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Research Article

A common mutation (G-455--> A) in the promoter region of the beta-fibrinogen gene has been associated with elevated plasma fibrinogen levels. Whether fibrinogen genotype affects plasma fibrinogen levels and risk of ischemic heart disease in the general population has not been studied. We investigated the association between fibrinogen genotype, plasma fibrinogen levels, and ischemic heart disease in a general population sample (n = 9,127). The A-allele (relative frequency, 0.20) was associated with elevated plasma fibrinogen levels in both genders ($P < 0.001$). While the effect of the A-allele on fibrinogen level was additive in men, the effect was dominant in postmenopausal women. The A-allele raising effect appeared to be two- to threefold greater in individuals with ischemic heart disease than in those without. An increase of 1 SD in plasma fibrinogen increased the odds ratio for ischemic heart disease by approximately 20% ($P < 0.01$ for women and < 0.005 for men). However, the frequency of the A-allele was similar in those with and without ischemic heart disease, and genotype was not a predictor of disease. These results demonstrate that the (G-455--> A) mutation in the promoter region of the beta-fibrinogen gene is associated with an increase in plasma fibrinogen in both genders in the general population. This increase does not appear to cause ischemic heart disease.

A Common Mutation ($G_{-455}\rightarrow A$) in the β-*Fibrinogen* Promoter is an Independent Predictor of Plasma Fibrinogen, but not of Ischemic Heart Disease

A Study of 9,127 Individuals Based on The Copenhagen City Heart Study

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Abstract

A common mutation ($G_{-455}\rightarrow A$) in the promoter region of the β-*fibrinogen* gene has been associated with elevated plasma fibrinogen levels. Whether *fibrinogen* genotype affects plasma fibrinogen levels and risk of ischemic heart disease in the general population has not been studied.

We investigated the association between *fibrinogen* genotype, plasma fibrinogen levels, and ischemic heart disease in a general population sample (n = 9,127).

The *A*-allele (relative frequency, 0.20) was associated with elevated plasma fibrinogen levels in both genders ($P <$ 0.001). While the effect of the *A*-allele on fibrinogen level was additive in men, the effect was dominant in postmenopausal women. The *A*-allele raising effect appeared to be two- to threefold greater in individuals with ischemic heart disease than in those without. An increase of 1 SD in plasma fibrinogen increased the odds ratio for ischemic heart disease by ~ 20% ($P <$ 0.01 for women and $<$ 0.005 for men). However, the frequency of the *A*-allele was similar in those with and without ischemic heart disease, and genotype was not a predictor of disease.

These results demonstrate that the ($G_{-455}\rightarrow A$) mutation in the promoter region of the β-*fibrinogen* gene is associated with an increase in plasma fibrinogen in both genders in the general population. This increase does not appear to cause ischemic heart disease. (*J. Clin. Invest.* 1997. 99:3034–3039.)
Key words: atherosclerosis • polymorphism • gene • genotype • menopause

Introduction

In large prospective studies in Europe (1–3) and the United States (4), plasma fibrinogen level has been shown to be an independent predictor of vascular disease, including ischemic heart disease (IHD),[1] in both women and men. Elevated plasma fibrinogen in an individual could either be the consequence of the presence of atherosclerosis or be due to the presence of predisposing genetic and/or environmental factors; evidence in favor of the latter has come from a recent report from the European Atherosclerosis Research Study (EARS) group (5).

There is little information on the relative contribution of genetic variation to the determination of plasma fibrinogen levels in the general population (6–8), although variation in the *fibrinogen* gene cluster on the long arm of chromosome 4 in itself is likely to be contributing. Of the polymorphisms studied to date, the ($G_{-455}\rightarrow A$) substitution in the 5′ flanking region of the β-*fibrinogen* gene is associated with the most consistent differences in plasma fibrinogen levels in both case-control studies, and in selected groups of healthy individuals (9–12). Whether *fibrinogen* genotype actually affects plasma fibrinogen levels and risk of IHD in the general population has not yet been studied.

This led us to initiate the present study to further elucidate the role of the ($G_{-455}\rightarrow A$) substitution in determining fibrinogen levels and susceptibility to IHD in a large general population sample. We conducted a study of 9,127 women and men from the Danish general population (The Copenhagen City Heart Study, reference 13), including 489 individuals with verified IHD. The primary study hypothesis was that genotype was an independent predictor of fibrinogen level in both genders, the *A*-allele being associated with the highest levels. The secondary study hypotheses were that plasma fibrinogen level and *fibrinogen* genotype both were independent predictors of ischemic heart disease.

Methods

Subjects. The Copenhagen City Heart Study (1991–1994) includes an almost equal number of women (55%) and men stratified into 10-yr age groups from 20 to 80 yr and above, drawn randomly from the Copenhagen Central Population Register, with the aim of drawing a sample representative of the adult general population in Copenhagen (13). Less than 1% were non-Caucasians and ~ 98.8% were of Danish descent. 9,127 individuals (93%) were genotyped for the present study. Within this sample, information on the prevalent events of IHD (WHO: 410–414; reference 14) and myocardial infarction (WHO: 410; reference 14) was collected and verified on 7,341 individuals by reviewing all hospital admissions and diagnoses (via The Danish National Hospital Discharge Register).

Laboratory analyses. Colorimetric and turbidimetric assays were used to measure nonfasting plasma levels of total and HDL cholesterol, triglycerides, apoB, apoAI, fibrinogen (all Boehringer Mannheim, Mannheim, Germany), and lipoprotein(a) (Dako A/S, Glostrup, Denmark). The ($G_{-455}\rightarrow A$) mutation was identified by PCR followed by restriction enzyme digestion of the amplified DNA (1,301 bp) with HaeIII as previously described (12).

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1. *Abbreviations used in this paper:* BMI, body mass index; HRT, hormonal replacement therapy; IHD, ischemic heart disease.

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Statistical methods. Data on women and men were analyzed separately using the SPSS for Windows program (release 7.0; Norusis MJ/SPSS Inc., Chicago, IL). $P < 0.05$ on a two-sided test was considered significant. Because of skewness, plasma triglycerides and lipoprotein(a) were logarithmically transformed ($\log_{10}$) before all analyses except the multiple logistic regression analysis (see below), to approach a normal distribution. Only post-hoc tests after ANOVA and tests of homogeneity of the effect of β-*fibrinogen* genotype on plasma fibrinogen levels (interaction) were corrected for multiple comparisons by the Bonferroni method.

For the primary study hypothesis (is β-*fibrinogen* genotype an independent predictor of plasma fibrinogen level?), as well as the derived exploratory hypothesis (does the effect of β-*fibrinogen* genotype differ in premenopausal and postmenopausal women, untreated or treated with hormonal replacement therapy?), ANOVA was used to test the effect of genotype on variation in plasma fibrinogen levels. If ANOVA revealed a significant main effect, all possible combinations of subgroup analyses (three for each ANOVA rejecting the null hypothesis) were tested by the Bonferroni test (a least-significant-difference test corrected for multiple comparisons by the Bonferroni method) to further explore the differences in fibrinogen levels between any two genotypes. For the second derived exploratory hypothesis (does the effect of β-*fibrinogen* genotype differ in women and men with and without IHD?), Student's *t* test was used for two group comparisons. For both the exploratory hypotheses mentioned above, the homogeneity of the association of genotype with plasma fibrinogen levels between the presence or absence of the discrete covariate was tested using interaction terms in an analysis of variance, including genotype and the covariate in question. The homogeneity of the association of genotype with plasma fibrinogen levels between tertiles of continuous covariates (age, cholesterol, HDL cholesterol, triglycerides, apoB, apoAI, lipoprotein(a), body mass index (BMI), waist–hip ratio), or between the presence or absence of smoking, diabetes mellitus, and hypertension were tested in the same way. ANOVAs exploring the effect of β-*fibrinogen* genotype on plasma fibrinogen level were performed with unadjusted fibrinogen values, as well as with values adjusted for age, apoB, apoAI, lipoprotein(a), BMI, and smoking habits by analysis of covariance (ANCOVA). The basis for choosing these covariates for the adjustment was that each of these covariates explained significant fractions ($P < 0.05$) of the remaining variance in plasma fibrinogen levels in both genders in the sample as a whole, as well as in individuals without IHD, when added as the only other covariate in an ANCOVA where age and BMI were already allowed for; diabetes mellitus and hypertension did not fulfill these criteria. Age and BMI are known to explain a substantial part of the variation in plasma fibrinogen levels, and in the present study explained ~ 17% of the total variation in plasma fibrinogen in both genders. Other continuous covariates like cholesterol, HDL cholesterol, triglycerides, and waist–hip ratio were all strongly correlated with covariates included in the adjustment model (apoB, apoAI, and BMI, respectively), and all explained significant, but smaller fractions of the remaining variance in fibrinogen level by the above method, and were therefore not included in the model. ANCOVA was also used to estimate the contribution of β-*fibrinogen* genotype to variation in fibrinogen levels, after adjustment by forced entry for the same covariates as above.

To test the secondary study hypotheses (is plasma fibrinogen or β-*fibrinogen* genotype an independent risk factor for IHD?), allele frequencies were estimated by gene counting, and odds ratios comparing patients with and without IHD within the general population cohort were calculated; Pearson's χ^2-test was used as a test of independence. Multiple logistic regression analysis, allowing for age only, or allowing for known major cardiovascular risk factors such as age, cholesterol, HDL cholesterol, triglycerides, lipoprotein(a), BMI, smoking habits, diabetes mellitus, and hypertension by forced entry, was performed to describe the relationship between the dependent variable (IHD) and the independent variables (plasma fibrinogen or β-*fibrinogen* genotype). Since cholesterol and apoB, HDL and apoAI, and BMI and waist–hip ratio are strongly positively correlated, only plasma cholesterol, HDL cholesterol, and BMI were included in the models. To allow for dependencies different from the linear on the logit scale, between the dependent (IHD) and independent variables, the following transformations were used: logarithmic transformation for plasma fibrinogen and inverse transformation for age, cholesterol, triglycerides, and lipoprotein(a). The transformations used were those that resulted in the best approximation to a linear dependency on the logit scale between the dependent and the independent variables. The methods used to asses this included plotting of the logit (IHD) for groups defined by the values of the independent continuous covariates and Box-Tidwell transformation (15, 16). Results for fibrinogen are given as odds ratios (e^{β}) for IHD with 95% confidence intervals ($e^{\beta \pm 1.96 \times SEM}$) for an increase of 1 SD in plasma fibrinogen level. Overall model fit was tested using the likelihood ratio test between models including and excluding the variable of interest.

Results

Basic characteristics of the general population sample are shown in Table I. Plasma fibrinogen concentrations increased with increasing age, plasma cholesterol, apoB, triglycerides, lipoprotein(a), BMI, and waist–hip ratio, and decreased with increasing HDL cholesterol and apoAI in both genders (Table II). Individuals with IHD, hypertension, or diabetes mellitus had higher plasma fibrinogen levels than those without, and smokers had higher levels than nonsmokers (Table III).

β-Fibrinogen genotype and plasma fibrinogen (primary study hypothesis). In both women and men, the *A*-allele was associated with the highest plasma fibrinogen concentrations (Fig. 1; ANOVA, $P < 0.001$ for both genders). There was no evidence for interaction between genotype and any of the following parameters: age, cholesterol, HDL cholesterol, apoAI, triglycerides, lipoprotein(a), BMI, waist–hip ratio, hypertension, diabetes mellitus, or smoking in either gender. However, there was evidence for interaction between *fibrinogen* genotype and apoB ($P = 0.02$) on plasma fibrinogen level in men, but not in women. This interaction in men was due to a larger fibrinogen-raising effect of the *A*/*A*-genotype in the lower tertile of apoB, compared with the middle and upper tertiles.

Table I. Characteristics of Individuals in the General Population Sample

	Women	Men
No.*	5049	4078
Age (yr)	58.3±0.2	56.8±0.2
Cholesterol (mmol/liter)	6.29±0.02	5.96±0.02
HDL cholesterol (mmol/liter)	1.73±0.01	1.38±0.01
Triglycerides (mmol/liter)	1.69±0.02	2.14±0.03
Lipoprotein(a) (mg/liter)	337±6	311±6
Fibrinogen (g/liter)	3.12±0.01	3.07±0.01
Body mass index (kg/m^2)	25.2±0.1	26.1±0.1
Hypertension (%)	19.3	21.0
Diabetes mellitus (%)	2.4	4.5
Smokers (%)	46.0	52.7
Exsmokers (%)	22.7	29.4
Nonsmokers (%)	31.2	17.8

*Number of individuals genotyped. The number for each characteristic varies slightly according to availability of data. Values in the top half of table are means±SEM.

Table II. Correlation Coefficients between Continuous Variables and Plasma Fibrinogen

	Women	Men
No.*	5049	4078
Age	0.34‡	0.38‡
Cholesterol	0.25‡	0.16‡
ApoB	0.31‡	0.23‡
HDL cholesterol	−0.22‡	−0.14‡
ApoAI	−0.17‡	−0.14‡
Triglycerides	0.26‡	0.11‡
Lipoprotein(a)	0.15‡	0.10‡
Body mass index	0.32‡	0.21‡
Waist–hip ratio	0.27‡	0.31‡

*Number of individuals genotyped. The number for each correlation varies slightly according to availability of data. Because of skewness, plasma triglycerides and lipoprotein(a) were logarithmically transformed before the analysis to approach a normal distribution. ‡$P < 0.001$.

On analysis of covariance, age, apoB, apoAI, lipoprotein(a), BMI, and smoking habits together explained ~ 25 and 20% of the total variation in plasma fibrinogen levels in both genders in the total general population sample and in individuals without IHD, respectively ($P < 0.001$ in all groups). After adjustment for these covariates, genotype explained ~ 1% of the remaining variance in plasma fibrinogen levels in the general population sample as a whole, as well as in individuals without IHD in both genders (Table IV; $P < 0.001$).

Except for plasma fibrinogen, there was no significant effect of *fibrinogen* genotype in either gender on any other parameters examined (cholesterol, apoB, HDL cholesterol, apoAI, triglycerides, lipoprotein(a), BMI, waist–hip ratio, systolic, or diastolic blood pressure [in untreated individuals only]) (data not shown).

β-Fibrinogen genotype and plasma fibrinogen in pre- and postmenopausal women, untreated or treated with hormonal replacement therapy (derived exploratory hypothesis). While the overall effect of the *A*-allele on plasma fibrinogen was additive in men, the overall effect in women appeared to be dominant (Fig. 1), with no additional effect on fibrinogen concentration in the homozygous state. This was supported statistically by a borderline significant interaction ($P = 0.06$) between gender and genotype on plasma fibrinogen level. Because of reports that hormonal replacement therapy (HRT) may lower plasma fibrinogen in postmenopausal women (17, 18), the data on women were stratified by menopausal status and by HRT in postmenopausal women. Postmenopausal women had significantly higher fibrinogen levels than premenopausal women (Student's *t* test, $P < 0.001$), but postmenopausal women treated with HRT had significantly lower levels than untreated postmenopausal women ($P < 0.001$). From a stratified analysis, it was apparent that the dominant effect of the *A*-allele on plasma fibrinogen found in women in the present study was due to a dominant effect in the majority of women (postmenopausal women not treated with HRT) (Fig. 2). In both premenopausal women and postmenopausal women treated with HRT, the effect of the *A*-allele appeared to be additive, although in premenopausal women the effect of the *A*-allele was smaller and differences only reached statistical significance (ANOVA, $P < 0.05$) when plasma fibrinogen was adjusted for age, apoB, apoAI, lipoprotein(a), BMI, and smoking habits.

β-Fibrinogen genotype and plasma fibrinogen in individuals with and without IHD (derived exploratory hypothesis). Because several studies have suggested variable effects of β-*fibrinogen* genotype on plasma fibrinogen levels in cases with IHD and in controls within the same study (9–11,19), the effect of β-*fibrinogen* genotype on plasma fibrinogen was examined in women and men with and without IHD. The *A*-allele was associated with significant increases in plasma fibrinogen in women and men both with and without IHD (Fig. 3); *G*/*A* and *A*/*A* genotypes were combined because of the relatively few individuals with the *A*/*A* genotype among those with IHD. Expressed as a percent increase, the *A*-allele raising effect was roughly threefold greater in women, and twofold greater in men with IHD compared with those without (Fig. 3). This was supported statistically by a significant ($P = 0.01$) interaction between genotype and disease status on unadjusted as well as adjusted plasma fibrinogen in women, and a similar but nonsignificant trend was found in men ($P = 0.34$).

Table III. Mean Fibrinogen Levels by Disease Status and Smoking Habits

	Women		Men	
	Plasma fibrinogen (g/liter)	No. of individuals	Plasma fibrinogen (g/liter)	No. of individuals
Without ischemic heart disease	3.20±0.01	3800	3.16±0.02	2834
With ischemic heart disease	3.57±0.08*	168	3.48±0.06*	302
Without hypertension	3.05±0.01	3937	3.01±0.02	3135
With hypertension	3.38±0.03*	947	3.29±0.03*	833
Without diabetes mellitus	3.10±0.01	4741	3.05±0.01	3766
With diabetes mellitus	3.66±0.09*	120	3.45±0.07*	180
Nonsmokers	3.05±0.02‡	1523	2.74±0.03‡	710
Exsmokers	3.09±0.03‡	1111	2.98±0.03‡	1160
Smokers	3.17±0.02‡	2230	3.23±0.02‡	2080

Values are mean±SEM. *$P < 0.001$, comparing individuals with and without disease by Student's *t* test. ‡$P < 0.001$, comparing individuals from the three smoking categories by ANOVA. Post-hoc test using the Bonferroni test, a modified least-significant-difference test, corrected for multiple comparisons by the Bonferroni method: women, smokers vs. nonsmokers, $P < 0.001$; smokers vs. exsmokers, $P < 0.05$. Men, smokers vs. nonsmokers, smokers vs. exsmokers, and exsmokers vs. nonsmokers, $P < 0.001$.

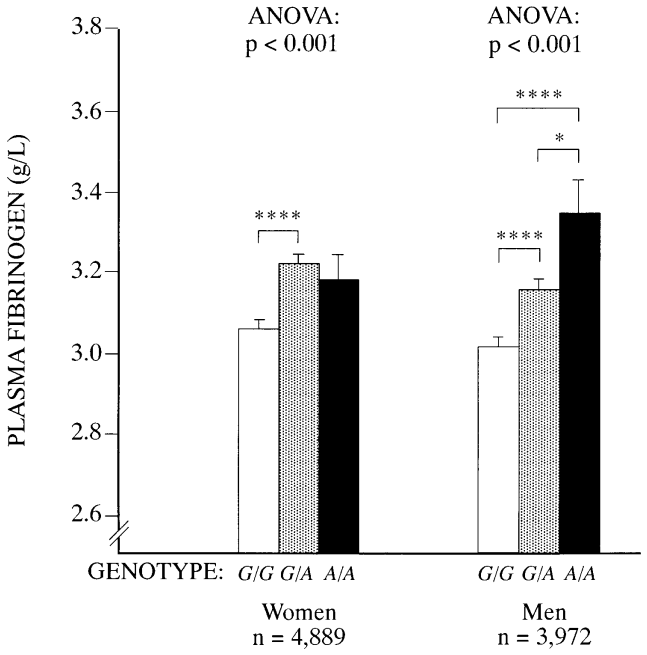

Figure 1. Plasma fibrinogen concentration as a function of β-*fibrinogen* ($G_{-455}\rightarrow A$) genotype in women and men in the total general population sample. Plasma fibrinogen is unadjusted for the effect of other covariates on plasma fibrinogen. $^{*}P \leq 0.05$, $^{****}P \leq 0.001$, on post-hoc tests between individual genotypes using the Bonferroni test, a modified least-significant-difference test, corrected for multiple comparisons by the Bonferroni method.

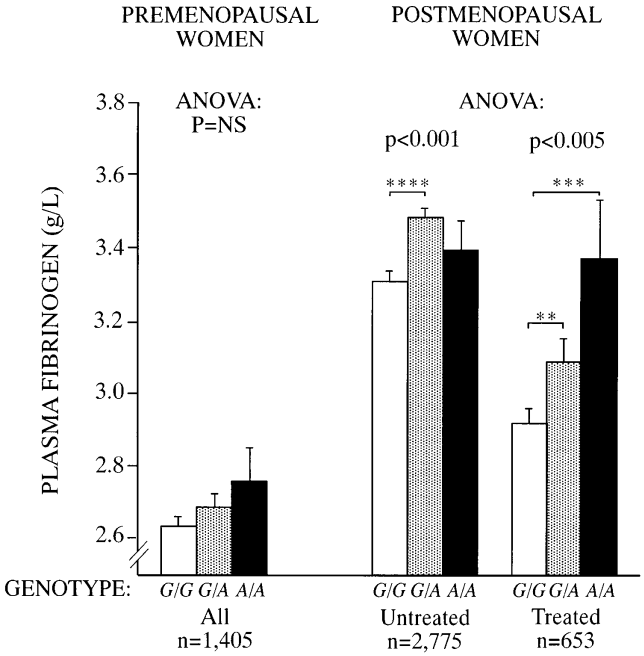

Figure 2. Plasma fibrinogen concentration as a function of β-*fibrinogen* ($G_{-455}\rightarrow A$) genotype in premenopausal women, in untreated postmenopausal women, and in postmenopausal women treated with hormonal replacement therapy. Plasma fibrinogen is unadjusted for the effect of other covariates on plasma fibrinogen. $^{**}P \leq 0.01$, $^{***}P \leq 0.005$, $^{****}P \leq 0.001$, on post-hoc tests between individual genotypes using the Bonferroni test, a least-significant-difference test corrected for multiple comparisons using the Bonferroni method.

Plasma fibrinogen and risk of IHD (secondary study hypothesis). Plasma fibrinogen concentration was significantly higher in women and men with IHD compared with those without (Table III, $P < 0.001$ in both genders by Student's *t* test). On multivariate logistic regression analysis (allowing for age only, or allowing for major cardiovascular risk factors such as age, cholesterol, HDL cholesterol, triglycerides, lipoprotein(a), BMI, smoking habits, diabetes mellitus, and hypertension), plasma fibrinogen concentration was an independent predictor of IHD in both genders (Table V; *P* values range from < 0.01 to < 0.001). An increase of 1 SD in plasma fibrinogen increased the odds ratio for IHD by 22% in women and 17% in men when the above-mentioned major cardiovascular risk factors were allowed for.

β-Fibrinogen genotype and risk of IHD (secondary study hypothesis). The relative frequencies of the *G*/*G*, *G*/*A*, and *A*/*A* genotypes were not significantly different from values predicted by the Hardy-Weinberg equilibrium in the total general population sample. The relative frequency of the *A*-allele in the general population sample was 0.20 in both genders (women versus men; χ^2, $P = 0.28$). There were no significant differences in relative genotype or allele frequencies between women and men with and without IHD in the general population sample as a whole (Table VI), or in women and men ≤ 60 or > 60 yr of age (data not shown). In agreement with this, the univariate odds ratios for IHD as a function of genotype were close to 1.0 and nonsignificant in both genders (data not shown). On multivariate logistic regression analysis, the odds ratios for IHD as a function of genotype were also not significant, (i.e., not different from 1.0) regardless of the effect assumed for the *A*-allele (additive or dominant; data not shown).

Table IV. Effect of Fibrinogen Genotype on Variation in Plasma Fibrinogen Level

	General population sample	General population sample without ischemic heart disease
Women	1.03%* (n = 4624)	0.98%* (n = 3610)
Men	1.03%* (n = 3676)	0.85%* (n = 2639)

Analysis of covariance adjusted for age, apoB, apoAI, lipoprotein(a), body mass index, and smoking habits. Because of skewness, plasma lipoprotein(a) was logarithmically transformed ($\log_{10}$) before the analysis to approach a normal distribution. $^{*}P < 0.001$; the value is expressed as a (partial correlation coefficient)^2.

Discussion

These results in a large general population sample strongly confirm the relationship in both genders between the β-*fibrinogen* ($G_{-455}\rightarrow A$) promoter polymorphism and plasma fibrinogen levels that has previously been reported in selected samples of healthy middle-aged men (12, 20), young healthy men and women (11), and in male survivors of myocardial infarction (9, 10, 19). The precise molecular mechanism of the fibrinogen-raising effect associated with the *A*-allele is not yet clear, but it is likely that the $G\rightarrow A$ change is either itself directly functional or is a marker for another functionally significant change that affects the strength of the β-*fibrinogen* promoter (11). Since synthesis of the β-*fibrinogen* protein in hepatocytes appears to be the rate-limiting step in the production of the

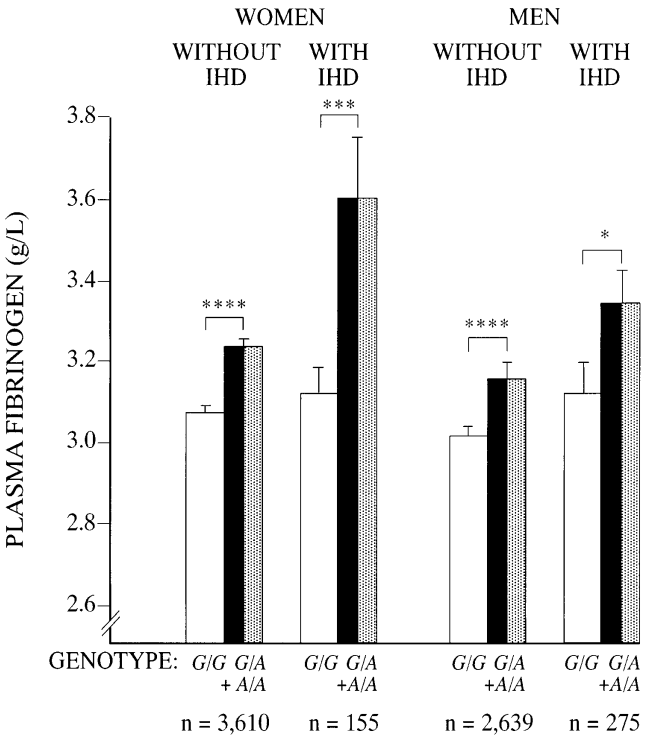

Figure 3. Plasma fibrinogen as a function of β-*fibrinogen* ($G_{-455}\rightarrow A$) genotype in women and men with and without ischemic heart disease. *G*/*A* and *A*/*A* genotypes were combined because there were only a few individuals with the *A*/*A* genotype among individuals with ischemic heart disease. Plasma fibrinogen is adjusted for age, apoB, apoAI, lipoprotein(a), body mass index, and smoking habits by analysis of covariance. Because of skewness, plasma lipoprotein(a) was logarithmically transformed ($\log_{10}$) before this analysis. *$P \leq 0.05$, ***$P \leq 0.005$, ****$P \leq 0.001$, between two individual genotypes on Student's *t* test.

mature 2α+2γ+2β-fibrinogen protein from the liver (21), even relatively small changes in the rate of transcription of the β-gene and thus the rate of β-protein synthesis would be expected to affect plasma fibrinogen levels.

Because the derived exploratory hypotheses were not proposed a priori (see Results), multiple testing may have resulted in some chance findings. Nevertheless, in this large sample, the pattern of the association between the *A*-allele and higher fibrinogen levels is different in the two genders; while the effect of the A-allele is additive in men, it appears to be dominant in women. It could be that women with the genotype *A*/*A* and fibrinogen levels as high as seen in men are no longer present in the population-based sample (i.e., dead or not able to participate). This seems unlikely as men with these levels are present in the sample and there is no significant deficiency of the *A*/*A* genotype group in women compared with men (prevalence of *A*/*A* genotype 3.7 vs. 4.2%, respectively). Another possibility is that hormones or other gender-specific factors modulate the genotype-elevated fibrinogen levels either by downregulating hepatic fibrinogen synthesis, or upregulating fibrinogen clearance. This seems a more likely explanation, since a dominant effect of the A-allele on plasma fibrinogen level was apparent only in untreated postmenopausal women, and neither in premenopausal women, nor in postmenopausal women on HRT.

Table V. Odds Ratio for Ischemic Heart Disease for a 1-SD Increase in Plasma Fibrinogen

	Allowed for age*		Allowed for major cardiovascular risk factors‡	
	Odds ratio	95% CI	Odds ratio	95% CI
Women (n = 3763)	1.27	(1.11–1.46)¶	1.22	(1.06–1.40)§
Men (n = 2912)	1.19	(1.09–1.30)¶	1.17	(1.06–1.29)‖

*Multivariate logistic regression analysis allowing for age only. ‡Multivariate logistic regression analysis allowing for age, cholesterol, HDL cholesterol, triglycerides, lipoprotein(a), body mass index, smoking habits, diabetes mellitus, and hypertension. To allow for dependencies different from the linear on the logit scale, between the dependent and independent variables, the following transformations were used: logarithmic ($\log_{10}$) transformation for plasma fibrinogen, inverse transformation for age, cholesterol, triglycerides, and lipoprotein(a). CI, confidence interval; §$P < 0.01$, ‖$P < 0.005$, ¶$P < 0.001$ on the likelihood ratio test between models including and excluding plasma fibrinogen.

Another finding in the present study is that the fibrinogen-raising effect associated with the *A*-allele is two- to threefold larger in those with IHD, especially in women, compared with those without. The data suggest that the *A*-allele in individuals with IHD may be associated with a greater fibrinogen response than in individuals without IHD, probably secondary to other changes resulting from IHD, and perhaps cytokine induced (11, 22, 23).

Using multiple logistic regression analysis, plasma fibrinogen levels were highly significantly associated with the presence of IHD in both genders, and this was independent of other classical cardiovascular risk factors. The odds ratios for IHD for a 1 SD increase in plasma fibrinogen in the present study were similar to values found for women and men in the

Table VI. Relative Frequencies of β-Fibrinogen Promoter Genotypes and Alleles in Women and Men from the General Population Sample with and without Ischemic Heart Disease

	Without ischemic heart disease	With ischemic heart disease
No. of women	3937	174
Genotype		
G/*G*	0.63 (2467)	0.58 (101)
G/*A*	0.34 (1323)	0.40 (70)
A/*A*	0.04 (147)	0.02 (3)
A-allele	0.21	0.22
No. of men	2915	315
Genotype		
G/*G*	0.64 (1877)	0.65 (204)
G/*A*	0.31 (913)	0.31 (98)
A/*A*	0.04 (125)	0.04 (13)
A-allele	0.20	0.20

Number of individuals in parentheses. Pearson's χ^2-test was used to test for differences in distribution of genotypes and alleles between individuals with and without ischemic heart disease. $P > 0.05$ for all comparisons.

Framingham Study (4). The present results in a large population-based sample studied cross-sectionally are therefore in support of the role of fibrinogen level as a risk factor for IHD as reported in prospective studies (1–4). Therefore, it is apparently paradoxical that in this sample plasma fibrinogen is independently associated with IHD, the β-promoter *A*-allele is associated with higher fibrinogen levels, but genotype is not in itself directly associated with IHD. The simplest interpretation of these data is that the increase in fibrinogen concentration resulting from the G_{-455}→A substitution does not cause IHD. Therefore, increased fibrinogen levels in IHD patients observed in this and other studies are most likely secondary to other "changes" resulting from or causing IHD. An alternative explanation is that the overall contribution of genotype to between-individual differences in plasma fibrinogen is too small to be detected as a factor increasing the risk of IHD in the present study. In a population-based sample such as this, age, apoB, apoAI, lipoprotein(a), BMI, and smoking habits explain 20–25% of the variance in fibrinogen levels, and the *G*/*A* genotype, only an additional 1%. Furthermore, the fibrinogen-raising effect of the *A*-allele is modest in absolute terms; men with the genotype *A*/*A* have mean levels 0.33 g/liter (11%) higher than those with the *G*/*G* genotype, and in women this effect is much smaller (0.12 g/liter, 4% higher). Finally, since *A*/*A* individuals represent only 4% of the population, the population-attributable risk associated with this genotype is also small, ~1% extrapolating from prospective studies (2). To detect effects of this size as a genotype or allele frequency difference between individuals with and without IHD, a larger study is needed.

In summary, our data demonstrate an association of the β-*fibrinogen* promoter A_{-455}-allele with higher fibrinogen levels in both genders in the general population, and suggest that the *A*-allele, either alone or through linkage disequilibrium with another mutation, causes elevated plasma fibrinogen levels. Though plasma fibrinogen was an independent predictor of IHD in both women and men, the increase in fibrinogen levels associated with the (G_{-455}→A) mutation was not associated with IHD in either gender.

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