosis Risk in Communities study were followed from 1987–1989 until 2010. Participants were categorized into three groups by ancestry and *APOL1* risk status: European Americans (n=10,206), African Americans with zero or one *APOL1* risk allele (n=2,733), and African Americans with two *APOL1* risk alleles (n=398). ESRD events were ascertained through linkage to the US Renal Data System. Cox regression was used to estimate the risk for ESRD associated with hemostatic factors (factor VIIc, factor VIIIc, fibrinogen, von Willebrand factor, protein C, and antithrombin III).

Results There were 232 cases of ESRD over 21.5 years (European Americans, 119; African Americans with zero or one *APOL1* risk allele, 94; African Americans with two *APOL1* risk alleles, 19). In unadjusted and adjusted analysis of the overall population, higher levels of all hemostatic factors, except antithrombin III, were significantly associated with ESRD (all $P<0.05$). Factor VIIc had the strongest association (per one interquartile range; adjusted hazard ratio, 1.46; 95% confidence interval, 1.21 to 1.76). With the exception of fibrinogen, the risk associated with each hemostatic factor was stronger in African Americans with two *APOL1* risk alleles compared with the other two groups. Statistically significant interactions were seen for factor VIIIc and protein C (interaction between those with two *APOL1* risk allele and the other two groups: $P<0.02$ for factor VIIIc and <0.04 for protein C).

Conclusions Higher levels of factor VIIc, VIIIc, fibrinogen, von Willebrand factor, and protein C were associated with ESRD risk, with a significantly stronger association of factor VIIIc and protein C in African Americans with two *APOL1* risk alleles.

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Introduction

ESRD is an important public health burden, affecting over half a million people (disproportionately African American) and costing an estimated $40 billion annually in public and private funds (1). Epidemiologic studies have linked higher levels of hemostatic factors in the coagulation cascade to faster kidney function decline, with stronger associations in African Americans than European Americans (2,3). The pathophysiology underlying the association of hemostatic factors to kidney function decline is unclear, although triggers of hemostatic activation, including vascular injury, endothelial dysfunction, and inflammation, have been proposed as potential mechanisms (4,5).

APOL1 genetic variants common in African Americans are known to be associated with progressive kidney disease (6–9). The *APOL1* high-risk genotype (two copies of the G1 or G2 alleles) has a population frequency of approximately 13% in African Americans and confers an approximately 2-fold higher risk for kidney function decline in cohort studies (8,9). Although the role of *APOL1* in relation to kidney function is largely unknown, environmental factors probably affect *APOL1*-associated renal risk (10). For example, HIV infection (and particularly an unsuppressed viral load) in individuals with the *APOL1* high-risk genotype appears to increase the susceptibility for progressive kidney disease (11,12). Other events that activate the innate immune response might similarly increase *APOL1*-associated susceptibility. Inflammatory cytokines induce the expression of the *APOL1* encoded protein, apolipoprotein L1 (apoL1) in macrophages, and endothelial and epithelial cells (13–15). Increased expression of the high-risk variants has been associated with organ damage in a transgenic mice study and reduced cell survival in *in vitro* studies (14–16).

Using the Atherosclerosis Risk in Communities (ARIC) study, a community-based prospective cohort of both

African Americans and European Americans, we investigated the association between levels of hemostatic factors (factor VIIc, factor VIIIc, fibrinogen, von Willebrand factor [vWF], protein C, and antithrombin III) and incident ESRD. Given the previously demonstrated interaction of ancestry and hemostatic factors with kidney function decline (2,3), and that *APOL1* expression and hemostatic activation may share risk factors, we hypothesized that the risk of ESRD would be higher in participants with higher levels of hemostatic factors, with a stronger association in those with two *APOL1* risk alleles.

Materials and Methods

Study Population

The ARIC Study is a community-based prospective observational study of 15,792 individuals age 45–64 years at the baseline visit from 1987 to 1989. Details of the ARIC cohort have been published elsewhere (19). For this study, we excluded individuals missing eGFR or with eGFR<15 ml/min per 1.73 m^2 (n=172), those who were not fasting at the time of phlebotomy (n=552), those receiving anticoagulant or hemostatic medications (warfarin, heparin, dipyridamole, aminocaproic acid, or pentoxifylline; n=293), those missing hemostatic factor values (n=420), African Americans missing *APOL1* genotype (n=343), those with self-reported race other than black or white (n=43), and those with missing data on covariates in the final model (n=633). Our final analyzed sample included 13,337 participants. For the purpose of this study, participants were followed until death, ESRD, or December 31, 2010, whichever came first. ESRD events were ascertained through linkage with the US Renal Data System.

Hemostatic Factor Measurements

The design of the hemostasis component of the ARIC study and the methods for the measurement of hemostatic factors have been described previously (18–20). Briefly, six hemostatic factors were measured in the overall population (factor VIIc, factor VIIIc, fibrinogen, vWF, protein C, and antithrombin III) from blood drawn from the antecubital vein after an 8-hour fast. Samples were processed according to a standardized protocol. Factor VII and VIII activity (factor VIIc and factor VIIIc, respectively) were measured by the coagulation test; fibrinogen by the thrombin-time titration method; vWF and protein C antigen by ELISA; and antithrombin III by a chromogenic substrate for thrombin. The reliability coefficients for repeated measures from a subsample of participants over 1–2 weeks were 0.78 for factor VIIc, 0.86 for factor VIIIc, 0.72 for fibrinogen, 0.68 for vWF, 0.56 for protein C, and 0.42 for antithrombin III (21).

Genotyping and *APOL1* Risk Group Definition

Taqman assays were used for direct genotyping of the *APOL1* risk variants in African Americans. The G1 risk variant consists of two missense mutations (rs73885319 [S342G] and rs60910145 [I384M]) that are in almost total linkage disequilibrium on the same haplotype, and the G2 risk variant (rs71785313) is a 6–base-pair deletion (22). The G1 and G2 variants are in high linkage disequilibrium on different haplotypes. The G1/G2 haplotypes were inferred using PLINK (23). All inferred haplotypes had a posterior probability of 1. Because both G1 and G2 variants are rare (minor allele frequency <1%) in European Americans (24), we assumed all European Americans had zero or one copy of the risk allele. Previous studies have shown that the G1 and G2 variants confer risks for ESRD in a recessive manner (11,25); therefore, we defined *APOL1* high-risk status as having two risk alleles (G1/G1, G1/G2, or G2/G2). The participants were categorized into three groups by ancestry and the number of *APOL1* risk alleles: European Americans, African Americans with zero or one *APOL1* risk allele (*APOL1* 0/1 risk allele), and African Americans with two *APOL1* risk alleles (*APOL1* 2 risk alleles).

Other Measurements

Prevalent diabetes mellitus was defined as having a fasting glucose level ≥126 mg/dl, nonfasting glucose level ≥200 mg/dl, self-reported diabetes medication use, or physician diagnosis of diabetes. BP measures were calculated as the average of the last two measures of three seated BP measures performed by certified technicians using a random-zero sphygmomanometer after the participant rested for 5 minutes. Medication use was determined on the basis of the inspection of medication bottles. Prevalent coronary heart disease was defined as a self-reported physician diagnosis or an electrocardiogram obtained during the baseline visit with signs of a previous myocardial infarction. Enzymatic methods were used to measure total plasma cholesterol and triglyceride levels. LDL cholesterol was calculated using the Friedewald equation (26) (excluding those with incalculable LDL cholesterol levels because of triglyceride values >400 mg/dl). Smoking status was based on self-report. eGFR was calculated using the CKD-Epidemiology Collaboration equation (27) with calibrated and standardized serum creatinine (28).

Statistical Analyses

Baseline characteristics of participants by ancestry-*APOL1* risk status (European American, African-American *APOL1* 0/1 risk allele, and African-American *APOL1* 2 risk alleles) were compared using t tests for nonskewed continuous variables, Wilcoxon tests for skewed continuous variable, and chi-squared tests for categorical variables.

For the analysis of the association between hemostatic factors and ESRD, the hemostatic variables were log-transformed (because of right skewness) and standardized to interquartile range (IQR), such that risk estimates represent an increase in one IQR. We estimated unadjusted and adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) using Cox regression. The covariates in the adjusted model were determined *a priori* on the basis of a literature review of CKD progression factors (29,30), medication or hematologic factors that may influence hemostatic factor levels, and their availability in the ARIC study. The covariates included ancestry-center-*APOL1* risk status, sex, and year of hemostatic marker measurement, as well as baseline age, systolic BP, diabetes status, hypertension medication use, prevalent coronary heart disease, smoking status, log-transformed triglycerides, HDL cholesterol, log-transformed LDL cholesterol, eGFR, serum albumin, use of salicylates, hematocrit, log-transformed white blood cell count, and log-transformed platelet count.

We assessed the proportional hazard assumption by testing the time interaction term of the hemostatic factors for ESRD. The time interaction terms of factor VIIIc, fibrinogen, and vWF were significant, demonstrating slightly weaker association

over time ($P<0.05$). Therefore, for these three hemostatic factors, Cox regression evaluated their average association during the follow-up period. We assessed the assumption of linearity for the continuous association of hemostatic factors with ESRD in two ways: first by adding a square term of the log-transformed hemostatic factor in the unadjusted and adjusted analysis, and second using piecewise linear splines with knots at each tertile of the hemostatic factor. Neither the square terms nor the slope differences between splines were significant ($P>0.05$). Therefore, we retained the model using the log-transformed hemostatic factors. For each ancestry-*APOL1* risk group, we estimated the risk for ESRD per one IQR of hemostatic factor levels using an interaction term between log-transformed hemostatic factor levels and ancestry-*APOL1* risk status.

For the two hemostatic factors (factor VIIIc and protein C) found to have significantly stronger association in African Americans with two *APOL1* risk alleles, we evaluated their association with mortality. The square terms of the log-transformed hemostatic factors were significant, indicating violation of the linear assumption. Therefore, we used linear splines with knots at each quintile for this analysis. Baseline characteristics were analyzed using R software. Other analyses were conducted using Stata/SE 13.1 (Stata Corp., College Station, TX).

Results

Study Population Characteristics

Over a median follow-up of 20.5 years, 232 ESRD events occurred in 13,337 individuals. Characteristics of the study populations are presented by European American, African-American *APOL1* 0/1 risk allele group, and *APOL1* 2 risk alleles group in Table 1. Compared with European Americans, both African-American *APOL1* groups had a higher prevalence of diabetes (7.9% in European Americans, 15.7% in the African-American *APOL1* 0/1 risk allele group, and 15.3% in the *APOL1* 2 risk alleles group) and higher proportions of individuals receiving hypertension medication (24.2% in European Americans, 41.9% in the African-American *APOL1* 0/1 risk allele group, and 43.0% in the *APOL1* 2 risk alleles group). Overall, the baseline characteristics of the two African-American *APOL1* groups were similar, except for factor VIIc levels (115% in the *APOL1* 0/1 risk allele group versus 110% in the *APOL1* 2 risk alleles group; $P=0.05$). The correlation between hemostatic factors varied from 0.01 between

Table 1. Baseline characteristics of the participants by ancestry-*APOL1* status

Characteristic	European American	African-American *APOL1* 0/1 Risk Allele	African-American *APOL1* 2 Risk Alleles
Participants (*n*)	10,206	2733	398
Age (yr)	54.3±5.7	53.5±5.9[a]	53.0±5.8[b]
Female, % (*n*)	53.7 (5479)	61.9 (1692)[a]	60.1 (243)[c]
Smoking, % (*n*)			
Current smoker	24.2 (2470)	28.8 (787)[a]	32.66 (130)[b]
Former smoker	35.3 (3598)	24.2 (661)[a]	22.6 (90)[b]
Never smoker	40.5 (4138)	47.0 (1285)[a]	44.7 (178)
Prevalent coronary heart disease, % (*n*)	4.1 (418)	3.3 (89)[d]	2.8 (11)
Prevalent diabetes, % (*n*)	7.9 (801)	15.7 (430)[a]	15.3 (61)[b]
eGFR (ml/min per 1.73 m^2)	93.2±12.6	103.7±17.8[a]	103.5±18.1[b]
Systolic BP (mmHg)	118.3±16.9	128.0±20.8[a]	129.0±20.7[b]
Diastolic BP (mmHg)	71.5±10.0	79.4±12.0[a]	80.6±12.4[b]
Hypertension medication, % (*n*)	24.2 (2468)	41.9 (1144)[a]	43.0 (171)[b]
Total cholesterol (mg/dl)	212 (187, 238)	212 (184, 241)	207 (179, 239)[c]
HDL cholesterol (mg/dl)	48 (39, 60)	53 (43, 65)[a]	52 (44, 65)[b]
LDL cholesterol (mg/dl)	135 (112, 160)	135 (109, 163)	132 (102, 160)
Fasting triglycerides (mg/dl)	113 (81, 161)	93 (70, 128)[a]	88 (68, 127)[b]
Serum albumin (g/dl)	3.9±0.3	3.8±0.3[a]	3.8±0.3[b]
Hemostatic factors			
Factor VIIc (%)	116 (100, 135)	115 (98, 134)[d]	110 (96, 134)[c]
Factor VIIIc (%)	122 (102, 144)	139 (114, 169)[a]	144 (117, 171)[b]
Fibrinogen (mg/dl)	289 (256, 327)	308 (270, 352)[a]	313 (273, 365)[b]
von Willebrand factor (%)	105 (81, 134)	122 (92, 162)[a]	126 (91, 162)[b]
Protein C (mg/L)	3.18±0.6	3.14±0.6[d]	3.10±0.6[c]
Antithrombin III[e] (%)	108 (96, 122)	113 (99, 129)[a]	115 (98, 130)[b]

Between *APOL1* 0/1 risk allele and 2 risk alleles groups, only factor VIIc had *P* value <0.05.
[a]$P<0.001$ for European Americans versus *APOL1* 0/1 risk allele.
[b]$P<0.001$ for European Americans versus *APOL1* 2 risk alleles.
[c]$0.001\leq P<0.05$ for European Americans versus *APOL1* 2 risk alleles.
[d]$0.001\leq P<0.05$ for European Americans versus *APOL1* 0/1 risk allele.
[e]The sample sizes for antithrombin III were 10203 in European Americans, 2732 in the African-American *APOL1* 0/1 risk allele group, and 398 in the *APOL1* 2 risk alleles group.

vWF and antithrombin III to 0.73 between vWF and factor VIIIc (Supplemental Table 1).

Association between Hemostatic Factors and Incident ESRD

In unadjusted and adjusted analyses of the overall population, higher levels of five hemostatic factors (factor VIIc, factor VIIIc, fibrinogen, vWF, and protein C) were associated with higher risk of incident ESRD ($P<0.05$) (Table 2). In adjusted analyses of the overall population, higher levels of factor VIIc had the strongest association with incident ESRD (per one IQR higher: HR, 1.46; 95% CI, 1.21 to 1.76; $P<0.001$). Supplemental Figure 1, A–F, presents the Kaplan–Meier estimates of the proportion free of ESRD by tertiles of the hemostatic factors.

In the adjusted analysis by subgroup of ancestry-*APOL1* status, factor VIIc was associated with incident ESRD in all three ancestry-*APOL1* risk groups (per one IQR higher: European American, HR, 1.32 [95% CI, 1.02 to 1.71]; *APOL1* 0/1 risk allele, HR, 1.46 [95% CI, 1.12 to 1.89]; *APOL*1 2 risk alleles, HR, 1.97 [95% CI, 1.29 to 3.02]; for interaction between European Americans and *APOL1* 2 risk alleles, $P=0.11$; for interaction between *APOL1* 0/1 risk alleles and 2 risk alleles, $P=0.22$) (Table 3). In contrast, higher levels of factor VIIIc and protein C were associated with significantly higher risk for ESRD in those with two *APOL1* risk alleles (factor VIIIc: European American, HR, 1.07 [95% CI, 0.83 to 1.38]; *APOL1* 0/1 risk allele, HR, 1.19 [95% CI, 0.92 to 1.53]; *APOL*1 2 risk alleles, HR, 2.48 [95% CI, 1.39 to 4.41]; *P* for interaction <0.02; protein C: European American, HR, 0.99 [95% CI, 0.79 to 1.25]; *APOL1* 0/1 risk allele, HR, 1.31 [95% CI, 1.03 to 1.67]; *APOL*1 2 risk alleles, HR, 2.61 [95% CI, 1.43 to 4.76]; *P* for interaction <0.04). The Kaplan–Meier estimates of the proportion free of ESRD by tertile 3 versus tertiles 1 and 2 of factor VIIIc and protein C in each ancestry-*APOL1* risk group are presented in Supplemental Figure 2, A and B. Antithrombin III had no significant association with incident ESRD.

With respect to mortality, we observed a total of 4632 events (European Americans, 3290; African Americans: *APOL1 0/1* risk allele, 1178; *APOL1* 2 risk alleles, 164). Higher factor VIIIc activity was associated with higher mortality risk, with a steeper gradient above the median value (per IQR change in the lowest quintile, adjusted HR, 1.05 [95% CI: , 0.86 to 1.24]; per IQR change in the highest quintile, adjusted HR, 1.49 [95% CI, 1.33 to 1.67]) (Supplemental Figure 3). Protein C had a slight U-shaped association with mortality. In the first quintile of protein C levels, higher protein C levels were associated with lower risk for death (per IQR change, adjusted HR, 0.78; 95% CI, 0.70 to 0.87) (Supplemental Figure 4). In the highest quintile of protein C levels, higher protein C levels were associated with higher risk for death (per IQR change, adjusted HR, 1.15; 95% CI, 0.99 to 1.33). The interaction between hemostatic factor and ancestry-*APOL1* risk status for mortality was not statistically significant for either factor VIIIc or protein C ($P>0.1$).

Discussion

This study of 13,337 persons from a population-based cohort with >20 years of follow-up demonstrates that higher factor VII activity (factor VIIc), factor VIII activity (factor VIIIc), fibrinogen, vWF, and protein C were associated with higher risk of developing ESRD, independent of baseline kidney function and other traditional risk factors. We also demonstrate a significant interaction of factor VIIIc and protein C with *APOL1* risk status for incident ESRD, with stronger associations observed in African Americans with two *APOL1* risk alleles. This might suggest that hemostatic activation (or its triggers) acts synergistically with the *APOL1* risk alleles to increase susceptibility for kidney disease.

The present study expands on previous studies of hemostatic factors and kidney disease risk. In the Multi-Ethnic Study of Atherosclerosis, higher factor VIII activity was significantly associated with rapid decline in eGFR, and higher levels of fibrinogen had a weak, nonsignificant association in the same direction (5). In the Cardiovascular Health Study, higher factor VII levels were significantly associated with annual increase in serum creatinine, with stronger association in blacks than in nonblacks (3). A prior investigation in the ARIC study demonstrated that higher levels of factor VIIIc, fibrinogen, and vWF were significantly associated with increased risk of incident CKD in both European Americans and African Americans, while the association of factor VIIc was significant only in African Americans with the same direction of association in European Americans (2). Although differences exist in the hemostatic factors tested and the statistical significance of associations among studies, the qualitative associations

Table 2. Unadjusted and adjusted hazard ratios for ESRD in the overall population

Variable (per IQR Change[a])	Unadjusted		Adjusted	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Factor VIIc	1.85 (1.56 to 2.18)	<0.001	1.46 (1.21 to 1.76)	<0.001
Factor VIIIc	2.12 (1.81 to 2.49)	<0.001	1.20 (1.00 to 1.44)	0.02
Fibrinogen	1.93 (1.66 to 2.24)	<0.001	1.35 (1.15 to 1.60)	<0.001
von Willebrand factor	2.18 (1.81 to 2.62)	<0.001	1.29 (1.06 to 1.56)	0.01
Protein C	1.17 (1.01 to 1.36)	0.04	1.19 (1.00 to 1.42)	0.03
Antithrombin III	1.19 (1.00 to 1.41)	0.05	1.07 (0.90 to 1.27)	0.43

Adjusted analysis covariates included age, ancestry-center-*APOL1* risk status, sex, systolic BP, diabetes status, hypertension medication use, prevalent coronary heart disease, smoking status, log-transformed triglycerides, HDL cholesterol, log-transformed LDL cholesterol, year of marker measurement, baseline eGFR, use of salicylates, hematocrit, log-transformed platelet count, log-transformed white blood cell count, and serum albumin. IQR, interquartile range; HR, hazard ratio; 95% CI, 95% confidence interval.
[a]The HRs and 95% CIs were estimated using log-transformed hemostatic factor for one IQR.

Table 3. Adjusted hazard ratios for ESRD for each ancestry-*APOL1* risk group

Variable (per IQR Change[a])	Adjusted HR (95% CI)		
	European American	African-American *APOL1* 0/1 Risk Allele	African-American *APOL1* 2 Risk Alleles
Factor VIIc	1.32 (1.02 to 1.71)	1.46 (1.12 to 1.89)	1.97 (1.29 to 3.02)
Factor VIIIc	1.07 (0.83 to 1.38)	1.19 (0.92 to 1.53)	2.48 (1.39 to 4.41)[b]
Fibrinogen	1.40 (1.12 to 1.75)	1.35 (1.04 to 1.75)	1.28 (0.74 to 2.20)
von Willebrand factor	1.20 (0.91 to 1.57)	1.30 (0.97 to 1.74)	1.64 (0.92 to 2.94)
Protein C	0.99 (0.79 to 1.25)	1.31 (1.03 to 1.67)	2.61 (1.43 to 4.76)[c]
Antithrombin III	1.07 (0.84 to 1.36)	1.03 (078 to 1.34)	1.30 (0.72 to 2.32)

Adjusted analysis covariates included age, sex, systolic BP, diabetes status, hypertension medication use, prevalent coronary heart disease, smoking status, log-transformed triglycerides, HDL cholesterol, log-transformed LDL cholesterol, year of marker measurement, baseline eGFR, use of salicylates, hematocrit, log-transformed platelet count, log-transformed white blood cell count, and serum albumin. IQR, interquartile range; HR, hazard ratio; 95% CI, 95% confidence interval.

[a]The HRs and 95% CIs were estimated using log-transformed hemostatic factor for one IQR.

[b]For interaction between factor VIIIc and ancestry-*APOL1* status: between European Americans and *APOL1* 2 risk alleles, P=0.008; between the *APOL1* 0/1 risk allele and 2 risk alleles, P=0.02.

[c]For interaction between protein C and ancestry-*APOL1* status: between European Americans and *APOL1* 2 risk alleles, P=0.003; between *APOL1* 0/1 risk allele and 2 risk alleles, P=0.03.

between higher hemostatic factor levels and kidney function decline were consistent across studies. The present study evaluated ESRD, arguably the most clinically meaningful kidney outcome, and found significant associations with factor VIIc, factor VIIIc, fibrinogen, vWF, and protein C in a population-based cohort, with stronger associations of factor VIIIc and protein C in participants with two *APOL1* risk alleles.

Multiple factors that increase *APOL1*-related renal susceptibility have been proposed or reported (10). The interaction of factor VIIIc activity and protein C levels with *APOL1* high-risk status may suggest an additional synergistic mechanism of action. Although speculative, *APOL1* expression and higher levels of factor VIIIc and protein C might be linked through vascular injury or infection. Both factor VIII and protein C are activated in response to vascular injury (31,32), with factor VIII serving as a procoagulant and protein C serving as an anticoagulant (33). Vascular injury can trigger the production of inflammatory cytokines (34), which can induce the expression of *APOL1* (35). Increased expression of the *APOL1* G1 or G2 risk variants causes organ damage in transgenic mice (16) and reduces cell survival (14,15). On the other hand, a recent study of HIV-infected individuals found that total plasma apoL1 levels had little cross-sectional correlation with inflammation biomarker levels or CKD status (36). Additional research is needed to prospectively evaluate the link between circulating apoL1 levels and kidney function decline.

The association between higher levels of protein C and ESRD risk is somewhat surprising because previous studies have shown an association of higher levels of protein C with lower risk of venous thromboembolism, atrial fibrillation, and ischemic stroke (37–39). We found not only an association of protein C with ESRD but also a slight U-shaped association between protein C and mortality. Higher protein C levels have been associated with prevalent hypertension or diabetes (40–42). Some have hypothesized that a reactive anticoagulatory response to hemostatic activity could increase protein C levels (42). We did note positive correlations between the anticoagulant protein C and the other procoagulant hemostatic factors, particularly factor VIIc. In addition, protein C levels may not perfectly correlate with activated protein C levels, a more direct measure of anticoagulant activity. Indeed, patients with diabetes and those undergoing hemodialysis may exhibit normal or higher protein C levels but lower activated protein C levels or thrombomodulin-induced anticoagulant activity (43,44).

Strengths of this study include a large community-based cohort of both African Americans and European Americans, a long follow-up period spanning >20 years, and careful measurement of hemostatic markers not obtained routinely in clinical care. Although the results from the present study are robust and biologically plausible, a few limitations should be noted. First, although many known risk factors of ESRD and hematologic variables were available to include as covariates, measures of albuminuria, an important marker of kidney damage, were not available at baseline. We cannot exclude the possibility that the observed interactions between hemostatic factor and *APOL1* status may be driven by albuminuria. Regarding hemostasis, the available hemostatic factors in this study provide only a partial view of the hemostasis process. The association of the hemostatic factors may represent the role of other factors in the hemostasis system in kidney function decline. Inflammatory biomarkers were not available at baseline to evaluate the relation between hemostatic activation and inflammation. Hemostatic activation could be a consequence of inflammation (45). Finally, the hemostatic factors were measured in blood and not in kidney tissues. Our results provide insight but not direct evidence on the pathogenesis of ESRD.

In conclusion, this study shows that higher levels of factor VII activity, factor VIII activity, fibrinogen, vWF, and protein C are associated with the development of ESRD in a middle-aged European American and African-American cohort, with higher levels of factor VIII activity and protein C having significantly stronger associations in African Americans with two *APOL1* risk alleles. These results suggest hemostatic activation

or its triggers may work synergistically to increase *APOL1*-associated renal risk. Further investigation into the pathways of apoL1 expression and hemostatic activation may help to unravel the *APOL1*-associated susceptibility for ESRD.

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Disclosures

None.

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