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Prospective study of γ′ fibrinogen and incident venous thromboembolism: The Longitudinal Investigation of Thromboembolism Etiology (LITE)

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ABSTRACT

Introduction: Epidemiological studies generally have not found plasma total fibrinogen to be a risk factor for venous thromboembolism (VTE), but several have reported associations between variants in the fibrinogen gamma gene (*FGG*) and VTE. A case-control study in whites suggested plasma γ′ fibrinogen concentration may be associated inversely with VTE, but this was not replicated in African Americans.
Objective: To examine the prospective association between γ′ fibrinogen concentrations and occurrence of VTE.
Methods: We used the Longitudinal Investigation of Thromboembolism Etiology (LITE), involving two pooled population-based cohorts in the United States including 16,234 participants. The cohorts comprised white and African American men and women, aged 50 years and older at study onset in the early 1990s. We identified VTEs during follow-up and documented they met standardized diagnostic criteria.
Results: During two decades of follow-up, neither γ′ fibrinogen nor total fibrinogen nor their ratio was associated with VTE overall (n = 521 VTEs), in subgroups defined by race, or in other subgroups. In both race groups, the minor allele of *FGG* rs2066865 was associated with lower γ′ fibrinogen concentrations, but this allele was not associated with VTE.
Conclusions: A lower plasma concentration of γ′ fibrinogen in healthy adults does not appear to increase VTE risk.

1. Introduction

Epidemiological studies have implicated a number of circulating procoagulant factors in the etiology of venous thromboembolism (VTE), that is, deep vein thrombosis (DVT) and pulmonary embolism (PE) [1]. However, existing studies generally have not found the concentration of fibrinogen associated with VTE [1]. This conclusion contrasts with consistent evidence that strongly associates higher fibrinogen concentrations with increased atherothrombotic events [1], although Mendelian randomization studies suggest the atherothrombotic association may not be causal [2,3].

Fibrinogen contains two copies each of Aα, Bβ, and γ chains. The γ chain, produced by the fibrinogen gamma chain gene (*FGG*), has two isoforms, γA and γ′. γ′ fibrinogen comprises approximately 10% of total plasma fibrinogen in the plasma, but the proportion varies among individuals and rises during an acute phase response. In the presence of factor XIII, clots made from γ′ fibrinogen are more resistant to lysis than normal clots; therefore the proportion of γ′ fibrinogen was hypothesized to be a risk factor for arterial thrombosis [4]. The Framingham Study reported that plasma γ′ fibrinogen was associated positively with prevalent arterial cardiovascular disease (odds ratio = 1.5 for the highest versus lowest γ′ fibrinogen tertiles), independent of total fibrinogen [5].

Despite a possible positive association of γ′ fibrinogen with atherothrombotic events in Framingham and several other epidemiological studies [4], studies of VTE have mostly reported the opposite association. For example, the Leiden Thrombophilia Study reported that having a *FGG*-H2 haplotype, which was strongly related to lower circulating γ′ fibrinogen, doubled the risk of VTE. Correspondingly,

Abbreviations: ARIC, Atherosclerosis Risk in Communities; BMI, body mass index; CHS, Cardiovascular Health Study; DVT, deep vein thrombosis; ELISA, enzyme-linked immunosorbent assay; *FGG*, fibrinogen gamma chain gene; GWAS, genome-wide association studies; HR, hazard ratio; LITE, Longitudinal Investigation of Thromboembolism Etiology; PE, pulmonary embolism; SD, standard deviation; SNP, single nucleotide polymorphism; VTE, venous thromboembolism.

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plasma γ′ fibrinogen (and the ratio of γ′ fibrinogen to total fibrinogen) were associated negatively with VTE, while plasma total fibrinogen was associated positively with VTE [6]. Another study reported that the minor allele of the *FGG* single nucleotide polymorphism (SNP) rs1049636, which is associated with increased mean γ′ fibrinogen levels, is associated with decreased risk of VTE [7].

Supporting a potential etiological role for lower γ′ fibrinogen in increasing VTE risk, three [8–10] of five [8–12] genome-wide association studies (GWAS) and some candidate-gene studies [6,13–15] have linked SNPs in *FGG* to VTE risk in whites. Our GWAS consortium of VTE found the top *FGG* SNP to be rs6536024 [8], which is in modest linkage disequilibrium ($r^2 = 0.25–0.53$) with *FGG* variants linked to VTE in other studies, namely, three tightly linked SNPs (rs7659024, rs2066865, and rs2066854, $r^2 = 1.0$ among them) and rs1049636 [6,7,9,10,13–15]. The minor allele of rs2066865 seems key to the *FGG*-H2 haplotype [6,16].

We recently measured γ′ fibrinogen in the entire study population of the Longitudinal Investigation of Thromboembolism Etiology (LITE) in order to examine the associations of γ′ fibrinogen concentrations and total fibrinogen with VTE occurrence. Our hypothesis was that γ′ fibrinogen concentration would be associated with VTE incidence. In the Atherosclerosis Risk in Communities (ARIC) Study, we also examined the association of a top *FGG* genetic variant (rs2066865) with incidence of VTE. The novel aspects of our study are that it is the first prospective study of γ′ fibrinogen and VTE, as well as its large size, biracial sample, wide age range, and long follow-up.

2. Methods

2.1. Study population

The LITE study is a prospective study of VTE occurrence in 2 pooled, multi-center, longitudinal population-based cohort studies: the ARIC Study [17] and the Cardiovascular Health Study (CHS) [18]. We reported the LITE study design, methods, and VTE incidence rates in detail elsewhere [19,20]. In brief, 15,792 men and women aged 45 to 64 years enrolled in the ARIC study in 1987–1989, and had subsequent examinations in 1990–92, 1993–95, 1996–98, and 2011–13, along with annual telephone contact. In CHS, 5201 men and women aged ≥65 years enrolled in 1989–1990. In 1992–1993, CHS recruited 687 new African American participants. CHS contacted participants every six months for follow-up, alternating between a telephone interview and clinic visit for the first 10 years and by telephone interview only after that. The institutional review committees at each study center approved the methods and staff obtained informed participant consent.

2.2. Plasma total and γ′ fibrinogen measurements and FGG genotyping

ARIC and CHS had measured plasma total fibrinogen at participants′ baseline visits using the method of Clauss [21]. In addition, ARIC had remeasured total fibrinogen in a stratified sample of participants (n = 999) in 1993–95. Because total fibrinogen was not associated with VTE in an early LITE analysis [20], we did not remeasure total fibrinogen along with γ′ fibrinogen, and used the baseline value of total fibrinogen for this report.

By the time we undertook γ′ fibrinogen measurement in 2014, ARIC and CHS had exhausted most baseline citrate plasma samples. Therefore, we measured γ′ fibrinogen concentrations on fasting citrate plasma collected in ARIC in 1993–95 (6 years after baseline) and CHS in 1992–93 (3 years after baseline for the original cohort and at baseline for the African American supplemental cohort) and stored unthawed at −70 °C until analysis in 2014. The Laboratory for Clinical Biochemistry Research at the University of Vermont used the assay developed by Lovely et al. [22], made available by Gamma Therapeutics (Portland, OR). It is a standard sandwich enzyme-linked immunosorbent assay (ELISA) using anti-γ′ monoclonal antibody. The coefficient of variation for control samples averages 10.3%.

Because of the large number of samples, requiring 12 months of laboratory measurement for γ′ fibrinogen, and based on priorities, the laboratory first analyzed ARIC samples from the three study centers other than the Jackson, MS center, then the CHS samples, and finally the ARIC Jackson center. In addition to the laboratory's standard assay quality assurance procedures, we instituted two other quality checks on γ′ fibrinogen measurement. First, ARIC included blinded duplicate samples split at the time of blood draw to check on reliability. Second, the laboratory included a normal pool to check for long-term drift. The analysis of 75 split specimen pairs during the early ARIC phase yielded a coefficient of variation of 27% for the first specimen in each pair and an intra-class reliability coefficient of 0.59, and the measured mean on a normal pool showed a downward drift of 31% over the time the early assays were run. The laboratory attributed this drift to its learning curve with the γ′ fibrinogen assay and the pipette technique for it. For the later CHS and Jackson, MS center periods, the normal pool showed no significant drift and the reliability coefficient for Jackson samples was 0.79 (n = 99 pairs). We therefore used the normal pool results to adjust participants' γ′ fibrinogen concentrations from the earlier period to the later period, done by multiplying the observed lab values by the ratio of the mean γ′ fibrinogen levels in the normal pool during the later period to mean γ′ fibrinogen levels in the normal pool during the earlier period. Prior to adjustment, we excluded 61 values in ARIC and 10 in CHS that were above the limit of quantification (>400 mg/dL). Samples that were below the limit of detection (8 in ARIC and 2 in CHS) were set equal to the limit. The mean ± standard deviation (SD) γ′ fibrinogen level in ARIC was 35.8 ± 10.6 mg/dL before adjustment and 30.8 ± 9.0 mg/dL after.

The ARIC DNA Laboratory at the University of Texas-Houston genotyped rs2066865 with the iPLEX multiplex assay which utilizes the MassARRAY system (Sequenom, Inc., San Diego, CA).

2.3. Measurement of risk factors

We analyzed risk factors for VTE in LITE, measured at the ARIC or CHS visits in which γ′ fibrinogen was measured. We calculated body mass index as weight (kg)/height $(m)^2$. We defined diabetes as a fasting blood glucose of 126 mg/dl or higher, non-fasting blood glucose of 200 mg/dl or higher, a physician diagnosis of diabetes, or use of antidiabetic medication in the past 2 weeks. Participants reported smoking status, and women reported whether or not they were taking hormone replacement therapy.

2.4. VTE occurrence

Staff contacted ARIC and CHS participants annually or semi-annually by phone and asked about all hospitalizations in the previous year. They retrieved hospital records for possible VTE events through 2011 in ARIC and through 2001 in CHS. To validate VTE events, two physicians reviewed the records using standardized criteria [19], requiring positive imaging tests for diagnosis of DVT and PE. We restricted DVTs for this analysis to those in the lower extremity or vena cava, because upper extremity DVTs were relatively few and almost always the result of venous catheters. The reviewers sub-classified VTEs as unprovoked (no obvious cause) or provoked (associated with cancer, major trauma, surgery, marked immobility).

2.5. Statistical analysis

Of the 12,887 ARIC and 5265 CHS participants who attended the relevant exam, we excluded those not white or African American (n = 38 ARIC, 34 CHS), those with a VTE prior to γ′ fibrinogen assessment (n = 302 ARIC, 323 CHS), those taking anticoagulants (n = 124 ARIC, 107 CHS), those without γ′ fibrinogen measurement (n = 355 ARIC, 633

CHS), and those with no further follow-up (n = 2 ARIC). This left a maximum of 16,234 participants (12,066 in ARIC and 4168 in CHS) for the present analyses of new VTE. Time at risk was computed from the date of blood collection for biomarker measurement to the earliest of the following: date of hospital admission for incident VTE, date of death, date of last follow-up contact, or end of follow-up (Dec 31, 2011 for ARIC, Dec 31, 2001 for CHS).

Our main hypothesis was that γ′ fibrinogen concentration would be associated with VTE incidence. We computed crude incidence rates of VTE by tertiles of both γ′ fibrinogen and total fibrinogen in the entire sample. For most regression analyses, we categorized γ′ fibrinogen into quintiles, but we also analyzed it as a continuous variable. We ran analyses separately for ARIC and CHS and pooled them only after verifying there was no γ′ fibrinogen by study interaction. We used Cox proportional hazards models to estimate hazard ratios (HR) and 95% confidence intervals of incident VTE and tested for trend using an ordinal variable to represent the quintiles. We verified that the proportional hazards assumption of the Cox models held by testing an interaction of γ′ fibrinogen by log follow-up time. Model 1 estimated the association between γ′ fibrinogen and VTE adjusted for age (continuous), sex, race, study (ARIC, CHS), and smoking status (never, former, or current); Model 2 additionally adjusted for characteristics previously associated with VTE in this cohort – diabetes status (yes or no), and body mass index (continuous) – as well as for total fibrinogen (continuous). We fit separate Cox models to estimate stratum-specific effects of γ′ fibrinogen by sex and race. We also fit separate Cox models for VTE subgroups: unprovoked versus provoked VTE and PE versus DVT without PE. As two sensitivity analyses, we (a) repeated the Cox regression analyses substituting the unadjusted γ′ fibrinogen measurements for the adjusted ones and (b) repeated the analyses also adjusting for hormone replacement therapy (coded: women taking hormones, women not taking hormones, or men).

In ARIC, we compared race-specific mean γ′ fibrinogen and total fibrinogen concentrations across the three *FGG* rs2066865 genotypes (CC, CT, and TT) using analysis of variance. We also computed race-specific hazard ratios of VTE for the three genotypes using Cox models. For each participant, we coded the SNP as having 0, 1, or 2 risk alleles and used an additive genetic model. For African Americans, we adjusted the hazard ratios for 10 principal components of ancestry.

3. Results

In the 16,234 participants without a prior VTE, the mean ± standard deviation γ′ fibrinogen concentrations were 30.8 ± 9.0 mg/dL for ARIC, 37.2 ± 11.0 mg/dL for CHS, and 32.5 ± 10.0 mg/dL overall. The mean total fibrinogen levels were 299 ± 62 mg/dL in ARIC, 329 ± 68 mg/dL in CHS, and 306 ± 65 mg/dL overall. The ratios of γ′ fibrinogen to total fibrinogen were 0.10 in ARIC, 0.11 in CHS, and 0.11 overall. As shown in Table 1, participants in higher quintiles of γ′ fibrinogen were older and more often from CHS, more often female, smokers, and diabetic, less likely to use female hormones, and had higher mean body mass index (BMI) and total fibrinogen. The proportion of African Americans was greater across successive quintiles of γ′ fibrinogen in ARIC, as was also the case across quintiles of total fibrinogen (not shown). In CHS, the proportion of African Americans also was greater across quintiles of total fibrinogen (not shown), but was lower across successive quintiles of γ′ fibrinogen.

The Pearson correlation coefficient between γ′ fibrinogen and total fibrinogen in ARIC was r = 0.43, overall, using total fibrinogen available from baseline. This correlation was larger for the subsets of participants

Table 1
Participant characteristics [mean ± SD or %] in relation to quintiles of γ′ fibrinogen, LITE.

Characteristic[a]	Quintile of γ′ fibrinogen (mg/dL)				
	8.0–24.4	24.4–28.9	28.9–33.4	33.4–39.8	39.8–82.5
Pooled					
N	3280	3290	3263	3235	3166
Age, years	60.8 ± 7.2	62.2 ± 7.7	63.4 ± 8.2	64.7 ± 8.4	66.8 ± 8.8
African American	17%	20%	22%	24%	26%
Women	51%	54%	57%	59%	59%
Diabetes	13%	15%	18%	19%	25%
BMI, kg/m^2	27.3 ± 4.7	27.7 ± 5.1	28.1 ± 5.2	28.1 ± 5.4	28.7 ± 6.1
Current smoker	13%	15%	15%	17%	18%
Hormone use (women)	19%	16%	16%	14%	10%
Total fibrinogen, mg/dL	266 ± 51	286 ± 48	303 ± 53	321 ± 54	359 ± 75
Ratio of γ′ to total fibrinogen	0.08 ± 0.02	0.10 ± 0.02	0.11 ± 0.02	0.012 ± 0.02	0.14 ± 0.03
ARIC					
N	2930	2700	2483	2210	1743
Age, years	59.3 ± 5.6	59.7 ± 5.7	60.0 ± 5.7	60.3 ± 5.7	60.7 ± 5.7
African American	16%	21%	23%	28%	35%
Women	51%	53%	57%	59%	60%
Diabetes	11%	13%	15%	17%	23%
BMI, kg/m^2	27.5 ± 4.8	28.0 ± 5.2	28.5 ± 5.4	29.0 ± 5.6	30.1 ± 6.6
Current smoker	14%	16%	18%	21%	24%
Hormone use (women)	20%	18%	18%	17%	13%
Total fibrinogen, mg/dL	266 ± 52	286 ± 49	301 ± 56	318 ± 58	346 ± 77
Ratio of γ′ to total fibrinogen	0.08 ± 0.02	0.10 ± 0.02	0.11 ± 0.02	0.12 ± 0.02	0.14 ± 0.03
CHS					
N	350	590	780	1025	1423
Age, years	73.8 ± 5.3	73.6 ± 5.0	74.1 ± 5.2	74.1 ± 5.1	74.4 ± 5.3
African American	24%	20%	17%	14%	15%
Women	56%	58%	58%	59%	58%
Diabetes	28%	24%	25%	24%	27%
BMI, kg/m^2	26.1 ± 4.2	26.6 ± 4.7	26.6 ± 4.3	26.4 ± 4.4	27.0 ± 4.8
Current smoker	7%	11%	8%	9%	11%
Hormone use (women)	9%	9%	9%	8%	6%
Total fibrinogen, mg/dL	263 ± 48	287 ± 44	307 ± 43	327 ± 45	375 ± 75
Ratio of γ′ to total fibrinogen	0.08 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	0.11 ± 0.02	0.14 ± 0.03

[a] γ′ fibrinogen and participant characteristics are from 1993–1995 for ARIC and 1992–1993 for CHS. Total fibrinogen for the CHS supplemental African American cohort also was from 1992–1993. Total fibrinogen for the remainder of CHS was from 1989–1990 and for ARIC was from 1987–1989.

Table 2
Incidence rates and hazard ratios (HRs) of venous thromboembolism in relation to quintiles of γ′ fibrinogen, LITE.

Outcome group[a]	Quintile of γ′ fibrinogen (mg/dL)					p-Trend
	8.0–24.4	24.4–28.9	28.9–33.4	33.4–39.8	39.8–82.5	
Total VTE						
N of VTEs	130	123	121	115	115	
Incidence rate (per 10^3 py)	2.8	2.6	2.7	2.6	3.0	
[95% CI]	[2.3, 3.3]	[2.2, 3.2]	[2.2, 3.2]	[2.2, 3.2]	[2.5, 3.7]	
Model 1 HR	1	0.9	1.0	0.9	1.1	0.64
[95% CI]	–	[0.7, 1.2]	[0.7, 1.2]	[0.7, 1.2]	[0.8, 1.4]	
Model 2 HR[b]	1	1.0	0.9	0.9	1.0	0.84
[95% CI]	–	[0.7, 1.2]	[0.7, 1.2]	[0.7, 1.2]	[0.7, 1.3]	
ARIC						
N of VTEs	125	115	101	92	88	
Model 1 HR	1	1.0	0.9	0.9	1.1	0.97
[95% CI]	–	[0.7, 1.2]	[0.7, 1.2]	[0.7, 1.1]	[0.8, 1.4]	
CHS						
N of VTEs	5	8	20	23	27	
Model 1 HR	1	0.9	1.6	1.4	1.4	0.42
[95% CI]	–	[0.3, 2.9]	[0.6, 4.2]	[0.5, 3.7]	[0.5, 3.5]	
Men						
N of VTEs	63	66	48	45	48	
Model 1 HR	1	1.1	0.9	0.9	1.2	0.93
[95% CI]	–	[0.8, 1.6]	[0.6, 1.3]	[0.6, 1.3]	[0.8, 1.7]	
Women						
N of VTEs	67	57	73	70	67	
Model 1 HR	1	0.8	1.0	1.0	1.1	0.51
[95% CI]	–	[0.6, 1.2]	[0.7, 1.4]	[0.7, 1.3]	[0.7, 1.5]	
Whites						
N of VTEs	95	90	91	82	56	
Model 1 HR	1	1.0	1.1	1.0	0.9	0.58
[95% CI]	–	[0.8, 1.4]	[0.8, 1.4]	[0.8, 1.4]	[0.6, 1.2]	
African Americans						
N of VTEs	35	33	30	33	59	
Model 1 HR	1	0.8	0.7	0.7	1.4	0.14
[95% CI]	–	[0.5, 1.3]	[0.4, 1.1]	[0.4, 1.1]	[0.9, 2.1]	
Unprovoked VTE						
N of VTEs	48	45	44	43	49	
Model 1 HR	1	1.0	1.0	1.0	1.3	0.24
[95% CI]	–	[0.6, 1.4]	[0.6, 1.4]	[0.6, 1.5]	[0.9, 2.0]	
Provoked VTEs						
N of VTEs	82	78	77	72	66	
Model 1 HR	1	0.9	0.9	0.9	1.0	0.76
[95% CI]	–	[0.7, 1.3]	[0.7, 1.3]	[0.7, 1.3]	[0.7, 1.3]	
Pulmonary Embolus (PE)						
N of PEs	71	65	55	56	51	
Model 1 HR	1	0.9	0.8	0.9	1.0	0.64
[95% CI]	–	[0.7, 1.3]	[0.6, 1.2]	[0.6, 1.2]	[0.7, 1.4]	
Deep vein thrombosis, no PE						
N of DVTs	59	58	66	59	64	
Model 1 HR	1	1.0	1.1	1.0	1.2	0.26
[95% CI]	–	[0.7, 1.4]	[0.8, 1.6]	[0.7, 1.5]	[0.9, 1.8]	

[a] Model 1: adjusted for age, race, sex, smoking status and study (ARIC, CHS), except where stratified by one of these variables.
[b] Model 2: adjusted for age, race, sex, study, diabetes, BMI, smoking status and total fibrinogen.

in whom we had γ′ fibrinogen and total fibrinogen from the same blood draw: CHS supplemental African American cohort (r = 0.64, n = 693), and ARIC subsample remeasured in 1993–95 (r = 0.63, n = 952).

Over a median of 17 years (maximum, 19 years) of follow-up in ARIC, we identified 521 VTEs. In CHS, we identified 83 VTEs over a median of 9 years (maximum, 10 years) of follow-up. We pooled ARIC and CHS for the main analyses, because interaction tests of study (ARIC, CHS) by γ′ fibrinogen (quintiles or continuous) in relation to VTE were not significant (p > 0.25). As shown in Table 2, the age, race, sex, and study adjusted hazard ratio of VTE was close to 1.0 for all quintiles of plasma γ′ fibrinogen, suggesting no association. This was true for various subgroups — ARIC and CHS, whites and African Americans, and men and women (all interactions not significant). After further adjustment for diabetes, BMI, smoking status, and total fibrinogen, γ′ fibrinogen remained unassociated with VTE incidence. In the sensitivity analysis that substituted the unadjusted γ′ fibrinogen for the adjusted γ′ fibrinogen (see Methods), there still was no association with VTE (data not shown). Likewise, further adjustment for hormone replacement therapy had no impact on the hazard ratios; for example, the p-trend for model 2 for total VTE in Table 2 went from 0.84 to 0.85.

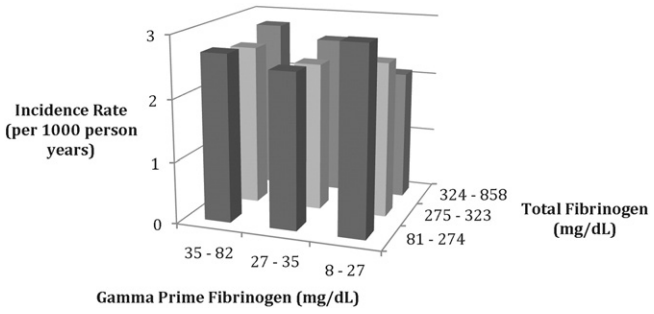

Fig. 1. Incidence rate of venous thromboembolism according to joint tertiles of γ′ fibrinogen and total fibrinogen, LITE.

Table 3
Race-specific unadjusted mean concentration γ′ fibrinogen in relation to *FGG* rs2066865 genotype, LITE.

	FGG genotype			p for difference[a]	Variance explained by genotype (r^2)
	CC	CT	TT		
African Americans					
N	1259	1096	236		
Mean ± SD γ′ fibrinogen, mg/dL	36.5 ± 9.3	31.0 ± 8.5	24.7 ± 6.8	<0.0001	0.14
N	1259	1096	236		
Mean ± SD total fibrinogen, mg/dL	318 ± 68	311 ± 68	299 ± 62	0.0009	0.007
Whites					
N	4913	3156	482		
Mean ± SD γ′ fibrinogen, mg/dL	32.8 ± 8.6	27.2 ± 7.2	21.8 ± 5.7	<0.0001	0.14
N	4913	3156	482		
Mean ± SD total fibrinogen, mg/dL	298 ± 61	291 ± 58	286 ± 55	<0.0001	0.004

[a] Analysis of variance.

Total fibrinogen was also not associated with VTE occurrence, whether or not γ′ fibrinogen was in the model. Using Model 2 adjustments, the hazard ratio of VTE per standard deviation (65 mg/dL) increment of fibrinogen was 0.93 (95% CI 0.84, 1.02). Fig. 1 shows the lack of association between incident VTE and joint tertiles of γ′ fibrinogen and total fibrinogen. Supplemental Table 1 illustrates that VTE also was not associated with the ratio of γ′ fibrinogen to total fibrinogen.

The T allele of the *FGG* rs2066865 SNP, which has been associated with increased risk of VTE in whites [14], was associated with lower concentrations of γ′ fibrinogen in ARIC in both African Americans and whites (Table 3). The rs2066865 genotype, by itself, explained 14% of the variance of γ′ fibrinogen concentrations in a regression model. Carriers of the T allele also had lower total fibrinogen concentrations, but genotype explained ≤ 1% of the variance of total fibrinogen.

There was no significant association of the *FGG* rs2066865 SNP with VTE. In ARIC whites, the unadjusted hazard ratio per T allele was 1.1 (95% CI 0.9, 1.3), p = 0.34. In ARIC African Americans, this hazard ratio, adjusted for 10 principal components of ancestry, was 0.9 (95% CI 0.7, 1.2), p = 0.61.

4. Discussion

This prospective study involving two cohorts showed no association between plasma γ′ fibrinogen concentration and incidence of VTE overall or any VTE subgroup. There also was no association between total fibrinogen and VTE, as reported by most other epidemiologic studies [1], including an early LITE publication based on only 159 VTE events [20]. The ratio of γ′ fibrinogen to total fibrinogen also was not associated with VTE. Novel aspects of our study are its prospective design involving a large biracial sample with a wide age range and long follow-up.

We studied γ′ fibrinogen and VTE for several reasons. First, γ′ fibrinogen rises in the acute phase response [4], and higher levels strengthen clots by altering fibrin formation and structure and making clots resistant to fibrinolysis [4,23,24]. These properties may help explain why some studies have found higher γ′ fibrinogen associated with increased risk of atherothrombotic vascular disease [4,5]. On the other hand, the γ′ isoform binds less strongly than does the γA isoform to platelets, possibly reducing platelet activation [25]. Some evidence suggests γ′ fibrinogen is antithrombotic due to its ability to sequester thrombin [26]. Second, various *FGG* SNPs that are significantly associated with γ′ fibrinogen levels also are associated with VTE occurrence [6–15]. Third, the Leiden Thrombophilia Study had reported that, among whites, γ′ fibrinogen concentrations were lower and total fibrinogen higher in VTE patients, compared with controls without VTE [6]. Yet, another study of African Americans reported no association between plasma γ′ fibrinogen and VTE [7]. Our study documented no association between total or γ′ fibrinogen and VTE in either African Americans or whites. Differences between our ARIC findings and those of the Leiden Thrombophilia Study may relate to differences in study design, such as: ARIC's long follow-up; some VTE precipitants and confounders captured less well in ARIC; and blood taken before versus after VTE. Presumably, blood taken before VTE in cohort studies offers a stronger causal inference than does blood in which γ′ fibrinogen may have changed after VTE.

Like the Leiden Study [6], we found that carriers of the T allele of *FGG* rs2066865 had modestly lower γ′ fibrinogen levels, but we found *FGG* genotype not significantly related to VTE risk in ARIC, although our statistical power was limited as the association between *FGG* rs2066865 and VTE was statistically significant in a recent, large VTE GWAS that included LITE [10]. Our findings suggest that the association of *FGG* variants with VTE likely operates through mechanisms other than influencing plasma levels of γ′ or total fibrinogen, perhaps such as influencing clot structure or interacting with other coagulation factors. rs2066865 is located within 500 bp downstream of the *FGG* gene. rs2066865 and two other highly linked *FGG* SNPs, rs2066864 and rs7659024 ($r^2 = 1$ with rs2066865), seem to have a role in altering regulatory motifs of other genes and influencing promoter and enhancer histone marks according to HaploReg v3 [27], an online functional annotation of non-coding sequences. Further investigations are needed to identify the mechanisms by which *FGG* variants influence VTE risk.

As described in the Methods, quality control data showed that our γ′ fibrinogen measurements for the ARIC whites had excessive imprecision and some downward drift, requiring us to adjust those γ′ fibrinogen values to be comparable to the stable and precise levels observed for ARIC African Americans and CHS. If our adjustment failed to fully correct the measurement error, there may be residual bias in the association of γ′ fibrinogen with VTE. Despite this, we believe our conclusions are likely valid, because there also was no association between γ′ fibrinogen and VTE for CHS alone (83% white) or for African American subgroups, whose γ′ fibrinogen measurements were satisfactory and required no adjustment.

Plasma γ′ fibrinogen is moderately correlated with plasma total fibrinogen, so we believed it to be important to consider them together when studying VTE. Except for CHS African Americans, we had measured total fibrinogen from a blood draw three to six years earlier than for γ′ fibrinogen. Although this is a weakness in our approach, it ultimately had little impact. Neither γ′ fibrinogen nor total fibrinogen nor their ratio was associated with VTE, making mutual adjustment or ratios superfluous.

Our large study had statistical power to ensure that we did not overlook clinically meaningful associations between γ′ fibrinogen and VTE. Yet, it had other potential limitations beyond those already mentioned. First, we measured γ′ fibrinogen on plasma samples that were stored at −70 °C approximately 20 years. Total fibrinogen is stable long-term at this temperature [28]. In addition, our pretests showed that γ′ fibrinogen was stable after several freeze–thaw cycles. Moreover, we found the ratio of γ′ fibrinogen to total fibrinogen to be approximately 10%, which is similar to the literature. Yet, any sample deterioration would have weakened the estimated association between γ′ fibrinogen and VTE. Second, a participant's γ′ fibrinogen concentration may have changed with age and other life circumstances during our long follow-

up. Third, high levels of γ′ fibrinogen or total fibrinogen may reflect an acute phase response. It therefore might have been desirable to exclude participants showing an acute phase response by another marker, such as elevated C-reactive protein. Unfortunately, ARIC had not measured C-reactive protein at visit 3. Fourth, although our sample size was large, for some informative subgroups, like unprovoked VTE, our statistical power to detect modest associations was limited. Fifth, we identified hospitalized VTE patients only. However, pilot data suggest that until recently the vast majority of patients with first VTEs in ARIC and CHS were hospitalized.

In conclusion, this cohort study documented that neither γ′ fibrinogen nor total fibrinogen nor their ratio was associated with incident VTE. Our results advance the field by providing novel prospective evidence that calls into question previous case-control findings of an inverse association between γ′ fibrinogen and VTE. At present, γ′ fibrinogen concentration should not be considered an important determinant of VTE.

Addendum

All authors contributed to critical revision of the manuscript and approved the final version.

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Conflict of interest statement

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.thromres.2016.01.008.

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