Association of Plasma γ′ Fibrinogen With Incident Cardiovascular Disease

The Atherosclerosis Risk in Communities (ARIC) Study

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Objectives—To prospectively examine the association of plasma γ′ fibrinogen with the incidence of multiple cardiovascular disease (CVD) end points, independent of established CVD risk factors, total fibrinogen, and other inflammatory markers.

Approach and Results—The Atherosclerosis Risk in Communities (ARIC) study measured γ′ fibrinogen by enzyme-linked immunosorbent assay in stored plasma samples from 1993 to 1995 and related levels in 10 601 adults to incident CVD end points (coronary heart disease [n=1603], ischemic stroke [n=548], peripheral artery disease [n=599], heart failure [n=1411], and CVD mortality [n=705]) through 2012 (median follow-up, 18 years). In Cox models accounting for established CVD risk factors and total fibrinogen levels, γ′ fibrinogen was associated positively with peripheral artery disease (hazard ratio [HR] per 1-SD [8.80 mg/dL] increment, 1.14 [1.04–1.24]), heart failure (HR, 1.06 [1.01–1.13]), and CVD deaths (HR, 1.12 [1.04–1.21]) but not with incident coronary heart disease (HR, 1.01 [0.96–1.07]) or ischemic stroke (HR, 0.98 [0.89–1.07]). Additional adjustment for C-reactive protein, however, eliminated the associations with peripheral artery disease and heart failure.

Conclusions—These findings do not lend support to the hypothesis that γ′ fibrinogen influences CVD events through its prothrombotic properties. Rather, γ′ fibrinogen concentrations seem to reflect general inflammation that accompanies and may contribute to atherosclerotic CVD, instead of γ′ fibrinogen being a causal risk factor. **(*Arterioscler Thromb Vasc Biol*. 2015;35:2700-2706. DOI: 10.1161/ATVBAHA.115.306284.)**

Key Words: cardiovascular ■ coronary disease diseases ■ epidemiology ■ fibrinogen ■ thrombosis

Plasma fibrinogen is a coagulation factor and an acute-phase inflammatory marker that has been implicated in the pathophysiology of cardiovascular disease (CVD).[1] Several epidemiological studies have shown an independent, positive association between elevated levels of fibrinogen and CVD, with hypothesized mechanisms relating to increased plasma viscosity or the size and strength of thrombi.[1–4] However, a study using Mendelian randomization suggests that the epidemiological association may not be causal.[5] Fibrinogen is also associated positively with several established CVD risk factors, so elevated fibrinogen may be one pathway by which these CVD risk factors exert their influence on the cardiovascular system.[4,6,7]

Fibrinogen is a 6-chain molecule containing 2 copies each of the Aα, Bβ, and γ chains, with the latter having 2 isoforms γA and γ′ arising from alternative mRNA processing.[8,9] γ′ fibrinogen constitutes ≈7% of plasma fibrinogen with higher levels found among individuals with pathological conditions..[9] Recent evidence suggests that higher plasma concentrations of γ′ fibrinogen yield thrombi that are resistant to fibrinolysis,[8,9] which provides novel hypotheses to explain the relationship between fibrinogen and CVD events.[10] Accordingly, some studies have observed a positive association between γ′ fibrinogen and atherothrombotic events independent of total plasma fibrinogen levels.[11–15] However, the retrospective or cross-sectional design of these studies that were conducted among individuals already diagnosed with CVD events hampers the determination of the temporality of this association, and a prospective design could reveal new perspectives on the association of γ′ fibrinogen with CVD events.

Therefore, the aim of this study was to prospectively investigate the association of plasma γ′ fibrinogen with the incidence of multiple CVD end points, independent of established CVD risk factors, total fibrinogen, and other inflammatory markers, among participants enrolled in the Atherosclerosis Risk in Communities (ARIC) study, a biracial cohort of white and black men and women.

Materials and Methods

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

Received on: July 24, 2015; final version accepted on: October 12, 2015.

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The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.115.306284/-/DC1.

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Nonstandard Abbreviations and Acronyms

ARIC	Atherosclerosis Risk in Communities
CHD	coronary heart disease
CI	confidence interval
CRP	C-reactive protein
CVD	cardiovascular diseases
HF	heart failure
HR	hazard ratio
PAD	peripheral artery disease

Results

Among the 10 601 participants free of CVD at ARIC visit 3, the mean age was 60 years, 57% were women, and 23% were blacks. Approximately one third of participants were on antihypertensive medications, 17% were current smokers, and 13% had diabetes mellitus. Median γ′ fibrinogen levels were modestly higher in women than in men (29.8 versus 28.6 mg/dL) and blacks than in whites (31.6 versus 28.7 mg/dL). The Spearman correlation coefficient between γ′ fibrinogen and total fibrinogen was 0.44. This correlation was larger for participants in whom γ′ fibrinogen and total fibrinogen were measured from the same blood drawn at ARIC visit 3 (r=0.60). The distribution of characteristics of participants stratified by quartiles of γ′ fibrinogen is presented in Table 1. The levels of γ′ fibrinogen showed positive associations with age, systolic blood pressure, body mass index, current smoking status, diabetes mellitus, total fibrinogen, and C-reactive protein (CRP), and negative associations with alcohol intake, sports-related physical activity, high-density lipoprotein cholesterol, and lipid-lowering medication use.

Kaplan–Meier cumulative incidence analysis showed higher incidence of all CVD outcomes with higher levels of crude γ′ fibrinogen quartiles (Figure 1). The incidence of CVD end points and their multivariable-adjusted associations with γ′ fibrinogen are shown in Table 2. In models adjusted for age, sex, race, and ARIC center (model 1), compared with participants in the lowest quartile of γ′ fibrinogen (8.0–24.34 mg/dL), those in the highest quartile (≥35.19 mg/dL) had elevated incidence rates of coronary heart disease (CHD; hazard ratio [HR], 1.40; 95% confidence interval [CI, 1.22–1.61), peripheral artery disease (PAD; HR, 1.87; 95% CI, 1.48–2.37), heart failure (HF; HR, 1.65; 95% CI, 1.42–1.92), and CVD mortality (HR, 1.96; 95% CI, 1.58–2.43) but not incident ischemic stroke (HR, 1.17; 95% CI, 0.93–1.48). These associations were attenuated but remained statistically significant when further adjustments were made for educational attainment and other established cardiovascular risk factors (model 2). Further adjustment for total fibrinogen (model 3) suggested that the positive association between γ′ fibrinogen and incident CHD was not independent of plasma concentrations of total

Table 1. Characteristics of Participants According to Quartiles of γ′ Fibrinogen, the Atherosclerosis Risk in Communities Study, 1993 to 1995

	γ′ Fibrinogen Quartiles, mg/dL			
Characteristics	Q1, 8.0–24.34	Q2, 24.35–29.26	Q3, 29.27–35.18	Q4, 35.19–80.28
n	2651	2648	2652	2650
Age, y	59.2 (5.6)	59.5 (5.6)	59.7 (5.7)	60.3 (5.7)
Women, %	52.4	55.2	58.9	62.3
Race, black, %	15.1	20.1	23.6	31.2
Education, %				
<high school	15.9	18.8	18.5	22.1
High school	42.7	42.2	41.6	41.4
>high school	41.4	39.0	39.9	36.5
Smoking status, %				
Former	43.5	42.2	38.8	35.0
Current	13.4	14.9	17.9	22.0
Alcohol intake, g/d	7.2 (16.0)	5.9 (14.3)	5.5 (16.8)	5.0 (15.5)
Systolic blood pressure, mm Hg	122.4 (17.4)	123.4 (18.6)	124.0 (19.2)	126.2 (19.9)
Hypertension meds, %	26.1	29.0	32.3	40.1
Diabetes mellitus, %	10.0	11.7	13.8	16.8
Body mass index, kg/m^2	27.3 (4.6)	27.9 (5.1)	28.5 (5.4)	29.5 (6.2)
Sports index	2.6 (0.8)	2.6 (0.8)	2.5 (0.8)	2.4 (0.8)
Total cholesterol, mg/dL	207.4 (38.0)	206.4 (36.4)	208.8 (36.0)	208.3 (39.1)
HDL cholesterol, mg/dL	54.1 (18.7)	53.6 (18.4)	53.2 (18.3)	51.6 (17.8)
Lipid-lowering meds, %	7.3	6.9	7.5	8.9
hs-CRP, mg/L	2.7 (4.9)	3.3 (5.1)	4.1 (6.6)	5.5 (8.6)
Total fibrinogen, mg/dL	264.9 (54.1)	285.4 (49.9)	302.5 (54.6)	330.6 (66.6)

Values are means (SDs) for continuous variables and percentages for categorical variables. HDL indicates high-density lipoprotein; and hs-CRP, high sensitive C-reactive protein.

fibrinogen (HR for the highest versus the lowest γ′ fibrinogen quartiles, 1.06; 95% CI, 0.91–1.24). In contrast, the elevated risk among participants in the upper quartile of γ′ fibrinogen compared with those in the lowest quartile persisted for the other CVD end points after accounting for total fibrinogen, with the risk most pronounced for CVD deaths (HR, 1.39; CI, 1.10–1.76), followed by PAD (HR, 1.36; CI, 1.05–1.75) and HF (HR, 1.21; CI, 1.02–1.42). Further adjustment for CRP concentrations (model 4) slightly attenuated the associations of γ′ fibrinogen with PAD (HR for the highest versus lowest quartiles, 1.26; CI, 0.96–1.66), HF (HR, 1.18; CI, 0.99–1.41) and CVD deaths (HR, 1.36; CI, 1.05–1.75). Similar patterns were observed when HRs were calculated using continuous values of γ′ fibrinogen.

Comparison of the associations of γ′ fibrinogen and total fibrinogen, adjusted for each other, with CVD end points is shown in Figure 2. With the exception of PAD, the HRs per 1-SD increment of total fibrinogen with each CVD end point were higher than those of γ′ fibrinogen. For all CVD end points, no significant interactions between γ′ fibrinogen and race, sex, CRP, and total fibrinogen were identified. For the end point that seemed most strongly associated with γ′ fibrinogen (CVD deaths), the risk was particularly elevated for participants who were in the highest tertiles of both γ′ fibrinogen and total fibrinogen (Figure 3). Finally, restricted cubic spline Cox regression analysis revealed that the relationships between γ′ fibrinogen and CVD end points were approximately linear (data not shown). In sensitivity analyses, we found no appreciable differences in the associations of corrected and uncorrected γ′ fibrinogen with CVD end points (Table I in the online-only Data Supplement). In a sensitivity analysis limiting follow-up to 5 years, we found that most associations were stronger, including a significant positive association between γ′ fibrinogen and incident CHD (Table II in the online-only Data Supplement). However, in an additional sensitivity analysis of the entire 20-year follow-up that excluded events that occurred in the first 3 years, we observed attenuation of the associations between γ′ fibrinogen and all CVD end points (Table III in the online-only Data Supplement).

Discussion

In this prospective observational cohort of middle-aged whites and blacks enrolled in the ARIC study, we found no independent association between plasma γ′ fibrinogen concentrations and the incidence of CHD and ischemic stroke. However, higher levels of γ′ fibrinogen were positively although modestly associated with PAD, HF, and CVD deaths, which seems to reflect a general contribution of inflammation to CVD, rather than a specific γ′ fibrinogen effect. Our findings suggest that γ′ fibrinogen is an inflammatory marker that adds little information to CVD prediction beyond total fibrinogen and high sensitivity CRP levels. To our knowledge, this is the first epidemiological prospective investigation of the association between γ′ fibrinogen and incident CVD events.

The underlying mechanism by which γ′ fibrinogen might affect cardiovascular health is debated. Previous experimental studies have suggested that γ′ fibrinogen promotes thrombosis by forming fibrin blood clots that have altered clot architecture that makes them mechanically stronger and highly resistant to fibrinolysis.[8,9,14,16] However, other studies

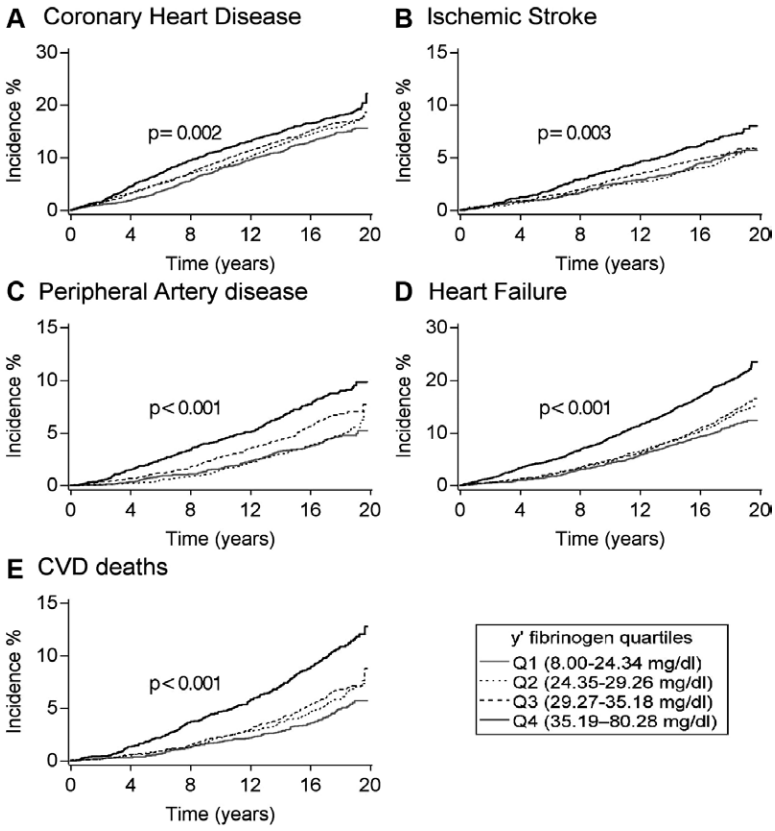

Figure 1. Kaplan–Meier cumulative incidence estimates of cardiovascular outcomes according to γ′ fibrinogen quartiles, the Atherosclerosis Risk in Communities study, 1993 to 2012. *P* values for the Log-rank tests represent differences among all groups (unadjusted).

Table 2. Hazard Ratios (95% Confidence Interval) of Incident Cardiovascular Outcomes in Relationship With Plasma γ′ Fibrinogen, the Atherosclerosis Risk in Communities Study, 1993 to 2012

	γ′ Fibrinogen Quartiles, mg/dL					Continuous
	Q1, 8.0–24.34	Q2, 24.35–29.26	Q3, 29.27–35.18	Q4, 35.19–80.28	P_{trend}	1-SD Increment*
CHD						
Events, n	359	393	407	444		1603
Incidence rate†	8.6 (7.8–9.6)	9.6 (8.7–10.6)	9.9 (9.0–10.9)	11.3 (10.3–12.4)		9.8 (9.4–10.3)
Model 1	1 (referent)	1.12 (0.97–1.30)	1.20 (1.04–1.38)	1.40 (1.22–1.61)	0.001	1.14 (1.08–1.19)
Model 2	1 (referent)	1.11 (0.96–1.28)	1.08 (0.93–1.25)	1.18 (1.02–1.37)	0.040	1.05 (1.00–1.11)
Model 3	1 (referent)	1.07 (0.93–1.24)	1.01 (0.87–1.17)	1.06 (0.91–1.24)	0.816	1.01 (0.96–1.07)
Model 4	1 (referent)	1.10 (0.94–1.28)	1.02 (0.88–1.20)	1.04 (0.89–1.23)	0.847	1.00 (0.94–1.06)
Ischemic stroke						
Events, n	127	120	132	169		548
Incidence rate†	2.9 (2.5–3.5)	2.8 (2.3–3.3)	3.1 (2.6–3.6)	4.1 (3.5–4.7)		3.2 (2.9–3.5)
Model 1	1 (referent)	0.90 (0.70–1.16)	0.95 (0.75–1.22)	1.17 (0.93–1.48)	0.049	1.11 (1.02–1.20)
Model 2	1 (referent)	0.89 (0.69–1.15)	0.86 (0.69–1.10)	0.96 (0.76–1.22)	0.884	1.00 (0.92–1.09)
Model 3	1 (referent)	0.86 (0.67–1.11)	0.84 (0.65–1.08)	0.89 (0.69–1.16)	0.397	0.98 (0.89–1.07)
Model 4	1 (referent)	0.90 (0.69–1.17)	0.82 (0.63–1.08)	0.87 (0.66–1.15)	0.352	0.97 (0.87–1.07)
PAD						
Events, n	111	117	160	211		599
Incidence rate†	2.5 (2.1–3.1)	2.7 (2.2–3.2)	3.7 (3.2–4.3)	5.1 (4.5–5.8)		3.5 (3.2–3.8)
Model 1	1 (referent)	1.02 (0.79–1.33)	1.40 (1.10–1.78)	1.87 (1.48–2.37)	0.001	1.30 (1.20–1.40)
Model 2	1 (referent)	0.97 (0.74–1.26)	1.20 (0.94–1.54)	1.43 (1.13–1.82)	0.001	1.16 (1.07–1.25)
Model 3	1 (referent)	0.95 (0.73–1.25)	1.17 (0.91–1.50)	1.36 (1.05–1.75)	0.042	1.14 (1.04–1.24)
Model 4	1 (referent)	1.00 (0.76–1.32)	1.16 (0.89–1.51)	1.26 (0.96–1.66)	0.050	1.09 (0.99–1.19)
Heart failure						
Events, n	272	317	336	486		1411
Incidence rate†	6.3 (5.6–7.1)	7.4 (6.7–8.3)	7.9 (7.1–8.8)	12.1 (11.0–13.2)		8.4 (7.9–8.8)
Model 1	1 (referent)	1.12 (0.95–1.32)	1.14 (0.97–1.34)	1.65 (1.42–1.92)	0.001	1.23 (1.17–1.30)
Model 2	1 (referent)	1.06 (0.90–1.25)	0.99 (0.84–1.16)	1.30 (1.11–1.52)	0.001	1.10 (1.04–1.15)
Model 3	1 (referent)	1.04 (0.88–1.23)	0.93 (0.79–1.11)	1.21 (1.02–1.42)	0.066	1.06 (1.01–1.13)
Model 4	1 (referent)	1.08 (0.91–1.28)	0.91 (0.76–1.09)	1.18 (0.99–1.41)	0.080	1.05 (0.99–1.12)
CVD deaths						
Events, n	123	151	166	265		705
Incidence rate†	2.7 (2.3–3.3)	3.4 (2.9–4.0)	3.7 (3.2–4.3)	6.2 (5.5–7.0)		4.0 (3.7–4.3)
Model 1	1 (referent)	1.19 (0.94–1.51)	1.27 (1.00–1.60)	1.96 (1.58–2.43)	0.001	1.30 (1.21–1.39)
Model 2	1 (referent)	1.16 (0.91–1.46)	1.17 (0.92–1.48)	1.66 (1.33–2.08)	0.001	1.19 (1.11–1.28)
Model 3	1 (referent)	1.10 (0.86–1.40)	1.04 (0.81–1.32)	1.39 (1.10–1.76)	0.014	1.12 (1.04–1.21)
Model 4	1 (referent)	1.12 (0.87–1.45)	1.00 (0.77–1.30)	1.36 (1.05–1.75)	0.015	1.10 (1.01–1.20)

Model 1: Cox proportional hazards model adjusted for age (continuous), sex, race (white and black), and ARIC center. Model 2: model 1 additionally adjusted for education (<high school, high school, and >high school), smoking (current, former, and never), alcohol intake (continuous), sports index (continuous), systolic blood pressure (continuous), body mass index (continuous), use of antihypertensive medications (yes and no), diabetes mellitus (yes and no), cholesterol medication (yes and no), high-density cholesterol (continuous), and total cholesterol (continuous). Model 3: model 2 additionally adjusted for total fibrinogen (continuous). Model 4: model 3 additionally adjusted for high sensitivity C-reactive protein (continuous). CHD indicates coronary heart disease; CVD, cardiovascular disease; and PAD, peripheral artery disease.

*1 SD=8.80 mg/dL.

†Unadjusted incidence rate per 1000 person-years with 95% confidence intervals.

suggest that γ′ fibrinogen is antithrombotic and exhibits anticoagulant properties because of its ability to sequester thrombin.[17] Recent studies observed that that γ′ fibrinogen has high-affinity binding sites for thrombin exosite II, which inhibits thrombin-mediated platelet activation and reduces fibrinopeptide B cleavage and factor VIII activation.[17–21] Accordingly, Walton et al[17] reported that γ′ fibrinogen did not promote acute arterial thrombosis in mice and revealed that the more dominant isoform of the fibrinogen γ chain, γA/γA, increased fibrin formation rates and shortened the time to

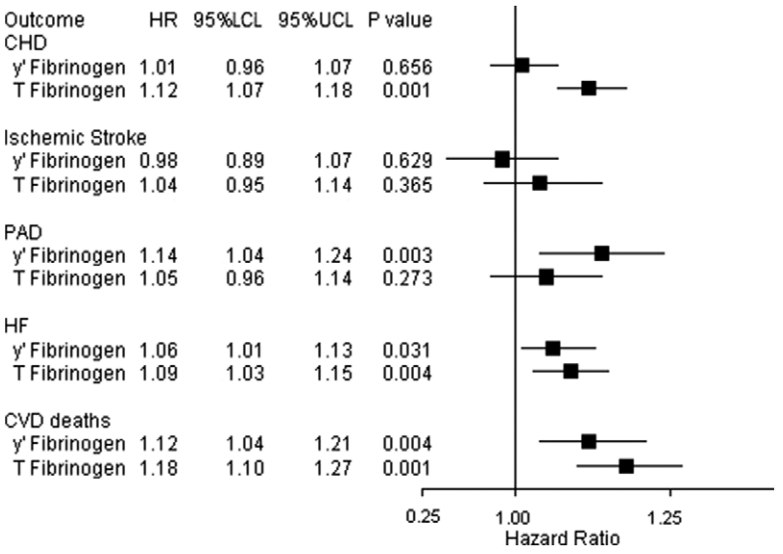

Figure 2. Hazard ratios (per 1 SD) and 95% confidence intervals of γ′ fibrinogen (8.80 mg/dL) and total fibrinogen (60.9 mg/dL) with Cardiovascular disease (CVD) end points, the Atherosclerosis Risk in Communities study, 1993 to 2012. HRs adjusted for age, sex, race, center, education, smoking, alcohol intake, sports index, systolic blood pressure, body mass index, antihypertensive meds use, high-density lipoprotein cholesterol, total cholesterol, lipid-lowering meds and diabetes. CHD indicates coronary heart disease; HF, heart failure; LCL, lower confidence limit; and UCL, upper confidence limit.

carotid artery occlusion, thereby promoting thrombosis to a greater extent than γA/γ′. Mosesson et al[18,22] also observed that γ′ fibrinogen is a constituent of a fibrin-dependent thrombin inhibitory system, and suggested that lower levels of γ′ fibrinogen may be associated with thrombotic events. These seemingly conflicting biochemical properties of γ′ fibrinogen make its role in the cause of CVD events unclear. In this study, we did not identify any association between γ′ fibrinogen and the 2 major arterial thrombotic events, CHD and ischemic stroke, after accounting for the effect of total fibrinogen concentrations. This suggests that γ′ fibrinogen does not influence major atherothrombotic diseases by means of unique prothrombotic properties.

Fibrinogen is an acute-phase reactant that increases in response to inflammation, and the inflammatory response has been reported to affect alternative splicing of the fibrinogen γ gene.[9,23] Levels of γ′ fibrinogen are associated positively with inflammatory markers.[9,23,24] Rein-Smith et al[24] reported that interleukin-6 preferentially upregulates hepatocyte production of γ′ fibrinogen, and CRP influences levels of γ′ fibrinogen.[13,25] Cheung et al[26] reported that CRP levels were positively correlated with the ratio of γ′ fibrinogen to total fibrinogen in the acute phase of ischemic stroke, providing further evidence that the mRNA processing of γ′ fibrinogen is altered in the presence of inflammation. We corroborated that higher levels of CRP are associated with higher γ′ fibrinogen. Because adjusting for the inflammatory makers, total fibrinogen and CRP, eliminated the associations of γ′ fibrinogen with CHD, ischemic stroke, PAD and HF, associations of γ′ fibrinogen seem to reflect a general contribution of inflammation to CVD, rather than a specific γ′ fibrinogen effect. In addition, the significant positive association of γ′ fibrinogen with the broad outcome of CVD deaths supports a nonspecific effect. It also suggests that the increased levels of γ′ fibrinogen found in individuals with CVD compared with controls in previous studies may rather be a consequence of CVD rather than a cause.[17]

It was interesting to note, in this study, that when we limited follow-up to 5 years, we found a significant positive association between γ′ fibrinogen and incident CHD. These short-term (5 year) associations especially for CHD may have been because of a reverse causal association, that is, subclinical disease elevating γ′ fibrinogen levels that resulted in participants with subclinical disease having elevated CHD risk. Alternatively, the stronger short-term associations could also mean that over time, a single value of γ′ fibrinogen becomes a less accurate representation of an individual's risk.

Our results contrast to previous epidemiological studies. Lovely et al[12] reported, cross-sectionally, 7-fold higher odds of coronary artery disease for the highest versus the lowest quartile of γ′ fibrinogen, adjusting only for age and sex, in a small case–control study of 133 patients undergoing elective, outpatient diagnostic cardiac catheterization. Another case–control study[13] comprised of 387 postmyocardial infarction patients and 387 healthy individuals from the Stockholm Coronary Artery Risk study reported a statistically significant 24% higher odds of myocardial infarction per 1-SD increment in γ′ fibrinogen after adjusting for traditional CVD risk

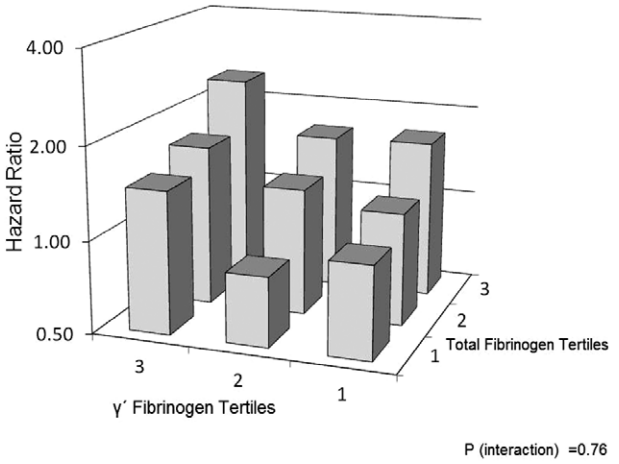

Figure 3. The joint associations of γ′ fibrinogen and total fibrinogen with cardiovascular disease deaths, Atherosclerosis Risk in Communities, 1993 to 2012. Hazard ratios adjusted for age, sex, race, center, education, smoking, alcohol intake, sports index, systolic blood pressure, body mass index, antihypertensive meds use, high-density lipoprotein cholesterol, total cholesterol, lipid-lowering meds, and diabetes mellitus.

factors and total fibrinogen. Similarly, cross-sectional results from the Framingham Heart Study Offspring Cohort[14] showed a statistically significant 76% higher odds of prevalent myocardial infarction among participants in the highest tertile of γ′ fibrinogen compared with the lowest tertile after adjusting for established CVD risk factors. Data on the relationship between γ′ fibrinogen and ischemic stroke are sparse and conflicting. van den Herik et al[27] observed that individuals with ischemic stroke had elevated levels of γ′ fibrinogen than age- and sex-matched stroke-free controls, and each unit increase in γ′ fibrinogen was associated with 48% higher odds for unfavorable stroke outcome. Cheung et al[15] identified elevated levels of γ′ fibrinogen in the acute phase of ischemic stroke that reduced to prestroke levels after 3 months. However, the Framingham Heart Study Offspring Cohort[14] found no association between γ′ fibrinogen and prevalent stroke (odds ratio, 1.42; CI, 0.68–2.95).

Possible explanations for these disparate results may be related to their study designs and inadequate control of the influence of total fibrinogen and other inflammatory markers. For instance, in the Framingham Heart Study Offspring Cohort,[14] γ′ fibrinogen was positively associated with prevalent CVD in models adjusted for established CVD risk factors, but additional control for total fibrinogen levels rendered the association nonsignificant. Furthermore, the cross-sectional or retrospective designs used by previous studies have greater likelihood of selection bias, as people who died of disease before the inception of such studies may have had different γ′ fibrinogen concentrations compared with survivors who were enrolled. Moreover, cross-sectional/retrospective studies lack the ability to determine whether elevated γ′ fibrinogen preceded or followed the CVD event, as it was measured among cases who already had CVD.

This study has several notable strengths, including the use of a large population-based biracial sample, extensive assessment of CVD risk factors, physician-adjudicated CHD, and stroke events using standardized criteria, and 20 years of follow-up with low attrition. In addition, validated procedures to measure γ′ fibrinogen levels were used.

Limitations of this study should be considered when interpreting our results. First, analyses were based on a single measure of γ′ fibrinogen. Second, our quality control data showed that our γ′ fibrinogen measurements for whites had some downward drift, requiring us to adjust those γ′ fibrinogen values to be comparable with the stable and precise levels observed for blacks. Our corrections in γ′ fibrinogen were an attempt to adjust for the drift seen in the normal data. Sensitivity analyses revealed that corrected and uncorrected results were similar. Because the ARIC study had only a single measure of γ′ fibrinogen on participants and there is no published long-term reliability coefficient in the literature, we could not address γ′ fibrinogen variation over time. Any misclassification of γ′ fibrinogen levels is likely to have been nondifferential with respect to our outcomes. Such errors would have biased our effect estimates toward the null (regression dilution), in expectation, and is one possible explanation for our findings. Third, we measured total fibrinogen and CRP from blood samples obtained 6 and 3 years, respectively, before the assessment of γ′ fibrinogen, and these may not represent actual levels at visit 3. Because γ′ fibrinogen is moderately correlated with total fibrinogen and CRP, we deemed it necessarily to adjust for these inflammatory markers in our models to enhance our understanding of any potential underlying mechanisms for the association of γ′ fibrinogen with CVD end points. Fourth, the statistically nonsignificant findings of CHD and ischemic stroke may be because of inadequate power to detect a small effect. However, our study had high power (>0.8) for HRs of 1.2 for CHD and 1.4 for stroke. Finally, some CVD end points (eg, HF, PAD, and CVD mortality) relied on International Classification of Disease codes. However, in ARIC, a high validity for International Classification of Disease codes in identifying these CVD end points has been demonstrated.[28]

In summary, γ′ fibrinogen was associated positively with PAD, HF, and CVD deaths but not independently with incident CHD and ischemic stroke, after accounting for total fibrinogen levels. With the exception of CVD deaths, these associations were attenuated to marginal statistical significance when high sensitivity CRP was added to the model. Our findings are consistent with γ′ fibrinogen concentration reflecting the inflammation that accompanies and may contribute to atherosclerotic CVD, rather than γ′ fibrinogen being a risk factor for CVD events.

Acknowledgments

We thank the staff and participants of the Atherosclerosis Risk in Communities (ARIC) study for their important contributions and Elaine Cornell for supervising γ′ fibrinogen measurements.

Sources of Funding

The Atherosclerosis Risk in Communities (ARIC) study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C). Dr Appiah was supported by National Heart, Lung, and Blood Institute training grant T32HL007779.

Disclosures

None.

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Significance

Cross-sectional and retrospective investigations have suggested that γ′ fibrinogen, a fibrinogen γ chain variant generated via alternative mRNA processing, is positively associated with atherothrombotic events. However, results from the Atherosclerosis Risk in Communities (ARIC) study, the first prospective study to assess this association, do not lend support to the hypothesis that γ′ fibrinogen influences cardiovascular disease events through its prothrombotic properties. Rather, γ′ fibrinogen concentrations seem to reflect general inflammation that accompanies and may contribute to atherosclerotic cardiovascular disease, instead of γ′ fibrinogen being a causal risk factor.