Published in final edited form as:
Thromb Haemost. 2011 April ; 105(4): 605–609. doi:10.1160/TH10-09-0626.

Association Between γ′ Fibrinogen Levels and Inflammation

Kristine S. Alexander[1], **Theresa E. Madden**[2], and **David H. Farrell**[3]

[1]Department of Molecular and Medical Genetics, School of Medicine, Oregon Health & Science University, Portland, OR, USA

[2]Dr. Theresa E. Madden & Associates, PLLC, Olympia, WA, USA

[3]Division of Cardiovascular Medicine, Department of Medicine, School of Medicine, Oregon Health & Science University, Portland, OR, USA

Summary

The γ′ fibrinogen isoform produces clots that are stiffer and more resistant to breakdown than the more common fibrinogen isoform, γA. Increased levels of γ′ fibrinogen are associated with several forms of cardiovascular disease. The purpose of this cross-sectional study is to investigate the relationship between γ′ fibrinogen, an emerging risk factor for cardiovascular disease, and inflammatory markers in subjects with a chronic inflammatory state. The 284 subjects for this study came from the Periodontitis and Vascular Events study, and γ′ fibrinogen and total fibrinogen in plasma were measured by ELISA. Information on patient demographics and health status, as well as levels of C-reactive protein, an inflammatory marker, have previously been collected for this study. The mean (SE) γ′ fibrinogen level in the subjects was 0.622 (0.017) mg/ml. Levels of γ′ fibrinogen were correlated with C-reactive protein ($p = 0.006$), with a one unit increase in C-reactive protein associated with a 1.9% increase in γ′ fibrinogen, after adjustment for potential confounders. Total fibrinogen was not correlated with γ′ fibrinogen in these subjects. The number of dental sites with evidence of tissue inflammation was also significantly associated with γ′ fibrinogen levels. These results provide an important step in the evolution of γ′ fibrinogen not only as a general risk factor for cardiovascular disease, but as a potentially useful biomarker for assessing a patient's inflammatory state and associated cardiovascular disease risk.

Keywords

cardiovascular disease; γ′ fibrinogen; inflammation; periodontitis; risk factor

Introduction

Fibrinogen is a large (340 kD) protein with a vital role in hemostasis. It has a dimeric structure, with each half of the fibrinogen molecule made up of three polypeptide chains: Aα, Bβ and γ. During coagulation, thrombin converts fibrinogen to fibrin, which polymerizes and forms the protein meshwork of the growing blood clot. The fibrin clot is

Correspondence to: David H. Farrell, PhD, Division of Cardiovascular Medicine, Mail code MQ280, Department of Medicine, School of Medicine, Oregon Health & Science University, 3181 S.W. Sam Jackson Park Road, Portland, OR 97239-3098, USA, Tel.: +1 503 494 8602, Fax: +1 503 494 8550, farrelld@ohsu.edu.

stabilized through the action of FXIIIa, a transglutaminase that crosslinks adjacent fibrin molecules.

The γ′ isoform of fibrinogen is formed by the incorporation of a splice variant of the fibrinogen γ chain called the γ′ chain. This alternative splicing event results in the extended carboxyl terminus of the γ′ chain, which contains 20 residues in place of the 4 carboxylterminal residues on the more common γA chain (1, 2). The γ′ fibrinogen isoform makes up approximately 10% of circulating fibrinogen, although this value can vary widely between individuals (3-5).

The unique sequence of the γ′ chain contains a high affinity binding site for thrombin (6-10). Clots formed with γ′ fibrin are more resistant to fibrinolysis (11, 12) and have altered clot architecture compared with γA/γA fibrin clots (13, 14). Because of these characteristics, γ′ fibrinogen has been studied as a possible risk factor for thrombosis (15). Research by our laboratory and others have linked levels of γA/γ′ fibrinogen with cardiovascular disease (CVD), including coronary artery disease (CAD) (3), heart attack (4, 16), and stroke (17).

The pathogenesis of cardiovascular disease has a number of inflammatory components. While it is well established that total fibrinogen is elevated during inflammation, it is not known whether γ′ fibrinogen is similarly increased. A recent report shows elevated γ′ fibrinogen levels in the acute phase of ischemic stroke and pulmonary embolism relative to the nonacute phase (18). To date, however, it is not known how γ′ fibrinogen levels relate to the presence of chronic, low-level inflammation.

In this cross-sectional study, we examined γ′ fibrinogen levels in subjects with both periodontal and cardiovascular disease to gain a better understanding of the relationship between γ′ fibrinogen and inflammation. We hypothesized that elevated γ′ fibrinogen levels would be associated with higher levels of the inflammatory marker high-sensitivity C-reactive protein (hs-CRP), and would similarly correlate with the degree of periodontal disease-associated inflammation.

Methods and Materials

Study subjects

The 284 subjects in this study were from the Periodontitis And Vascular Events (PAVE) cohort. These subjects had both periodontal disease and history of recent cardiovascular disease. The human subjects research was approved by the relevant institutional review boards, and all participants gave written informed consent. The PAVE pilot study, conducted from January 2003 to June 2005, was originally designed to determine the feasibility of a randomized secondary prevention trial to investigate the effects of periodontal treatment on inflammation and risk of future cardiovascular events. This was a multicenter trial conducted at the University at Buffalo, the University of North Carolina at Chapel Hill, Boston University, Kaiser Permanente Center for Health Research/Oregon Health and Science University, and the University of Maryland. Written informed consent was obtained from subjects prior to enrollment in the PAVE study. Complete information on the PAVE pilot study can be found in several previous publications (19-21).

Inclusion criteria for the PAVE study included mild to moderate periodontitis and recent evidence of coronary heart disease (20). Mild to moderate periodontitis was defined as having a minimum of 6 natural teeth with at least 3 teeth having a pocket depth of ≥ 4 mm, at least 2 teeth having interproximal attachment loss of ≥ 2 mm, and at least 10% of dental sites exhibiting bleeding on probing. Cardiovascular criteria were 50% or more blockage of a coronary artery or a history of a coronary event within 3 to 36 months prior to enrollment. Coronary events included myocardial infarction, coronary artery bypass graft surgery, and coronary transluminal angioplasty with or without a stent. Participation was limited to those age 75 and below.

Data collection

All data and laboratory samples used in this study were collected at baseline, prior to dental treatment. As a part of the PAVE study, serum levels of hs-CRP were measured using latex-enhanced nephelometry as described (21). This automated assay has been approved for use in assessing cardiovascular risk by the United States Food and Drug Administration.

Measures of periodontal inflammation

Thorough periodontal examinations were performed on PAVE subjects as previously described (21). Periodontal data was missing for 3 subjects. Estimates of periodontal disease severity used for these analyses came from the PAVE dataset and included extent of pocket depth greater than or equal to 5mm, extent of bleeding on probing, and number of sites with evidence of gingivitis, as indicated by a Gingival Index score of 1 or more.

We examined whether γ′ fibrinogen level was associated with the severity of the periodontal disease, particularly the inflammatory component, using the clinical data collected for the PAVE study. A study by Beck and Offenbacher indicates that the useful clinical variables for estimating systemic inflammatory exposure relevant to cardiovascular disease include the extent of bleeding on probing and an extent of pocket depth ≥ 5mm (23). In addition to these variables, we examined whether the number of dental sites with tissue inflammation, identified as sites with a gingival index > 0, was associated with γ′ fibrinogen or CRP.

Total and γ′ fibrinogen measurements

Plasma γ′ fibrinogen levels were measured by enzyme-linked immunosorbent assay using a modification of the method described by Lovely *et al.* (5). Briefly, the γ′ fibrinogen in subject plasma samples and standards were captured in 96 well plates using the 2.G2.H9 monoclonal antibody and detected using horseradish peroxidase (HRP)-conjugated sheep anti-human fibrinogen (Accurate Chemical, Westbury, NY, USA) along with high sensitivity HRP colorimetric substrate and stop solution (BioFX Laboratories, Owings Mills, MD, USA). Total fibrinogen levels were measured in a similar fashion as previously described (3).

Statistical analysis

Statistical analyses were performed using SPSS (17.0). CRP, γ′ fibrinogen, and total fibrinogen levels were log transformed when analyzed as dependent variables by linear regression. Differences in mean γ′ fibrinogen levels between groups were assessed by t-test

or ANOVA after log-transformation. The relationship between γ′ fibrinogen and CRP was assessed using a multivariate linear regression model. Further analyses were performed using logistic regression with CRP as a dichotomized variable, with a cutoff of > 3 mg/L defined as "high CRP" according to the three-level definition by Ridker (22), with adjustment for BMI and total fibrinogen levels. Correlations between γ′ fibrinogen, CRP, or total fibrinogen, and periodontal disease variables were examined using linear regression. Missing data were very limited, at less than 2.2% for all analyzed variables, and were excluded from analyses.

Results

We measured plasma levels of γ′ fibrinogen in a group of 284 subjects from the PAVE study with both periodontitis and CVD. The average age of the subjects was 59 and they were predominantly white and male (Table 1). There was a relatively large proportion of current (16.2%) and former (52.7%) smokers, which is not unexpected as smoking is a major risk factor for both cardiovascular and periodontal disease.

The mean (SE) plasma γ′ fibrinogen level in the PAVE subjects was 0.622 (0.017) mg/ml, with females having a slightly higher level than males, 0.690 (0.036) *vs*. 0.597 (0.019) mg/ml, respectively (p=0.022). The mean (SE) plasma total fibrinogen level was 3.70 (0.074) mg/ml. In this cohort, γ′ fibrinogen represented on average 18.6 (0.62) % of total plasma fibrinogen.

We investigated the relationship between γ′ fibrinogen, total fibrinogen, and several cardiovascular risk factors previously measured in this cohort, including hs-CRP. The γ′ fibrinogen concentration was significantly correlated with CRP level, while there was no significant association between γ′ fibrinogen and total fibrinogen, BMI, age, smoking status, or type II diabetes in this model or when evaluated separately (Table 2). The Pearson correlation coefficient between γ′ fibrinogen and total fibrinogen was 0.098 (p = 0.099) after log transformation of both variables.

Further evaluation of the relationship between γ′ fibrinogen and hs-CRP showed significant correlation (p = 0.006) after adjustment for age, gender, smoking status, BMI, type 2 diabetes, and total fibrinogen, with a one unit increase in CRP associated with a 1.9% increase in γ′ fibrinogen. Additionally, the γ′ fibrinogen level was highly associated (p < 0.001) with having a high CRP level of > 3 mg/L, which indicates an elevated risk for future cardiovascular events (22). A 0.1 mg/ml increase in γ′ fibrinogen raised the odds of being in this high-risk group by 20% (p<0.001, OR 95% CI: 1.086 – 1.321) in a model that included BMI and total fibrinogen.

Analysis of the relationship between these markers and clinical measures of periodontitis demonstrated that the extent of pocket depth ≥ 5 mm was associated with CRP (p = 0.042) but not with γ′ fibrinogen, while the number of sites with gum inflammation was highly associated with γ′ fibrinogen (p = 0.004) but not CRP (Table 3). There was no significant association between total fibrinogen and any of the disease variables.

Discussion

Little is known about the relationship between γ′ fibrinogen, an emerging CVD risk factor, and inflammation. Therefore, we measured γ′ and total fibrinogen levels in a study sample consisting of 284 subjects from the PAVE study cohort, all with mild to moderate periodontal disease and a recent history of coronary artery disease or CVD event. Our results demonstrate a link between γ′ fibrinogen and inflammation as represented by hs-CRP and gingivitis.

The mean γ′ fibrinogen level in these subjects, 0.622 mg/ml, was the highest seen in any published study to date with the exception of a study by Cheung *et al.* that noted a mean of 0.79 mg/ml in a group of 16 acute phase pulmonary embolism patients (18). Our finding, in conjunction with the relatively normal mean total fibrinogen of 3.70 mg/ml, supports the concept that γ′ fibrinogen is particularly elevated in the setting of chronic inflammation. A striking finding is that γ′ fibrinogen represented, on average, 18.6 % of total plasma fibrinogen in this population. This is over twice the normal percentage of γ′ fibrinogen in healthy blood donors, in which γ′ fibrinogen represents only 7.2% of total plasma fibrinogen (3), and in individuals in the Framingham Offspring Study, in which γ′ fibrinogen represents 6.8% of total plasma fibrinogen (5). These results suggest that γ′ fibrinogen may display differential regulation from total fibrinogen in the presence of combined cardiovascular and periodontal disease.

While γ′ fibrinogen was significantly associated with hs-CRP as both a continuous and a dichotomized variable, no significant correlation was found with other cardiovascular risk factors measured. This contrasts with the findings of our recent study of γ′ fibrinogen in the Framingham Offspring cohort, in which γ′ fibrinogen was associated with a number of traditional cardiovascular risk factors, including age, gender, BMI, smoking status, diabetes and total fibrinogen (5). These disparate results are likely due to the major differences between the PAVE cohort and the community-based Framingham Offspring cohort, particularly the fact that all of the PAVE subjects had significant cardiovascular and periodontal disease at the time of data collection, while only a relatively small fraction of community subjects would be expected to have similar conditions.

Our investigation into the relationship between γ′ fibrinogen and periodontal disease-associated inflammation was hampered by a lack of validated measures of this inflammation. Periodontal disease has been the subject of extensive study, but until fairly recently this research has focused on the disease as an outcome, and not as a risk factor for other conditions, such as cardiovascular disease. Beck and Offenbacher attempted to address this problem by looking at correlation between dental variables and markers of cardiovascular risk (23). They saw an association between the extent of bleeding on probing and serum soluble intercellular adhesion marker, and between extent of pocket depth ≥5 mm and serum CRP level. In our study we also examined correlations between the number of sites with gingivitis and the analytes CRP, total and γ′ fibrinogen. We did this with the idea that the number of dental sites with tissue inflammation may be a better marker of current (and not historic) inflammatory burden. Our findings replicated the association between the

extent of pocket depth ≥5 mm and CRP levels, and additionally we saw correlation between γ′ fibrinogen and the number of sites with gingivitis.

One of the main limitations of the present study is the lack of a control group of subjects. This makes it impossible to assess the individual contributions of CVD and periodontitis on the variables measured. Another major difficulty is the lack of validated clinical measures of periodontal disease severity that are known to associate with cardiovascular risk. Additionally, the discontinuation of the PAVE pilot study has prevented analysis of the effect of periodontal treatment on inflammation and γ′ fibrinogen levels, and assessment of the relationship between these variables and future cardiovascular events.

In conclusion, the link between γ′ fibrinogen and inflammation is intriguing, particularly in light of the growing literature associating γ′ fibrinogen with thrombotic disease (3-5, 16-18, 24-27). The lack of association between γ′ fibrinogen and total fibrinogen levels seen in this study and a previous case-control study of CAD (3) contrasts with the correlation between both fibrinogen isoforms seen in a large community-based cohort (5), and this may indicate a dysregulation of these isoforms under pathological conditions. Further work will be needed to determine whether γ′ fibrinogen is indeed regulated independently of total fibrinogen in an inflammatory setting, and, if so, what mechanisms are involved.

Acknowledgments

We thank Drs. Robert Genco, David Cooper and Lloyd Chambless for access to the PAVE plasma samples and data.

Supported by the National Heart, Lung, and Blood Institute (R21HL097298 to D.H.F.) and by the Oregon Clinical and Translational Research Institute TL1 grant (1TL1RR024159 to K.S.A.) from the National Center for Research Resources, a component of the National Institutes of Health, and the NIH Roadmap for Medical Research.

References

1. Chung DW, Davie EW. γ and γ′ chains of human fibrinogen are produced by alternative mRNA processing. Biochemistry. 1984; 23:4232–6. [PubMed: 6091742]
2. Fornace AJ Jr, Cummings DE, Comeau CM, et al. Structure of the human γ-fibrinogen gene. Alternate mRNA splicing near the 3′ end of the gene produces γA and γB forms of γ-fibrinogen. J Biol Chem. 1984; 259:12826–30. [PubMed: 6092346]
3. Lovely RS, Falls LA, Al-Mondhiry HA, et al. Association of γA/γ′ fibrinogen levels and coronary artery disease. Thromb Haemost. 2002; 88:26–31. [PubMed: 12152671]
4. Mannila MN, Lovely RS, Kazmierczak SC, et al. Elevated plasma fibrinogen γ′ concentration is associated with myocardial infarction: effects of variation in fibrinogen genes and environmental factors. J Thromb Haemost. 2007; 5:766–73. [PubMed: 17263791]
5. Lovely RS, Kazmierczak SC, Massaro JM, et al. γ′ fibrinogen: evaluation of a new assay for study of associations with cardiovascular disease. Clin Chem. 2010; 56:781–8. [PubMed: 20348406]
6. Meh DA, Siebenlist KR, Mosesson MW. Identification and characterization of the thrombin binding sites on fibrin. J Biol Chem. 1996; 271:23121–5. [PubMed: 8798504]
7. Lovely RS, Moaddel M, Farrell DH. Fibrinogen γ′ chain binds thrombin exosite II. J Thromb Haemost. 2003; 1:124–31. [PubMed: 12871549]
8. Pospisil CH, Stafford AR, Fredenburgh JC, et al. Evidence that both exosites on thrombin participate in its high affinity interaction with fibrin. J Biol Chem. 2003; 278:21584–91. [PubMed: 12682049]

9. Sabo TM, Farrell DH, Maurer MC. Conformational analysis of γ′ peptide (410-427) interactions with thrombin anion binding exosite II. Biochemistry. 2006; 45:7434–45. [PubMed: 16768439]
10. Pineda AO, Chen ZW, Marino F, et al. Crystal structure of thrombin in complex with fibrinogen γ′ peptide. Biophysical Chem. 2007; 125:556–9.
11. Falls LA, Farrell DH. Resistance of γA/γ′ fibrin clots to fibrinolysis. J Biol Chem. 1997; 272:14251–6. [PubMed: 9162058]
12. Collet JP, Nagaswami C, Farrell DH, et al. Influence of γ′ fibrinogen splice variant on fibrin physical properties and fibrinolysis rate. Arterioscler Thromb Vasc Biol. 2004; 24:382–6. [PubMed: 14656741]
13. Cooper AV, Standeven KF, Ariëns RA. Fibrinogen γ-chain splice variant γ′ alters fibrin formation and structure. Blood. 2003; 102:535–40. [PubMed: 12663453]
14. Gersh KC, Nagaswami C, Weisel JW, et al. The presence of γ′ chain impairs fibrin polymerization. Thromb Res. 2009; 124:356–63. [PubMed: 19138790]
15. de Willige SU, Standeven KF, Philippou H, et al. The pleiotropic role of the fibrinogen γ′ chain in hemostasis. Blood. 2009; 114:3994–4001. [PubMed: 19687509]
16. Drouet L, Paolucci F, Pasqualini N, et al. Plasma γA/γ′ fibrinogen ratio, a marker of arterial thrombotic activity: a new potential cardiovascular risk factor? Blood Coagul Fibrinolysis. 1999; 10:S35–9. [PubMed: 10070816]
17. Cheung EY, Uitte de Willige S, Vos HL, et al. Fibrinogen γ′ in ischemic stroke: a case-control study. Stroke. 2008; 39:1033–5. [PubMed: 18239174]
18. Cheung EY, Vos HL, Kruip MJ, et al. Elevated fibrinogen γ′ ratio is associated with cardiovascular diseases and acute phase reaction but not with clinical outcome. Blood. 2009; 114:4603–4. author reply 4604-5. [PubMed: 19965709]
19. Beck JD, Couper DJ, Falkner KL, et al. The Periodontitis and Vascular Events (PAVE) pilot study: adverse events. J Periodontol. 2008; 79:90–6. [PubMed: 18166097]
20. Couper DJ, Beck JD, Falkner KL, et al. The Periodontitis and Vascular Events (PAVE) pilot study: recruitment, retention, and community care controls. J Periodontol. 2008; 79:80–9. [PubMed: 18166096]
21. Offenbacher S, Beck JD, Moss K, et al. Results from the Periodontitis and Vascular Events (PAVE) Study: a pilot multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary prevention model of cardiovascular disease. J Periodontol. 2009; 80:190–201. [PubMed: 19186958]
22. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003; 107:363–9. [PubMed: 12551853]
23. Beck JD, Offenbacher S. Relationships among clinical measures of periodontal disease and their associations with systemic markers. Ann Periodontol. 2002; 7:79–89. [PubMed: 16013220]
24. Mosesson MW, Hernandez I, Raife TJ, et al. Plasma fibrinogen γ′ chain content in the thrombotic microangiopathy syndrome. J Thromb Haemost. 2007; 5:62–9. [PubMed: 17038160]
25. Uitte de Willige S, de Visser MC, Houwing-Duistermaat JJ, et al. Genetic variation in the fibrinogen γ gene increases the risk for deep venous thrombosis by reducing plasma fibrinogen γ′ levels. Blood. 2005; 106:4176–83. [PubMed: 16144795]
26. Uitte de Willige S, Rietveld IM, De Visser MC, et al. Polymorphism 10034C>T is located in a region regulating polyadenylation of *FGG* transcripts and influences the fibrinogen γ′/γA mRNA ratio. J Thromb Haemost. 2007; 5:1243–9. [PubMed: 17403086]
27. de Willige SU, Pyle ME, Vos HL, et al. Fibrinogen γ gene 3′-end polymorphisms and risk of venous thromboembolism in the African-American and Caucasian population. Thromb Haemost. 2009; 101:1078–84. [PubMed: 19492150]

Table 1

Characteristics of the PAVE cohort.

Characteristic	Value
Age (n=284)	59.2 ± 8.9 yrs
Gender (n=284)	
Female	78 (27.5%)
Male	206 (72.5%)
Ethnicity (n=281)	
Non-Hispanic	275 (96.8%)
Hispanic	6 (2.1%)
Race (n=282)	
White	238 (84.4%)
Black	33 (11.7%)
Other	11 (3.9%)
Smoking (n=283)	
Current smoker	46 (16.2%)
Past smoker	149 (52.7%)
Never smoked	88 (31.1%)

Table 2

Relationship between γ′ fibrinogen and cardiovascular disease risk factors.

Risk factor	Mean γ′ fibrinogen concentration (SD)	*P* value*
Gender		0.022
Female [n=78]	0.690 (0.320)	
Male [n=206]	0.597 (0.270)	
Age		0.631
≤ 55 [n=92]	0.629 (0.287)	
56 – 64 [n=104]	0.613 (0.310)	
≥ 65 [n=88]	0.627 (0.262)	
BMI		0.391
< 25 [n=38]	0.554 (0.201)	
25 - 29.99 [n=118]	0.614 (0.266)	
≥ 30 [n=122]	0.651 (0.326)	
Smoking status		0.394
Nonsmoker [n=88]	0.595 (0.257)	
Former smoker [n=149]	0.620 (0.282)	
Current smoker [n=46]	0.686 (0.351)	
CRP (mg/dl)		<0.001
< 1 [n=94]	0.583 (0.282)	
1 – 3 [n=95]	0.552 (0.228)	
> 3 [n=90]	0.731 (0.323)	
Total fibrinogen (mg/ml)		0.091
< 3.077 [n=94]	0.575 (0.261)	
3.077 – 4.050 [n=96]	0.627 (0.279)	
> 4.050 [n=94]	0.665 (0.287)	
Type 2 diabetes		0.937
Absent [n=221]	0.623 (0.285)	
Present [n=59]	0.628 (0.301)	

* *P* value for group differences in mean log-transformed γ′ fibrinogen. All variables were assessed independently.

Table 3

Relationships between periodontal disease variables and log-transformed CRP, γ′ fibrinogen, and total fibrinogen by univariate regression.

	Periodontal Disease Variable (n = 281)	Parameter Estimate	SE	*P* Value
γ′ Fibrinogen	Extent of PD ≥ 5mm	0.002	0.002	0.383
	Extent of BOP	-0.002	0.002	0.266
	# sites with gingivitis	0.004	0.001	**0.004**
CRP	Extent of PD ≥ 5mm	0.012	0.006	**0.042**
	Extent of BOP	0.003	0.005	0.558
	# of sites with gingivitis	-0.002	0.003	0.577
Total Fibrinogen	Extent of PD ≥ 5mm	0.000	0.002	0.931
	Extent of BOP	-0.002	0.002	0.224
	# of sites with gingivitis	0.000	0.001	0.516