Published in final edited form as:

Thromb Res. 2016 July ; 143: 50–52. doi:10.1016/j.thromres.2016.04.023.

Lack of association of plasma gamma prime (γ') fibrinogen with incident cardiovascular disease

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Abstract

Introduction—The association of gamma prime (γ') fibrinogen; a fibrinogen γ chain variant generated via alternative mRNA processing, with cardiovascular disease (CVD) remains equivocal. We prospectively examine the association of plasma γ' fibrinogen with the incidence of multiple cardiovascular disease (CVD) endpoints, independent of established CVD risk factors and total fibrinogen.

Materials and Methods—We measured plasma γ' fibrinogen on plasma samples collected in 1992–1993 from adults ≥ 65 years ($n=3219$) enrolled in the Cardiovascular Health Study, who were followed through 2013 for incident CVD events.

Results and Conclusions—In multivariable Cox models adjusted for traditional CVD risk factors and total fibrinogen, the hazard ratio per 1 standard deviation (10.7 mg/dl) increment of γ' fibrinogen was 1.02 (95%CI: 0.95–1.10) for coronary heart disease; 0.88 (0.77–1.00) for ischemic stroke; 1.07 (0.87–1.32) for peripheral artery disease; 1.00 (0.92–1.08) for heart failure and 1.01 (0.92–1.10) for CVD mortality. Likewise, we failed to show a statistically significant association of γ' /total fibrinogen ratio with any CVD endpoint. These results suggest that among the elderly, γ' fibrinogen does not add much to CVD prediction beyond traditional risk factors and total fibrinogen level.

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Conflict of interest
None

Keywords

Cardiovascular Diseases; Epidemiology; Fibrinogen; Hemostasis; Thrombosis

Introduction

Gamma prime (γ') fibrinogen, a fibrinogen γ chain variant generated via alternative mRNA processing, constitutes approximately 7% of plasma fibrinogen and is elevated among individuals with certain pathological conditions (1). By forming fibrin blood clots that are highly resistant to fibrinolysis, γ' fibrinogen has been hypothesized to promote thrombosis (1-3). Cross-sectional and retrospective investigations suggest that γ' fibrinogen level is positively associated with atherothrombotic events (3-5), although the underlying mechanisms are debated (6). A recent prospective study in middle-aged adults (7) failed to find a positive association of plasma γ' fibrinogen concentration with the incidence of several cardiovascular disease (CVD) endpoints. This observation casts doubts on the hypothesis that γ' fibrinogen importantly influences CVD occurrence, but additional studies are needed. We investigated the association of γ' fibrinogen with incidence of coronary heart disease (CHD), ischemic stroke, peripheral artery disease (PAD), heart failure (HF) and CVD death in the Cardiovascular Health Study (CHS), a prospective study of older adults.

Materials and Methods

The methodology and design of CHS have been previously described (8). Briefly, the CHS is a population-based biracial cohort of 5888 white and black men and women aged ≥ 65 years who were recruited from Medicare eligibility lists in four US communities beginning in 1989. At each of the 9 annual follow-up clinic visits, standardized protocols were used to collect information on demographics, anthropometrics, lifestyle and behavioral factors, medical history, biomarkers, and medication use from participants. We measured γ' fibrinogen by enzyme-linked immunosorbent assay developed by Lovely et al (2) in stored fasting citrated plasma collected in 1992-1993. The coefficient of variation for control materials averaged 10.3%. Follow-up for clinical events occurred every 6 months. Incident CVD events (CHD, ischemic stroke, PAD, CHF and CVD deaths) were adjudicated by the CHS Cardiac Events Committee, using standardized criteria (9, 10). Multivariable Cox proportional hazard models were used to assess the relation of γ' fibrinogen with these CVD endpoints. Person-time accrued from 1992-1993 until CVD event, loss-to follow-up, death, or June 30, 2013, whichever came first.

Results

Of the 3219 participants (mean age 74.4 years) without CVD at 1992-1993, who were not on anticoagulant therapy, 16.3% were blacks and 63.1% were women. Approximately 41% were on antihypertensive medications, 9.8% were current smokers, and 12.9% had diabetes. γ' fibrinogen was moderately correlated with total fibrinogen ($r = 0.59$). Levels of γ' fibrinogen were higher in blacks, showed positive associations with age, total cholesterol, C-reactive protein (CRP) and interleukin-6 and negative associations with HDL cholesterol, systolic blood pressure, alcohol intake and leisure-time physical activity. Levels of γ' /total

fibrinogen were lower in blacks, diabetics, current smokers, and showed positive associations with age and years of education, as well as negative associations with BMI and CRP.

In multivariable Cox models adjusted for traditional CVD risk factors and total fibrinogen, we were unable to show a statistically significant association of γ' fibrinogen with the incidence of CHD, ischemic stroke, PAD, HF, or CVD death (Table 1).

Likewise, we failed to show a statistically significant association of γ' /total fibrinogen ratio (per 1 standard deviation of 0.028) with any CVD endpoints in unadjusted or models adjusted for CVD risk factors (CHD: Hazard ratio (HR) =1.03, 95%CI: 0.97-1.10; ischemic stroke: HR=0.92, CI:0.83-1.02; PAD:HR=1.04, CI:0.88-1.23; HF:HR=1.02, CI:0.96-1.09; CVD death: HR=1.00, CI: 0.93-1.07).

Discussion

In this large prospective cohort of adults 65 years who were followed up for approximately 21 years, we were unable to show a statistically significant association between plasma γ' fibrinogen concentrations and the incidence of any of four major types of CVD events. This result suggests that γ' fibrinogen does not add much to CVD prediction beyond traditional risk factors and total fibrinogen level.

Whether γ' fibrinogen promotes atherothrombosis continues to be a subject of controversy. γ' fibrinogen has been hypothesized to form fibrin blood clots that have altered clot architecture which are mechanically stronger and resistant to fibrinolysis (1-3). Accordingly, some experimental studies have reported that γ' fibrinogen may increase the length of time for thrombin to remain active on the clot surface, thereby promoting thrombus formation (3). However, others have also reported that γ' fibrinogen exhibits antithrombotic properties. For example, γ' fibrinogen has been observed to be a constituent of a fibrin-dependent thrombin inhibitory system (6), and has a high affinity for binding sites for thrombin exosite II which limits thrombin-mediated platelet activation and reduces factor VIII activation (6, 11). These apparently contradicting mechanistic properties of γ' fibrinogen render its role in the etiology of CVD unclear.

On the one hand, our results contrast with several cross-sectional and retrospective studies (3, 5, 12, 13) that report that γ' fibrinogen is positively associated with CVD events, potentially by means of its prothrombotic properties. On the other hand, they corroborate our previous report from the Atherosclerosis Risk in Communities (ARIC) Study, the only previous prospective investigation of γ' fibrinogen and CVD. In ARIC, we found no association between γ' fibrinogen and the incidence of CVD events after accounting for traditional CVD risk factors, total fibrinogen and CRP concentrations. Levels of γ' fibrinogen are positively associated with inflammatory markers, and recent evidence has shown that the inflammatory response affects alternative splicing of the fibrinogen γ gene (1, 14). Therefore, higher concentrations of γ' fibrinogen may be a consequence of the inflammation associated with CVD rather than a cause of CVD. This observation may explain the disparate results between prospective and non-prospective studies as the latter

cannot determine whether elevated γ' fibrinogen preceded or followed CVD events. Other possible explanations for the disparate findings may relate to other differences in study design, or inadequate control of the influence of total fibrinogen and other inflammatory markers in previous studies.

Some potential limitations of this analysis include the use of a single measure of γ' fibrinogen in plasma samples that were stored for almost 20 years at -70°C and the absence of information on the long-term reliability coefficient for γ' fibrinogen, and the within person variation in γ' fibrinogen. The possibility of regression dilution bias from such variation could have contributed to the observed results.

In conclusion, we were unable to show a statistically significant association of plasma γ' fibrinogen with the incidence of CVD events in this large elderly cohort.

Acknowledgments

Funding sources:

This research was supported by contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grant U01HL080295 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. Dr. Appiah was supported by NHLBI training grant T32HL007779.

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Highlights

- The relation of gamma prime (γ') fibrinogen with cardiovascular disease (CVD) remains equivocal
- Prospective associations of γ' fibrinogen with CVD are limited
- We failed to show associations between γ' fibrinogen and CVD endpoints beyond CVD risk factors.

Table 1

Hazard ratios (95% CI) of incident cardiovascular outcomes in relation to plasma γ' fibrinogen, the CHS study, 1992-2013

	γ fibrinogen quartiles, mg/dl				P _{TREND}	Continuous
	Q1 (8.00 – 29.33)	Q2 (29.34 – 35.31)	Q3 (35.32- 42.44)	Q4 (42.45 – 82.53)		1-SD (10.7) increment
CHD						
Events, n	279	278	269	313		
Incidence rate ‡	30.2 (26.8-33.9)	29.6 (26.3-33.3)	29.0 (25.7-32.6)	37.1 (33.2-41.5)		
Model 1	1 (Referent)	0.96 (0.81-1.13)	0.93 (0.79-1.10)	1.18 (1.00-1.39)	0.036	1.06 (1.00-1.13)
Model 2	1 (Referent)	0.92 (0.77-1.09)	0.86 (0.73-1.02)	1.09 (0.92-1.29)	0.251	1.04 (0.98-1.10)
Model 3	1 (Referent)	0.90 (0.76-1.07)	0.84 (0.69-1.01)	1.03 (0.85-1.26)	0.614	1.02 (0.95-1.10)
Ischemic stroke						
Events, n	124	106	111	107		
Incidence rate ‡	12.4 (10.4-14.8)	10.4 (8.6-12.5)	11.1 (9.3-13.4)	11.1 (9.2-13.5)		
Model 1	1 (Referent)	0.80 (0.62-1.04)	0.85 (0.66-1.10)	0.85 (0.66-1.11)	0.349	0.91 (0.83-1.01)
Model 2	1 (Referent)	0.80 (0.61-1.07)	0.82 (0.63-1.07)	0.80 (0.60-1.03)	0.144	0.89 (0.80-0.98)
Model 3	1 (Referent)	0.80 (0.61-1.05)	0.84 (0.63-1.12)	0.82 (0.60-1.13)	0.341	0.88 (0.77-1.00)
PAD						
Events, n	19	36	37	47		
Incidence rate ‡	1.8 (1.2-2.8)	3.4 (2.5-4.7)	3.6 (2.6-4.9)	4.8 (3.6-6.4)		
Model 1	1 (Referent)	1.89 (1.08-3.29)	1.91 (1.10-3.32)	2.52 (1.47-4.31)	0.001	1.28 (1.10-1.48)
Model 2	1 (Referent)	1.76 (0.98-3.01)	1.75 (1.00-3.06)	2.32 (1.32-3.92)	0.004	1.26 (1.07-1.47)
Model 3	1 (Referent)	1.52 (0.87-2.69)	1.39 (0.78-2.50)	1.51 (0.80-2.84)	0.377	1.07 (0.87-1.31)
Heart failure						
Events, n	225	256	261	283		
Incidence rate ‡	23.2 (20.4-26.4)	26.2 (23.2-29.6)	27.1 (24.0-30.6)	31.6 (28.1-35.5)		
Model 1	1 (Referent)	1.08 (0.90-1.29)	1.13 (0.95-1.36)	1.31 (1.09-1.60)	0.002	1.10 (1.04-1.17)
Model 2	1 (Referent)	1.03 (0.86-1.23)	1.08 (0.90-1.30)	1.19 (1.00-1.43)	0.037	1.08 (1.01-1.15)
Model 3	1 (Referent)	0.97 (0.80-1.17)	0.96 (0.79-1.17)	0.99 (0.80-1.22)	0.953	1.00 (0.92-1.08)
CVD deaths						
Events, n	209	195	205	226		
Incidence rate ‡	19.7 (17.2-22.5)	18.1 (15.8-20.9)	19.3 (16.9-22.2)	22.5 (19.8-25.7)		
Model 1	1 (Referent)	0.85 (0.70-1.04)	0.93 (0.76-1.12)	1.06 (0.88-1.28)	0.273	1.07 (1.00-1.14)
Model 2	1 (Referent)	0.85 (0.70-1.04)	0.89 (0.73-1.08)	0.99 (0.82-1.20)	0.762	1.05 (0.98-1.12)
Model 3	1 (Referent)	0.81 (0.66-0.99)	0.80 (0.65-0.99)	0.85 (0.67-1.07)	0.311	1.00 (0.92-1.10)

Model 1: Cox proportional hazards model adjusted for age, sex, race, years of education and CHS center

Model 2: Model 1 plus smoking status, alcohol intake, physical activity, systolic blood pressure, BMI, antihypertensive medications use, diabetes, cholesterol medication use, HDL cholesterol and total cholesterol

Model 3: Model 2 plus total fibrinogen

$\ddagger$ Unadjusted incidence rate per 1,000 person-years with 95% confidence intervals.