 

# Association of γA/γ' Fibrinogen Levels and Coronary Artery Disease

Rehana S. Lovely[1], Lisa A. Falls[3], Hamid A. Al-Mondhiry[4], Charles E. Chambers[4], Gary J. Sexton[2], Hanyu Ni[5], David H. Farrell[1]

[1]Department of Pathology and [2]Clinical Research Center, Oregon Health & Science University, Portland, OR; [3]Center for Hemostasis and Thrombosis Research, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [4]Department of Medicine, The Pennsylvania State University College of Medicine, Hershey, PA; [5]Division of Health Interview Statistics, National Center for Health Statistics, Hyattsville, MD, USA

**Keywords**

Age, coronary, epidemiology, fibrinogen, gender

**Summary**

γA/γ' fibrinogen is a fibrinogen isoform that constitutes about 15% of total plasma fibrinogen. This isoform contains an additional binding site for zymogen factor XIII and for active thrombin, and forms fibrin clots that are resistant to fibrinolysis in vitro. Little is known about the variability of γA/γ' fibrinogen levels in human populations, whereas total fibrinogen levels are known to increase with age and are higher in women than in men. In this report, evidence is presented that, in contrast to total fibrinogen levels, γA/γ' fibrinogen levels showed no significant association with age or gender in a population of normal blood donors. A study of γA/γ' fibrinogen levels in patients undergoing coronary angiography also showed that γA/γ' fibrinogen levels were higher on average in coronary artery disease patients than in patients without coronary artery disease, and that this association was independent of total fibrinogen levels.

## Introduction

Fibrinogen consists of three polypeptide chains, α, β, and γ, arranged as a dimer with the stoichiometry $(\alpha, \beta, \gamma)_2$. In approximately 15% of fibrinogen molecules, one γ chain, termed γ' (1) γB (2), or $\gamma^{57.5}$ (3) has the twenty amino acid sequence VRPEHPAETEYDSLY-PEDDL (4) substituted for the carboxyl terminal four amino acids AGDV found in the more common γ chain, sometimes termed γA (2) or $\gamma^{55}$ (3). This gives rise to a heterodimeric molecule termed γA/γ' fibrinogen (also known as "peak 2" fibrinogen) (5) with the stoichiometry (α, β, γA) (α, β, γ'). The γ' extension results from alternative mRNA processing (6, 7) and disrupts the binding site for platelet integrin $\alpha_{IIb}\beta_3$ (8, 9, 10). This alternative mRNA processing event (Fig. 1) is also found in other species (11) and is tissue-specific (12). Furthermore, the γ' extension is highly anionic, containing sulfotyrosine residues (13) and seven Asp and Glu residues (1), and mediates binding of γA/γ' fibrinogen to zymogen coagulation factor XIII (14, 15) and thrombin (16, 17). Previous studies (18) have shown that γA/γ' fibrinogen forms clots that are more extensively crosslinked by factor XIIIa, a plasma transglutaminase (19), and are therefore resistant to breakdown by fibrinolytic enzymes. In addition, γA/γ' fibrin binds thrombin more avidly, and the increased binding of thrombin to this form of fibrin provides an additional source of clot-bound thrombin (16, 17). Clot-bound thrombin is active even in the presence of heparin, since clot-bound thrombin is resistant to heparin-catalyzed inhibition by antithrombin III (20, 21). These unique characteristics of γA/γ' fibrinogen have lead to the hypothesis that γA/γ' fibrinogen levels may contribute to thrombosis (22).

Although many studies have been performed that measured levels of total fibrinogen in plasma (23-28), little is known about the variability of γA/γ' fibrinogen levels in human populations. Indeed, little is known about the regulation of γA/γ' synthesis, although there is compelling evidence that the mRNA processing that gives rise to the γ' mRNA is a tissue-specific and regulated event (12). In contrast, much is known about factors that regulate total fibrinogen levels in humans. Fibrinogen is an acute phase protein whose synthesis increases during inflammation (29). Polymorphisms in the fibrinogen genes have been shown to affect levels of total fibrinogen (30-32). Studies have shown that levels of total fibrinogen increase with age (33). Studies have also shown that total fibrinogen levels tend to be higher overall in women than in men (34, 35). The levels of total fibrinogen in plasma correlate strongly with myocardial infarction and stroke (23). In this report, evidence is presented that in contrast to total fibrinogen levels, γA/γ' fibrinogen levels showed no significant association with age in a population of normal blood donors. In addition, γA/γ' fibrinogen levels showed no significant association with gender. A study of γA/γ' fibrinogen levels in patients with coronary artery disease (CAD) also showed that γA/γ' fibrinogen levels were higher on average in CAD patients than in non-CAD patients.

## Materials and Methods

*Plasma Samples*

Blood samples were obtained from anonymous blood donors between the ages of 41 and 80 at the Hershey Medical Center blood bank at the Pennsylvania State University College of Medicine. Blood samples were also obtained from patients between the ages of 41 and 80 at the Hershey Medical Center who were referred for elective, outpatient diagnostic cardiac catheterizations. Indications for catheterization included anginal chest pain, positive stress test, valvular heart disease, and preoperative clearance prior to non-cardiac surgery in patients suspected of ischemic heart disease. Patients

Correspondence to: Prof. David H. Farrell, Department of Pathology, School of Medicine, Oregon Health & Science University, 3181 S. W. Sam Jackson Park Road, Portland, OR 97201-3098, USA – Tel.: 503-494-8602; Fax: 503-494-2025; E-mail: farrelld@ohsu.edu

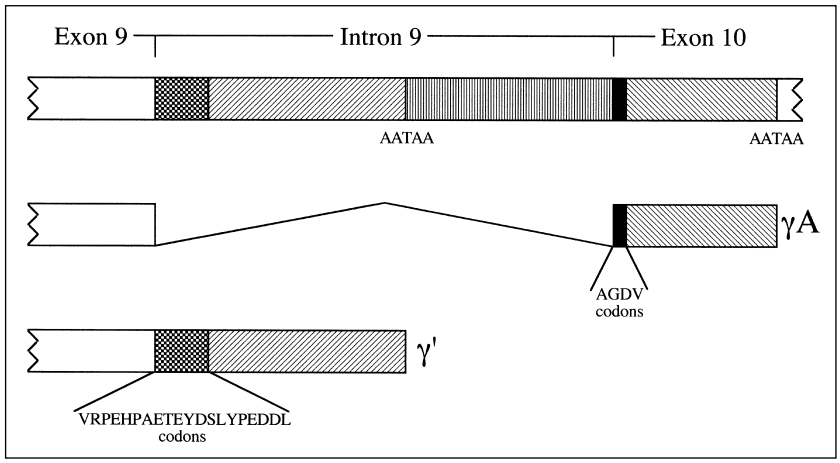


*Fig. 1* Alternative mRNA processing of the γ chain mRNAs. In about 90% of transcripts, the ninth intron is spliced out and the ninth exon and tenth exon are joined in frame, giving rise to the γA mRNA. But in the remaining transcripts, polyadenylation and cleavage occurs within the ninth intron before the ninth intron can be removed, giving rise to the γ' mRNA (5). The tenth exon encodes the γA chain four amino acid carboxyl terminal sequence AGDV, whereas the ninth intron encodes the γ' chain twenty amino acid carboxyl terminal sequence VRPEHPAETEYDSLYPEDDL

were entered sequentially during two different sampling intervals; April through August, 1996 and October through December, 1997. All patients signed an informed consent form approved by the Pennsylvania State University College of Medicine Institutional Review Board and the study was done in accordance with the Helsinki Declaration.

All cardiac catheterizations were performed via the femoral approach using the Judkin's technique, with arterial blood samples obtained immediately after arterial access before the administration of heparin. Coronary cineangiography was performed in standard projections. All studies were interpreted qualitatively by two angiographers, with a third angiographer reviewing films as required. All discrepancies in interpretation were settled by consensus. A patient was considered to have significant CAD if the patient had a luminal narrowing lesion of 50% or greater in at least one major coronary artery or branch. CAD was diagnosed in 91 cases; 42 patients had no angiographic evidence of disease and constituted the non-CAD group.

*Laboratory Studies*

Blood was collected in standard citrate anticoagulant. Plasma was prepared by centrifugation at 10,000 × g for 10 min at room temperature and was stored at –70° C. Samples were thawed only once for use in the assay.

A monoclonal antibody directed against the γ' chain (2.G2.H9) was developed at the Pennsylvania State University Biotechnology Institute, University Park, PA by Dr. William Scheuchenzuber. The immunogen was a synthetic peptide corresponding to the carboxyl terminal twenty amino acids of the γ' chain, VRPEHPAETEYDSLYPEDDL, coupled to keyhole limpet hemocyanin as a carrier protein. 2.G2.H9 recognized γA/γ' fibrinogen exclusively, and did not cross-react measurably with γA/γA fibrinogen (data not shown). 2.G2.H9 was purified from 30 ml of a concentrated bioreactor preparation by precipitation with 50% ammonium sulfate followed by DEAE-cellulose chromatography in 0.01 M potassium phosphate, pH 8.0.

Monoclonal antibody 2.G2.H9 was used to develop an ELISA assay specific for γA/γ' fibrinogen, using a buffer and blocking system described previously for the measurement of annexin V in plasma (36). 2.G2.H9 (1.5 μg/ml) in 120 mM NaCl/2.7 mM KCl/10 mM sodium phosphate, pH 7.4 (PBS) was used as the capture antibody, and 50 μl was coated per well overnight at 4° C in 96 well MaxiSorp plates (Nunc, Naperville, IL). Plates were blocked with 230 μl 1% bovine serum albumin (BSA) in PBS for 90 min at 37° C, and 50 μl plasma samples diluted 1:1,000 in PBS/5 mM EDTA/0.1% BSA/0.1% Triton X-100 were incubated for 1 h at 37° C. Bound γA/γ' fibrinogen was detected with 50 μl of a 1.25 μg/ml solution of a biotinylated rabbit anti-human fibrinogen immunoglobulin fraction (Accurate Chemical & Scientific Corp., Westbury, NY) in PBS/0.1% BSA/0.1% Triton X-100 for 1 h at 37° C. The biotinylated immunoglobulin was detected with 50 μl of a 1.25 μg/ml solution of streptavidin-alkaline phosphatase conjugate (Life Technologies, Gaithersburg, MD) in PBS/0.1% BSA/0.1% Triton X-100 for 1 h at 37° C, and incubated with 50 μl of a 1 mg/ml solution of *p*-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO) in 5 mM $MgCl_2$/0.1 mM Tris pH 8.8 for 30-60 min at 37° C. The reaction was quenched with 150 μl of 0.1 N NaOH, and the absorbance at 405 nm was read using a microplate reader (Dynatech, Chantilly, VA).

γA/γ' fibrinogen was purified as described previously using DEAE-cellulose chromatography (5, 18). Pooled human plasma was heat-defibrinated for 30 min at 56° C and centrifuged at 100,000 × g for 30 min at 4° C. ELISA

*Table 1* Characteristics and γA/γ' fibrinogen levels of the normal blood donors

| **Variable** | **% (*n*)** | **[γA/γ' fibrinogen] (mg/ml ± SE)** |
|---|---|---|
| **Gender** | | |
| Female | 38.3 (*46*) | 0.298 ± 0.015 |
| Male | 61.7 (*74*) | 0.277 ± 0.009 |
| **Age** | | |
| 41-50 | 20.0 (*24*) | 0.282 ± 0.021 |
| 51-60 | 28.3 (*34*) | 0.290 ± 0.012 |
| 61-70 | 33.3 (*40*) | 0.286 ± 0.015 |
| 71-80 | 18.3 (*22*) | 0.278 ± 0.020 |

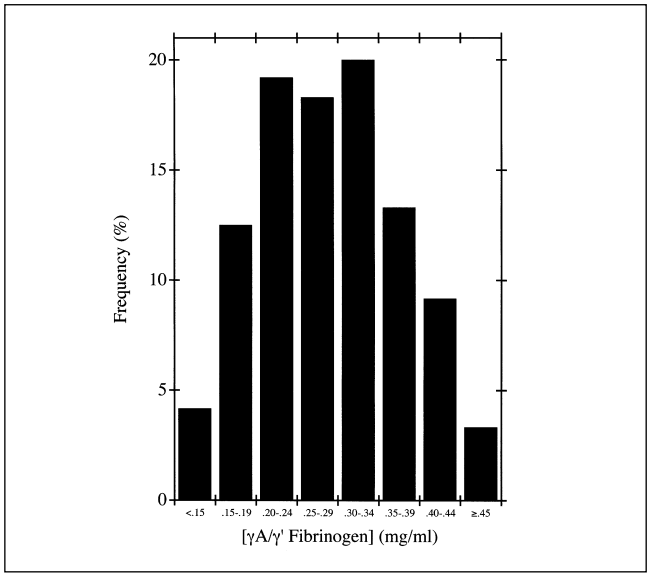


*Fig. 2* Distribution of γA/γ' fibrinogen levels in normal blood donors. The frequency of the indicated γA/γ' fibrinogen levels are shown for normal blood donors aged 41-80. The γA/γ' fibrinogen levels showed a normal distribution about the mean of 0.285 ± 0.088 mg/ml (mean ± SD)

standards were prepared by reconstituting defibrinated plasma with purified γA/γ' fibrinogen. Plasma samples and standards were diluted 1:1000 for the assay.

Total fibrinogen was assayed similarly, except that rabbit anti-human fibrinogen was used as capture antibody, heat-defibrinated plasma was reconstituted with unfractionated fibrinogen rather than γA/γ' fibrinogen, and samples were diluted 1:10,000 for the assay.

*Statistical Analysis*

Each variable was summarized for each subgroup by calculating mean values and associated standard deviations (SD) and standard errors (SE). Data are presented with associated SD or SE as noted. Differences between patients with angiographic confirmation of CAD and those without CAD were assessed using a t-statistic, as were differences in the ratio (case/control). Gender differences between the two patient groups was assessed using a Pearson's Chi-Square statistic. Regression analysis was used to assess the relationship between total fibrinogen and γA/γ' fibrinogen.

## Results

Little is known about the distribution of γA/γ' fibrinogen levels in adult human populations. We therefore assayed γA/γ' fibrinogen levels in 120 plasma samples obtained from normal blood donors aged 41-80. This population (Table 1) had a mean age of 60.0 ± 9.9 years (mean ± SD) and was predominantly male (61.7%). The mean level of γA/γ' fibrinogen in this population was 0.285 ± 0.088 mg/ml (mean ± SD), which is consistent with previous estimates that γA/γ' fibrinogen constitutes about 15% of total fibrinogen (17). The γA/γ' fibrinogen levels showed a normal distribution about the mean (Fig. 2).

Since total fibrinogen levels vary with age and gender, the association between γA/γ' fibrinogen levels and either age or gender was examined. Mean γA/γ' fibrinogen levels were slightly higher in females than in males (Table 1), but this did not reach statistical significance (P = 0.207), indicating that γA/γ' fibrinogen levels did not vary significantly between men and women. This result is in contrast to the levels of total fibrinogen in females, which have been shown to be significantly higher than in males (34, 35).

The relation between γA/γ' fibrinogen levels and age was then examined. Mean γA/γ' fibrinogen levels were determined for each age group and analyzed by decade. γA/γ' fibrinogen levels showed no obvious trend with age (Table 1); in no instance did the mean γA/γ' fibrinogen levels for any age group vary significantly from the mean of 0.285 ± 0.008 mg/ml (mean ± SE). This result is also in contrast to the levels of total fibrinogen, which increase significantly with age (33). These combined results indicate that γA/γ' fibrinogen levels did not show gender and age differences that are found with total fibrinogen levels.

Since total fibrinogen levels are correlated with ischemic heart disease (23-28), a study of 91 CAD patients and 42 patients without CAD aged 41-80 was subsequently conducted using blood samples obtained during cardiac catheterization on patients investigated for ischemic heart disease. The number of CAD patients was higher than the number of non-CAD patients (Table 2), since in patients who present with symptoms suggestive of ischemic heart disease, cardiac catheterization typically confirms the diagnosis of CAD in about two thirds of cases, while the remaining third show no demonstrable coronary lesion. The CAD patients tended to be slightly older than non-CAD patients (62.8 ± 1.01 years vs. 59.1 ± 1.62 years (mean ± SE); P = 0.0522), but no significant association (P = 0.0732) was found

*Table 2* Characteristics of the CAD and non-CAD patients

| **CAD** | **% (*n*)** | **non-CAD** | **% (*n*)** |
|---|---|---|---|
| **Gender** | | **Gender** | |
| Female | 33.0 (*30*) | Female | 64.3 (*27*) |
| Male | 67.0 (*61*) | Male | 35.7 (*15*) |
| **Age** | | **Age** | |
| 41-50 | 13.2 (*12*) | 41-50 | 19.0 (*8*) |
| 51-60 | 25.3 (*23*) | 51-60 | 42.9 (*18*) |
| 61-70 | 35.2 (*32*) | 61-70 | 16.7 (*7*) |
| 71-80 | 26.4 (*24*) | 71-80 | 21.4 (*9*) |

between age and γA/γ' fibrinogen levels in this population, similar to the normal blood donors. The CAD patients also tended to be male (61 vs. 30) while the non-CAD patients tended to be female (27 vs. 15), but no significant association was found between gender and γA/γ' fibrinogen levels (0.373 ± 0.016 for males, 0.383 ± 0.024 for females (mean ± SE); P = 0.714), as was found for the normal blood donors. When γA/γ' fibrinogen levels were compared between CAD patients and non-CAD patients (Fig. 3), γA/γ' fibrinogen levels were significantly higher in CAD patients (0.413 ± 0.016 mg/ml vs. 0.299 ± 0.024 mg/ml (mean ± SE); P < 0.0001), both in men and women (Table 3). Mean γA/γ' fibrinogen levels in the non-CAD patients (0.299 ± 0.024 mg/ml) were also similar to those found in the normal blood donors (0.285 ± 0.008 mg/ml (mean ± SE), who were from the same age range of 41-80 years.

Total fibrinogen levels were also significantly higher (P < 0.0001) in CAD patients than non-CAD patients (data not shown), consistent with many previous studies of coronary heart disease (37). It was possible, therefore, that γA/γ' fibrinogen levels were simply a surrogate marker for total fibrinogen levels. In order to address this possibility, total fibrinogen levels were compared to γA/γ' fibrinogen levels (Fig. 4). There was no significant association (P = 0.200) between total fibrinogen levels and γA/γ' levels in this population, indicating that the association between CAD and γA/γ' fibrinogen levels was independent of total fibrinogen levels. In addition, the ratio of γA/γ' fibrinogen to total fibrinogen was not significantly associated with CAD (P = 0.330), contrary to a previous report (22). From these results, it is clear that γA/γ' fibrinogen levels were not simply a surrogate for total fibrinogen levels, but showed an independent association with CAD. This result was confirmed by multivariate analysis. The odds ratio for the association between CAD and γA/γ' fibrinogen was 7.16 (95% CI = 1.82 - 27.7) per γA/γ' fibrinogen quartile (Fig. 5), adjusted for age, gender, and total fibrinogen levels.

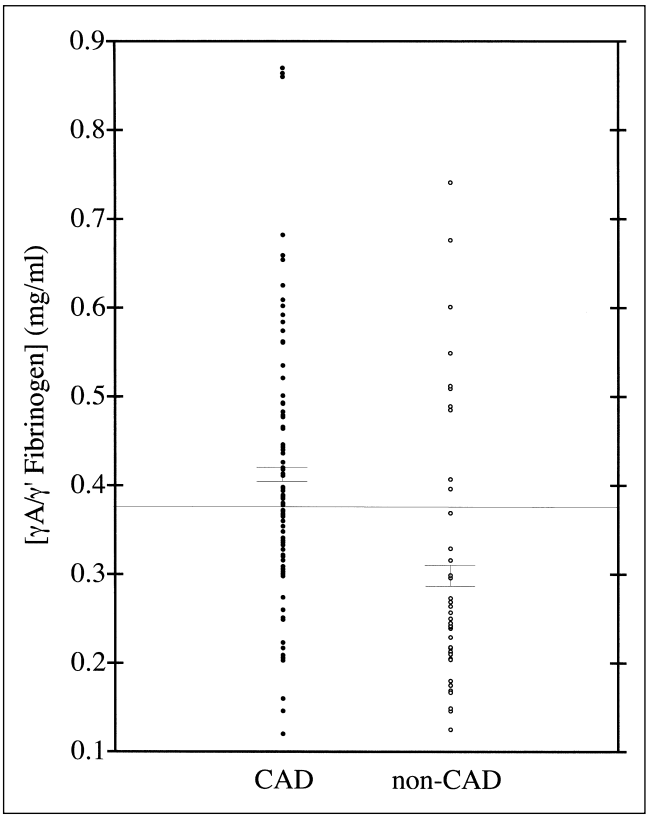


*Fig. 3* Elevation of γA/γ' fibrinogen levels in CAD patients vs. non-CAD patients. γA/γ' fibrinogen levels were determined in 133 cardiac catheterization patients aged 41-80, of whom 91 were diagnosed with CAD; bars indicate SE. Mean γA/γ' fibrinogen levels were significantly higher in CAD patients (0.413 ± 0.016 mg/ml vs. 0.299 ± 0.024 mg/ml, P < 0.0001). The mean γA/γ' fibrinogen levels for the combined CAD and non-CAD patient groups of 0.377 ± 0.014 mg/ml is shown by the horizontal line

## Discussion

The factors that regulate the levels of γA/γ' fibrinogen are, for the most part, uncharacterized. The levels of total fibrinogen have been correlated with gene polymorphisms (30-32); however, as shown in the present study, γA/γ' fibrinogen levels can vary independently of total fibrinogen levels. This may be explained by the fact that synthesis of the β chain mRNA is a rate-limiting factor in total fibrinogen expression (38), whereas expression of the γ' chain mRNA is more likely to be affected by the cleavage of intron/exon boundaries and polyadenylation sites in the mRNA by spliceosomes and cleavage/polyadenylation enzymes, respectively (6, 7). The processing events that give rise to the γ' mRNA are also liver-specific, since the γ' mRNA is not found in other tissues that express γA mRNA (12). Mechanistically, the relative levels of γA vs. γ' mRNA may be the result of direct competition between spliceosomes that remove the ninth intron encoding the carboxyl terminus of the γ' chain vs. enzymes that cleave and polyadenylate the 3' end of the γ' mRNA within the ninth intron (6). Presumably, elevated levels of spliceosomes would favor removal of the ninth intron, thus increasing the level of γA mRNA. In contrast, elevated levels of cleavage and polyadenylation activity would favor termination of the mRNA within the ninth intron, increasing the level of γ' mRNA. Therefore, an understanding of the regulation of γA/γ' fibrinogen levels will likely come from future investigations of ninth intron boundary polymorphisms, polyadenylation recognition sites, and liver-specific mRNA spliceosome and cleavage/polyadenylation enzymes rather than the fibrinogen gene promoters.

*Table 3* γA/γ' fibrinogen levels of the CAD and non-CAD patients

| Variable | [γA/γ' fibrinogen] (mg/ml ± SE) |
|---|---|
| **Group** | |
| CAD | 0.413 ± 0.016 |
| non-CAD | 0.299 ± 0.024 |
| **Gender** | |
| Male | 0.373 ± 0.016 |
| Female | 0.383 ± 0.024 |
| **Group and Gender** | |
| CAD male | 0.391 ± 0.018 |
| CAD female | 0.458 ± 0.027 |
| Non-CAD male | 0.300 ± 0.029 |
| Non-CAD female | 0.299 ± 0.033 |

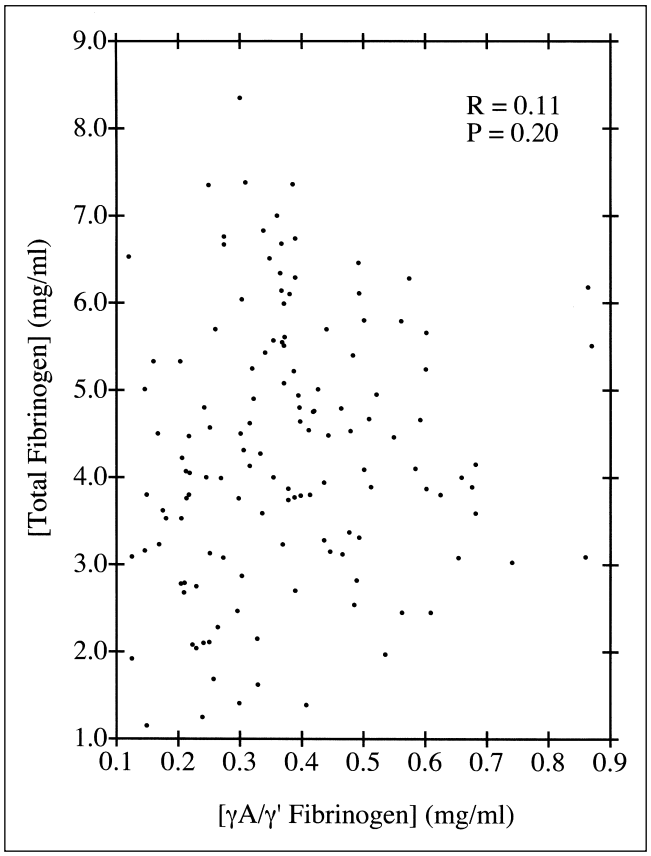


*Fig. 4* Lack of correlation between total fibrinogen levels vs. γA/γ' fibrinogen levels. A scatter plot is shown of the total fibrinogen levels vs. γA/γ' fibrinogen levels for each of the 133 patients enrolled in the study. No significant association was found (P = 0.200) between total fibrinogen levels and γA/γ' fibrinogen levels

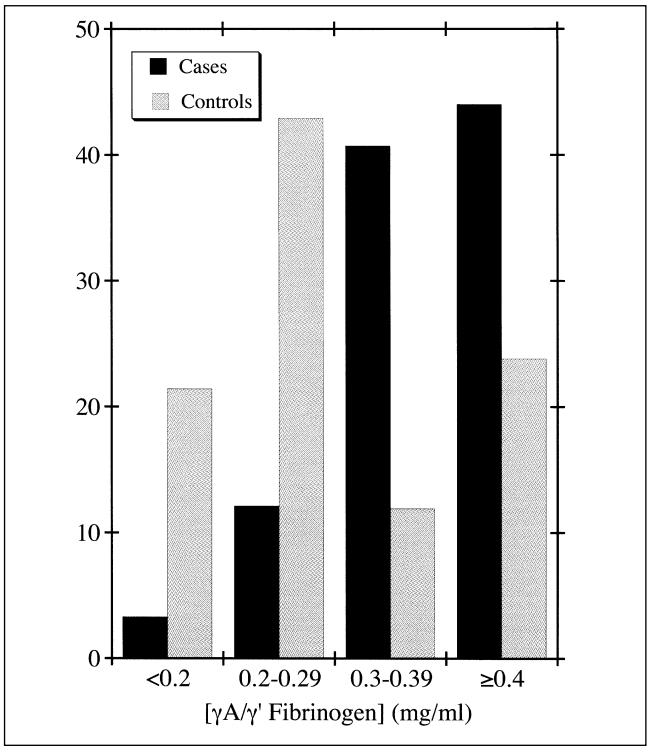


*Fig. 5* Distribution of γA/γ' fibrinogen levels in CAD patients vs. non-CAD patients by quartile. The CAD patients (black bars) and non-CAD patients (stippled bars) were stratified in quartiles according to the γA/γ' fibrinogen level. The odds ratio for the association between CAD and γA/γ' fibrinogen was 7.16 (95% CI = 1.82 - 27.7) per γA/γ' fibrinogen quartile, adjusted for age, gender, and total fibrinogen levels

The data also indicate that the level of γA/γ' fibrinogen in plasma correlates with CAD. This conclusion differs significantly from that of Drouet et al. (22) who hypothesized that only the ratio of γA/γ' fibrinogen to total fibrinogen may be a potential marker for cardiovascular risk. In addition, the study of Drouet et al. (22) showed an unexplained bimodal distribution of γA/γ' fibrinogen to total fibrinogen ratio that was not apparent in the present study. Furthermore, no odds ratios were presented to substantiate their hypothesis that the ratio of γA/γ' fibrinogen to total fibrinogen may be a marker of cardiovascular risk.

A limitation of the present study is that it does not include an extensive number of potential confounding factors. It is possible that other variables (such as smoking, exercise, hypertension, alcohol intake, and obesity) that influence total fibrinogen levels may also have an impact on γA/γ' fibrinogen levels. Furthermore, it is not known if γA/γ' fibrinogen levels are associated with other thrombotic disorders that have been correlated with total fibrinogen levels, including myocardial infarction and stroke. A larger, prospective study will be necessary to definitively address these issues.

Presently, it is not clear which, if any, of the properties of γA/γ' fibrinogen are responsible for its association with CAD. It is possible that elevated γA/γ' fibrinogen levels in CAD are simply an epiphenomenon unrelated to the mechanisms that lead to the disease. It is also possible that elevated γA/γ' fibrinogen levels are a consequence of the disease, rather than a causative factor. However, γA/γ' fibrinogen has several unique biologic activities that may be causally related to CAD. Fibrin clots made from γA/γ' fibrinogen are more extensively cross-linked by factor XIIIa and are resistant to fibrinolysis (18). In addition, γA/γ' fibrin(ogen) serves as a carrier protein for factor XIII (14, 15), and acts as a cofactor in factor XIII activation (39). Furthermore, γA/γ' fibrin(ogen) binds thrombin more avidly (16, 17), providing an additional source of clot-bound thrombin. Clot-bound thrombin is particularly difficult to manage clinically, since it is resistant to heparin-mediated inhibition by antithrombin III (20, 21). However, irrespective of the mechanism, the finding that γA/γ' fibrinogen levels were elevated in the CAD patients is provocative and bears further investigation.

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## References

1. Wolfenstein-Todel C, Mosesson MW. Human plasma fibrinogen heterogeneity: evidence for an extended carboxyl-terminal sequence in a normal γ chain variant (γ'). Proc Natl Acad Sci USA 1980; 77: 5069-73.
2. Francis CW, Marder VJ, Martin SE. Demonstration of a large molecular weight variant of the γ chain of normal human plasma fibrinogen. J Biol Chem 1980; 255: 5599-604.

3. Francis CW, Kraus DH, Marder VJ. Structural and chromatographic heterogeneity of normal plasma fibrinogen associated with the presence of three γ-chain types with distinct molecular weights. Biochim Biophys Acta 1983; 744: 155-64.
4. Wolfenstein-Todel C, Mosesson MW. Carboxy-terminal amino acid sequence of a human fibrinogen γ-chain variant (γ'). Biochemistry 1981; 20: 6146-9.
5. Mosesson MW, Finlayson JS, Umfleet RA. Human fibrinogen heterogeneities: identification of γ chain variants. J Biol Chem 1972; 247: 5223-7.
6. Chung DW, Davie EW. γ and γ' chains of human fibrinogen are produced by alternative mRNA processing. Biochemistry 1984; 23: 4232-6.
7. Fornace AJ, Cummings DE, Comeau CM, Kant JA, Crabtree GR. 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.
8. Farrell DH, Thiagarajan P, Chung, DW, Davie EW. Role of fibrinogen α and γ chain sites in platelet aggregation. Proc Natl Acad Sci USA 1992; 89: 10729-32.
9. Lawrence SO, Wright TW, Francis CW, Fay PJ, Haidaris PJ. Purification and functional characterization of homodimeric γB-γB fibrinogen from rat plasma. Blood 1993; 82: 2406-13.
10. Farrell DH, Thiagarajan P. Binding of recombinant fibrinogen mutants to platelets. J Biol Chem 1994; 269: 226-31.
11. Crabtree GR, Kant JA. Organization of the rat γ-fibrinogen gene: alternative mRNA splice patterns produce the γA and γB (γ') chains of fibrinogen. Cell 1982; 31: 159-66.
12. Haidaris PJ, Courtney MA. Tissue-specific and ubiquitous expression of fibrinogen γ chain mRNA. Blood Coagul Fibrinolysis 1990; 1: 433-7.
13. Farrell DH, Mulvihill ER, Huang S, Chung DW, Davie EW. Recombinant human fibrinogen and sulfation of the γ' chain. Biochemistry 1991; 30: 9414-20.
14. Siebenlist KR, Meh DA, Mosesson MW. Plasma factor XIII binds specifically to fibrinogen molecules containing γ' chains. Biochemistry 1996; 35: 10448-53.
15. Moaddel M, Farrell DH, Daugherty MA, Fried MG. Interactions of human fibrinogens with factor XIII: roles of calcium and the γ' peptide. Biochemistry 2000; 39: 6698-705.
16. Meh DA, Siebenlist KR, Mosesson MW. Identification and characterization of thrombin binding sites on fibrin. J Biol Chem 1996; 271: 23121-5.
17. Meh D, Siebenlist KR, Brennan SO, Holyst T, Mosesson MW. The amino acid sequence in fibrin responsible for high affinity thrombin binding. Thromb Haemost 2001; 85: 470-4.
18. Falls LA, Farrell DH. Resistance of γA/γ' fibrin clots to fibrinolysis. J Biol Chem 1997; 272: 14251-6.
19. Lorand L. Sol Sherry Lecture on Thrombosis: Research on Clot Stabilization Provides Clues for Improving Thrombolytic Therapies. Arterioscler Thromb Vasc Biol 2000; 20: 2-9.
20. Hogg PJ, Jackson CM. Fibrin monomer protects thrombin from inactivation by heparin-antithrombin III: implications for heparin efficacy. Proc Natl Acad Sci USA 1989; 86: 3619-23.
21. Weitz JI, Hudoba M, Massel D, Maraganore J, Hirsh J. Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors. J Clin Invest 1990; 86: 385-91.
22. Drouet L, Paolucci F, Pasqualini N, Laprade M, Ripoll L, Mazoyer E, Bal dit Sollier C, Vanhove N. Plasma γA/γ' fibrinogen ratio, a marker of arterial thrombotic activity: a new potential cardiovascular risk factor? Blood Coagul Fibrinolysis 1999; 10 (Suppl 1): S35-S39.
23. Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med 1984; 311: 501-5.
24. Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WR, Haines AP, Stirling Y, Imeson JD, Thrompson SG. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. Lancet 1986; 2: 533-7.
25. Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease: the Framingham Study. JAMA 1987; 258: 1183-6.
26. Yarnell JWG, Baker IA, Sweetnam PM, Bainton D, O'Brien JR, Whitehead PJ. Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease: the Caerphilly and Speedwell Collaborative Heart Disease Studies. Circulation 1991; 83: 836-44.
27. Heinrich J, Balleisen L, Schulte H, Assmann G, van de Loo J. Fibrinogen and factor VII in the prediction of coronary risk: results from the PROCAM study in healthy men. Arterioscler Thromb 1994; 14: 54-9.
28. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. Circulation 1997; 96: 1102-8.
29. Grieninger G, Hertzberg KM, Pindyck J. Fibrinogen synthesis in serum-free hepatocyte cultures: stimulation by glucocorticoids. Proc Natl Acad Sci USA 1978; 75: 5506-10.
30. Thomas AE, Green FR, Kelleher CH, Wilkes HC, Brennan PJ, Meade TW, Humphries SE. Variation in the promoter region of the beta fibrinogen gene is associated with plasma fibrinogen levels in smokers and non-smokers. Thromb Haemost 1991; 65: 487-90.
31. Fowkes FG, Connor JM, Smith FB, Wood J, Donnan PT, Lowe GD. Fibrinogen genotype and risk of peripheral atherosclerosis. Lancet 1992; 339: 693-6.
32. Green FR. Fibrinogen polymorphisms and atherothrombotic disease. Ann NY Acad Sci 2001; 936: 549-59.
33. Folsom AR, Wu KK, Rasmussen M, Chambless LE, Aleksic N, Nieto FJ. Determinants of population changes in fibrinogen and factor VII over 6 years: the Atherosclerosis in Communities (ARIC) study. Arterioscler Thromb Vasc Biol 2000; 20: 601-6.
34. Kannel WB, D'Agostino RB, Belanger AJ, Silbershatz H, Tofler GT. Long-term influence of fibrinogen on initial and recurrent cardiovascular events in men and women. N Engl J Med 1996; 78: 90-2.
35. Folsom AR, Wu K, Sharrett A, Chambless L. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. Circulation 1997; 96: 1102-8.
36. Flaherty MJ, West S, Heimark RL, Fujikawa K, Tait JF. Placental anticoagulant protein-I: measurement in extracellular fluids and cells of the hemostatic system. J Lab Clin Med 1990; 115: 174-81.
37. Lowe GDO, Rumley A. Fibrinogen and its degradation products as thrombotic risk factors. Ann NY Acad Sci 2001; 936: 560-5.
38. Yu S, Sher B, Kudryk B, Redman CM. Fibrinogen precursors: order of assembly of fibrinogen chains. J Biol Chem 1984; 259: 10574-81.
39. Moaddel M, Falls LA, Farrell DH. The role of γA/γ' fibrinogen in plasma factor XIII activation. J Biol Chem 2000; 275: 32135-40.

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