

# Elevated Plasma Fibrinogen

## Cause or Consequence of Cardiovascular Disease?

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**Abstract**—An association between increased plasma fibrinogen and an increased risk for myocardial infarction (MI) is well established, but the nature of this association is subject to debate. Our aim was to shed light on the potentially causal nature of this association. We examined whether increased plasma fibrinogen, due to a condition that is independent of cardiovascular events, also increases the risk for MI. A case-control study was performed in 139 subjects with a history of MI and 287 control subjects selected from the Rotterdam Study, a population-based cohort of 7983 subjects aged 55 years and older. The genotype of the  $-455G/A$  polymorphism in the fibrinogen  $\beta$ -gene was determined by polymerase chain reaction. Functional plasma fibrinogen levels were determined according to von Clauss. The plasma level of fibrinogen was significantly higher in subjects with one or two *A* alleles compared with subjects with the *GG* genotype: 3.8 (95% confidence interval [CI], 3.6 to 3.9) g/L and 3.6 (3.5 to 3.7) g/L, respectively. With increasing plasma fibrinogen level, the risk for MI increased gradually; a rise in fibrinogen of 1 g/L was associated with a 45% increased risk (odds ratio adjusted for age, sex, and smoking, 1.45; 95% CI, 1.12 to 1.88). There was no association between the genotype of the  $-455G/A$  polymorphism and the risk for MI. The  $-455G/A$  polymorphism is therefore associated with increased plasma fibrinogen levels but not with an increased risk for MI. These findings indicate that an increased plasma fibrinogen level due to this genetic factor does not increase the risk for MI. (*Arterioscler Thromb Vasc Biol.* 1998;18:621-625.)

**Key Words:** cardiovascular disease risk ■ thrombotic tendency ■ *HaeIII* polymorphism ■  $\beta$ -fibrinogen gene ■  $-455G/A$  polymorphism

High plasma fibrinogen levels are associated with an increased risk for myocardial infarction (MI).<sup>1-3</sup> To date, it is not clear whether an elevated plasma fibrinogen level itself increases the risk for MI (risk factor) or whether the elevated level is merely a reflection of the presence of preclinical atherosclerosis or of an association with a true risk factor (risk indicator).

Fibrinogen is related to both preclinical atherosclerosis and to most of the cardiovascular risk factors. To elucidate whether high plasma fibrinogen per se increases the risk for MI, one should study the association of fibrinogen, increased due to a factor not related to MI, with the risk for MI. When such an “independently” raised fibrinogen level is associated with an increased risk for MI, then this indicates that plasma fibrinogen is causally related to the risk for MI. Unfortunately, no intervention to influence plasma fibrinogen level specifically is known. Drugs known to influence fibrinogen levels, such as fibric acid derivatives, also alter many other factors related to the risk of MI.<sup>4</sup>

An alternative approach is to study genetic markers that are associated with elevated fibrinogen levels. Several poly-

morphisms in the genes for fibrinogen have been associated with increased plasma fibrinogen, such as the  $-455G/A$  polymorphism.<sup>5</sup> An individual's genetic status is changed neither by the presence of disease nor by risk factors for the disease. Therefore, we presume that a genetically determined increase in fibrinogen level is independent of other factors related to MI. We propose that if a certain genotype for fibrinogen is associated with an increased plasma fibrinogen level and this increased plasma fibrinogen level causes an increased risk for MI, then that genotype should also be associated with an increased risk for MI.

The *A* allele of the  $-455G/A$  polymorphism in the  $\beta$ -fibrinogen gene has been associated with increased levels of plasma fibrinogen.<sup>6</sup> Whether possession of the *A* allele is associated with an increased risk for MI is not clear. In two studies, the  $-455G/A$  polymorphism was not associated with the risk for MI, but the authors reported that bias in selection of subjects or the weak association between genotype and plasma fibrinogen level might explain the absence of an association.<sup>7,8</sup>

The present study was conducted to explore whether the *A* allele of the  $-455G/A$  polymorphism in the fibrinogen

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$\beta$ -gene is associated with raised fibrinogen levels on the one hand and with an increased risk for MI on the other.

## Methods

### Population

A cross-sectional, case-control study was performed among subjects selected from the Rotterdam Study. The Rotterdam Study is a prospective population-based study of 7983 subjects. Between March 1990 and July 1993, all subjects aged 55 years and older living in Ommoord, a suburb of Rotterdam, the Netherlands, were invited to participate. The overall response rate was 78%. The study was approved by the Medical Ethics Committee of Erasmus University, and written, informed consent was obtained from all participants. The rationale and design of the study have been described elsewhere.<sup>9</sup>

Cases ( $n=150$ ) were selected from the cohort on the basis of the presence of an infarction pattern on the electrocardiogram (ECG) and using the diagnostic classification system of the modular ECG analysis system,<sup>10,11</sup> independent of a history of chest pain. Two control subjects per case were drawn from the same 5-year age stratum wherefrom the cases of MI were drawn, and these control subjects constituted a sample of study participants who had no history of cardiovascular disease, ie, no history of MI, angina pectoris, stroke, or abnormal ECG and no peripheral arterial disease (ankle/arm index  $>0.9$ ). We excluded subjects who were using anticoagulant drugs.

### Measurements

All subjects were first visited at their home. Information on current health status, medical history, drug use, and smoking behavior was obtained by a computerized questionnaire, which included the Dutch version of the Rose cardiovascular questionnaire.<sup>12</sup> The home interview was followed by two visits at the research center. Subjects were not asked to fast or to refrain from smoking. During the clinic visits, several cardiovascular risk indicators were determined. Height and weight were measured, and body mass index was calculated as weight in kilograms divided by the square of height in meters squared. Sitting blood pressure was measured at the right upper arm with a random-zero sphygmomanometer. The average of two measurements obtained on one occasion was used. Systolic blood pressure at the ankles (posterior tibial artery) was measured with an 8-MHz Doppler transducer while the subject was in the supine position with an adult-size regular cuff just above the malleoli.<sup>13</sup> The ankle/arm index is the ratio of the systolic blood pressure at the ankle to that at the arm. Peripheral arterial disease was defined as a right or left ankle/arm index  $\leq 0.9$ . Blood sampling and storage have been described elsewhere.<sup>14</sup> Blood samples were collected in evacuated tubes containing CTAD (Vacutainers; 0.11 mol/L citrate, 15 mmol/L theophylline, 3.7 mmol/L adenosine, and 0.198 mmol/L dipyrindamole; Diatube H, Becton-Dickinson).<sup>15</sup> Fibrinogen was measured as described by von Clauss.<sup>16</sup> The  $-455G/A$  polymorphism restriction fragment length polymorphism of the fibrinogen  $\beta$ -gene was determined by amplification of the polymorphic region by polymerase chain reaction, followed by digestion with the restriction enzyme *HaeIII* as described by Thomas et al.<sup>6</sup>

### Statistical Analysis

Means and proportions of cardiovascular risk factors for men and women were calculated. Because homozygosity for the *A* allele was rare and heterozygosity for the  $-455G/A$  polymorphism was also associated with increased levels of fibrinogen, we combined both genotypes into one group. Subjects with a genetically increased fibrinogen level, ie, those possessing one or two *A* alleles, were categorized as the exposed group and compared with those homozygous for the *G* allele (*G/G* genotype; the nonexposed group).

We examined whether increased plasma fibrinogen level was a risk indicator in our study by comparing the risk for MI for different levels of fibrinogen by using a logistic regression model with fibrinogen level as a continuous variable. The number of cases and

TABLE 1. Baseline Characteristics of the Study Population

	Men	Women
n	189	237
Age, y	70 (8)	74 (9)
Systolic blood pressure, mm Hg	137 (21)	142 (12)
Diastolic blood pressure, mm Hg	74 (12)	74 (12)
Body mass index, kg/m <sup>2</sup>	26 (2.7)	27 (4.2)
Current smoking, %	28	13
Total cholesterol, mmol/L	6.30 (1.11)	6.63 (1.13)
HDL cholesterol, mmol/L	1.25 (0.34)	1.42 (0.35)
Fibrinogen, g/L	3.57 (0.83)	3.72 (0.84)

Values are percentages or means and SDs.

control subjects for the exposed and nonexposed groups were determined, and logistic regression was used to calculate the relative risk (estimated as the odds ratio) for MI in the exposed compared with the nonexposed group. To examine the presence of confounding in the study population, we compared means and proportions of cardiovascular risk factors for exposed and nonexposed subjects. Differences were tested with Student's *t* test and Pearson's  $\chi^2$  test. Additional adjustments for the established cardiovascular disease risk factors that might confound the association were made by adding them to the logistic model.

To examine the potentially modifying effects of age, sex, smoking behavior, body mass index, serum total and HDL cholesterol, and systolic and diastolic blood pressure on the association between the  $-455G/A$  polymorphism and the risk for MI, we stratified the study population of men and women by age categories (cutpoint at 75 years); in current smokers (subjects who answered the question "Do you smoke?" with "yes") versus nonsmokers (former and never-smokers); and according to increasing levels of body mass index (cutpoints at 25 and 27 kg/m<sup>2</sup>), systolic blood pressure (cutpoints at 130 and 145 mm Hg), total cholesterol (cutpoints at 5.5 and 7.0 mmol/L), and HDL cholesterol (cutpoints at 1.0 and 1.5 mmol/L).

## Results

After exclusion of those with missing data, the remaining study population consisted of 139 cases and 287 control subjects. General characteristics are presented in Table 1. The frequency of the *A* allele was .20, and the distribution of the genotypes was in Hardy-Weinberg equilibrium in both cases and control groups.

Plasma fibrinogen level was higher in the subjects carrying one or two *A* alleles compared with those homozygous for the

TABLE 2. Plasma Fibrinogen (g/L) in the *GG* and the *GA+AA* Genotypes for Cases, Control Subjects, Current Smokers, and Noncurrent Smokers

	<i>GG</i>	<i>GA+AA</i>	<i>P</i>
Cases of myocardial infarction*	3.83 (3.65, 4.01)	3.82 (3.59, 4.05)	.94
Control subjects*	3.47 (3.36, 3.58)	3.72 (3.57, 3.87)	.009
Current smokers†	3.97 (3.70, 4.24)	3.96 (3.65, 4.28)	.79
Noncurrent smokers†	3.50 (3.40, 3.60)	3.71 (3.57, 3.84)	.02

*GG*=homozygous for the *G* allele; *GA+AA*=carriers of the *A* allele.

\*Values are means and (95% confidence intervals) adjusted for age, sex, and smoking.

†Values are means and (95% confidence intervals) adjusted for age, sex, and case/control status.

*G* allele: 3.8 g/L (95% confidence interval [CI], 3.6 to 3.9) and 3.6 (3.5 to 3.7), respectively. The association between fibrinogen and the presence of the *A* allele was restricted to control subjects and nonsmokers (Table 2). Fibrinogen level was associated with the risk for MI; this risk increased by 45% for each 1 g/L increase in fibrinogen level (odds ratio, 1.45; 95% CI, 1.12 to 1.88).

Among the 139 cases, 86 subjects (62%) were homozygous for the *G* allele. Similarly, among the 287 control subjects, 183 subjects (64%) were homozygous for the *G* allele. The risk for MI was not different for *A* allele carriers compared with non-*A* allele carriers (Table 3). To check whether the genetic status was associated with other cardiovascular disease risk factors (confounders), we compared these risk factors across genetic categories (Table 4). The *G/G* genotype was associated with somewhat higher total serum cholesterol levels, higher HDL cholesterol levels, and possibly also with a lower proportion of smokers and a somewhat higher systolic and diastolic blood pressure. Additional adjustments for smoking behavior, total and HDL cholesterol, and systolic and diastolic blood pressure did not substantially change the association between the polymorphism and the risk for MI (Table 3).

Because the association between the presence of the *A* allele and plasma fibrinogen level was restricted to nonsmokers, we examined the association between the presence of the *A* allele and MI in nonsmokers (102 cases and 241 control subjects). The association did not substantially differ from the overall results: odds ratio after adjustment for age and sex, 0.93; 95% CI, 0.56 to 1.53.

No evidence for an effect modification of the association between the -455G/A polymorphism and the risk for MI by age, sex, body mass index, serum total cholesterol, serum HDL cholesterol, systolic blood pressure, or diastolic blood pressure was observed. Results for age, sex, and smoking behavior are presented in Table 5.

## Discussion

The main finding of this study is that the *A* allele of the -455G/A polymorphism in the fibrinogen  $\beta$ -gene was found to be associated with increased plasma fibrinogen but not with an increased risk for nonfatal MI.

To appreciate these findings, some aspects of the study design need to be discussed. To address our etiologic question, we performed a population-based, case-control study. Cases of nonfatal MI and control subjects were drawn from

**TABLE 4. Means and Proportions of Cardiovascular Disease Risk Factors for the Different Genotypes**

	<i>GG</i>	<i>GA+AA</i>	<i>P</i>
n	269	157	
Age, y	71.2 (0.5)	73.0 (0.7)	.14
Women, %	58	50	.16
Systolic blood pressure, mm Hg	140 (1.3)	138 (1.7)	.19
Diastolic blood pressure, mm Hg	74 (0.7)	73 (0.9)	.20
Body mass index, kg/m <sup>2</sup>	26.4 (0.2)	26.4 (0.3)	.93
Current smoking, %	17	22	.18
Total cholesterol, mmol/L	6.53 (0.07)	6.40 (0.09)	.26
HDL cholesterol, mmol/L	1.38 (0.02)	1.31 (0.03)	.04
Fibrinogen, g/L	3.59 (0.05)	3.76 (0.07)	.03

*GG*=homozygous for the *G* allele; *GA+AA*=heterozygous or homozygous for the *A* allele.

Values are percentages or means and (SEs).

Adjusted for case/control status and when appropriate, for age and sex.

one large, single-center study. All subjects participating in the study were white, and allele frequencies were similar to those reported by others.<sup>6</sup> The observed distribution of genotypes was identical to the expected distribution (Hardy-Weinberg equilibrium) in cases as well as control subjects. Also, allele frequencies for each genotype were similar in case and control groups. This confirms that case and control subjects originated from the same source population and provides support for the absence of an association between the -455G/A polymorphism and MI. A source of bias in a cross-sectional design might be that the risk profile of cases has changed after their MI. Because the aim of the present study was primarily to investigate the influence of possession of the *A* allele on the risk for MI, this potential source of bias does not play a role. Plasma fibrinogen level may be different after an MI, but genotypes are not changed by an infarct. Finally, by virtue of its design, a cross-sectional study is limited to nonfatal cases of MI. If the *A* allele were associated with an increased proportion of fatal cases of MI, then we would underestimate the true risk for MI associated with possession of the *A* allele. To examine whether the *A* allele is associated with an increased proportion of fatal cases of MI, a longitudinal study is needed.

**TABLE 5. Risk for Myocardial Infarction (Odds Ratio Adjusted for Age and Sex) for the *GA* and *AA* Genotypes Compared With the *GG* Genotype, Stratified for Age, Sex, and Smoking Behavior**

Group	Relative Risk for Myocardial Infarction	95% CI
All study participants	1.01	0.66, 1.55
Age <75 years	0.80	0.47, 1.36
Age >75 years	1.31	0.69, 2.50
Men	0.85	0.48, 1.50
Women	1.09	0.61, 1.97
Smokers	1.01	0.42, 2.41
Nonsmokers	0.94	0.59, 1.50

**TABLE 3. Relative Risk (Odds Ratios and 95% Confidence Intervals [CIs]) for Myocardial Infarction Associated With the *GA* and *AA* Genotypes Compared With the *GG* Genotype**

	Relative Risk	95% CI
Crude risk	1.08	(0.71, 1.65)
Adjusted for age and sex	1.01	(0.66, 1.55)
Adjusted for age, sex, and smoking	0.95	(0.62, 1.47)
Adjusted for all of the above and HDL cholesterol	0.87	(0.55, 1.35)
Adjusted for all of the above and systolic and diastolic blood pressure	0.97	(0.61, 1.52)



**TABLE 6. Results of Studies That Reported the Association Between the -455 G/A Polymorphism and the Risk of Myocardial Infarction (Odds Ratio and 95% Confidence Interval [95% CI] of Subjects With an A Allele Compared With Those Without an A Allele**

Study	Cases (n)	Controls (n)	Relative Risk and (95% CI)
ECTIM <sup>7</sup>	533	648	0.89 (0.69, 1.13)
Gardemann et al <sup>8</sup>	450	473	0.94 (0.72, 1.22)
Rotterdam	139	287	1.01 (0.66, 1.55)

Assessment of causality of the association between fibrinogen and risk for MI has been difficult. Fibrinogen, as an acute-phase protein, is strongly associated with (preclinical) atherosclerosis and also with a number of cardiovascular risk factors, notably smoking.<sup>17-19</sup> Therefore the link between fibrinogen and cardiovascular events may have been due to the association of fibrinogen with these other factors. Examining the association between a determinant of fibrinogen that is independent of these other factors and the risk for MI provides an estimate of the association between fibrinogen and MI that is unbiased with respect to the other determinants of fibrinogen. Our "independent determinant" of plasma fibrinogen was the -455G/A polymorphism. Nevertheless, the question remains whether fibrinogen measurement with the clotting rate-based method as described by von Clauss is specific enough to determine the association between plasma fibrinogen and the risk for MI. At least three molecular forms of fibrinogen have been identified, which may have different effects on the risk for MI.<sup>20</sup> Recent studies on fibrates and ticlopidine have shown reductions in fibrinogen, as measured with a functional clotting rate-based method, but unchanged fibrinogen molecular mass values.<sup>21</sup> We cannot exclude the possibility that the increase in fibrinogen level due to the -455G/A polymorphism reflects a form other than the "risk-carrying" form of fibrinogen.

Quantitative studies in humans are, by their nature, limited in precision, and it is never possible to achieve the degree of control possible in a laboratory. Perhaps the most persuasive evidence to support a judgment of a cause-effect relationship arises when a number of studies show similar results. The association between the -455G/A polymorphism and MI has been studied in two other studies.<sup>5,7,8</sup> In both studies, this polymorphism was associated with plasma fibrinogen levels but not with the risk for MI. Results of these studies are presented in Table 6. Thus, although it is difficult to prove the absence of an association, three studies that show the same absence of association suggest that an increased fibrinogen level due to the presence of the A allele is truly not associated with the risk for MI.

Stratified analyses did not support the presence of modification of the strength of the (absent) association for any of the factors mentioned. It remains possible that some unknown factor modifies the effect of possession of the A allele on the risk for MI. Still, one expects an overall trend to an increase in risk for MI. As the risk for nonfatal MI did not even tend to be increased in the A allele carriers, we

presume that possession of the A allele is not associated with an increased risk for nonfatal MI.

The association between the -455G/A polymorphism and plasma fibrinogen was not found in current smokers, nor in cases with a history of MI. A possible explanation for this is that in smokers and in cases, other factors increase the fibrinogen level and thereby obscure the contribution of the A allele. In the ECTIM Study, the association between the -455G/A polymorphism and fibrinogen level was, however, more pronounced in smokers and in cases.<sup>5</sup> A difference between the ECTIM Study and our study is that in our study, smoking status and fibrinogen level were assessed at the same time, whereas in the ECTIM Study, blood samples were taken 3 to 9 months after the MI and cigarette consumption was defined as the daily consumption just before the event.

For clinical practice the question of causality is important. The role of fibrinogen as an indicator for an increased risk for MI is well established. Our finding suggests that directly lowering fibrinogen levels may not decrease the risk for MI. Increased plasma fibrinogen level may, however, change after reductions in risk by other measures.

In conclusion, the finding that, in our population of men and women aged 55 years and over, the A allele of the -455G/A polymorphism in the fibrinogen  $\beta$ -gene is associated with increased plasma fibrinogen levels but not with an increased risk for MI does not support the view that this increased plasma fibrinogen level is causally related to the risk for MI.

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