Fibrinogen Genes Modify the Fibrinogen Response to Ambient Particulate Matter

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Rationale: Ambient particulate matter has been associated with systemic inflammation indicated by blood markers such as fibrinogen, implicated in promoting atherothrombosis.

Objectives: This study evaluated whether single-nucleotide polymorphisms (SNPs) within the fibrinogen genes modified the relationship between ambient particles and plasma fibrinogen.

Methods: In 854 myocardial infarction survivors from five European cities plasma fibrinogen levels were determined repeatedly (n = 5,082). City-specific analyses were conducted to assess the impact of particulate matter on fibrinogen levels, applying additive mixed models adjusting for patient characteristics, time trend, and weather. City-specific estimates were pooled by meta-analysis methodology.

Measurements and Main Results: Seven SNPs in the FGA and FGB genes shown to be associated with differences in fibrinogen levels were selected. Promoter SNPs within FGA and FGB were associated with modifications of the relationship between 5-day averages of particulate matter with an aerodynamic diameter below 10 μm (PM10) and plasma fibrinogen levels. The PM10-fibrinogen relationship for subjects with the homozygous minor allele genotype of FGB rs1800790 compared with subjects homozygous for the major allele was eight-fold higher (P value for the interaction, 0.037).

Conclusions: The data suggest that susceptibility to ambient particulate matter may be partly genetically determined by polymorphisms that alter early physiological responses such as transcription of fibrinogen. Subjects with variants of these frequent SNPs may have increased risks not only due to constitutionally higher fibrinogen concentrations, but also due to an augmented response to environmental stimuli such as ambient particulate matter.

Keywords: air pollution; inflammation; genetic susceptibility; epidemiology; particulate matter

Fibrinogen plays a key role in the clotting cascade, where its conversion to fibrin stabilizes blood clots on injury. Fibrinogen has procoagulant as well as proinflammatory properties and thereby promotes atherothrombosis. Elevated concentrations of circulating fibrinogen in the blood have been consistently associated with an increased risk for myocardial infarction in epidemiologic studies (1). Fibrinogen is a large fibrous glycoprotein (340 kD) composed of two subunits with three protein chains each (α, β, and γ chains). We found, in a sample of 895 survivors of myocardial infarction, that polymorphisms in the fibrinogen genes encoding the fibrinogen protein chains are associated with variations in fibrinogen plasma levels (2). However, whether polymorphisms in the fibrinogen genes are independent risk factors for coronary artery disease has not been established. van der Krabben and colleagues (3) found no association between coagulation factor polymorphisms per se, previously associated with plasma levels, and the risk of recurrent cardiovascular events in survivors of myocardial infarction.

Ambient air pollution has been linked to exacerbation of cardiovascular disease morbidity and mortality (4). Systemic inflammation induced by particle deposition in the lung is one of the potential mechanisms linking ambient air pollution to myocardial infarctions (4, 5). Evidence has been reported that fibrinogen concentrations increase in association with high ambient air pollution concentrations during the days preceding myocardial infarction (6–8).

The aim of the study presented here was to assess whether genetic polymorphisms within the fibrinogen genes (FGA [α

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Ambient particulate air pollution has been associated with inflammatory responses, but little is known about the extent to which individual responses vary due to genetic predisposition.

What This Study Adds to the Field

Subjects with variants in the fibrinogen gene cluster may have increased risks not only due to constitutionally higher fibrinogen concentrations, but also due to an augmented response to environmental stimuli such as ambient particulate matter.

* A complete list of members may be found before the beginning of the REFERENCES.

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chain], FGB [β chain], and FGG [γ chain]) modify the associations between changes in ambient particle concentrations and plasma fibrinogen levels. The study focused on survivors of myocardial infarction (9), a potentially susceptible subgroup of the general population with respect to air pollution (10).

Some of the results of this study have been previously reported in the form of an abstract (11).

METHODS

Field Study

Fibrinogen concentrations were assessed in a multicenter longitudinal study of myocardial infarction survivors from five European cities—Augsburg, Germany; Barcelona, Spain; Helsinki, Finland; Rome, Italy; and Stockholm, Sweden—the so-called AIRGENE Study. A detailed description of the design of the study is published elsewhere (9). Study protocols were approved by the local human subjects committees and written informed consent was obtained from all patients.

Fibrinogen was analyzed by immunonephelometry (Dade Behring Marburg GmbH, Marburg, Germany) in 5,327 ethylenediaminetetraacetic acid–plasma samples collected between May 2003 and July 2004 from 895 survivors of myocardial infarction. Data on particulate matter with an aerodynamic diameter less than 10 or 2.5 μm (PM10 or PM2.5, respectively), particle number concentrations, temperature and relative humidity were collected for each city (9) and aggregated to daily means according to standard procedures (12).

Genotyping

DNA was extracted from ethylenediaminetetraacetic acid–anticoagulated blood, using a salting-out procedure. Twenty-one single-nucleotide polymorphisms (SNPs) for FGA, FGB, and FGG were selected and genotyped (9). Genotyping success rates ranged between 97.9% for rs2070011 and 99.8% for rs1800791.

SNP Selection

All SNPs were within Hardy-Weinberg equilibrium (P > 0.05) as tested by means of a χ² test or Fisher’s exact test depending on allele frequency for all cities combined (2). We selected SNPs that were associated with fibrinogen levels in our sample; P values smaller than 0.05/10 = 0.005 were considered statistically significant, because we estimated, according to Li and Ji (13), that the 21 SNPs represented about 10 effective uncorrelated loci. This criterion was met by a total of 7 SNPs from FGA (rs2070006 and rs2070011) and FGB (rs1800790, rs1800791, rs2223799, rs6056, and rs4220) and these were selected for the analysis of gene–environment interactions.

Linkage disequilibrium and correlation between all 21 SNPs are shown in Figure E1 in the online supplement.

Statistical Analysis of SNPs Selected for Investigating Gene–Environment Interaction

The main effect of the SNPs on fibrinogen was estimated using linear mixed models with random subject-specific intercepts adjusting linearly for the variables age, body mass index, ratio of high-density lipoprotein to total cholesterol, pack-years of smoking, N-terminal–pro-B–type natriuretic peptide, and hemoglobin A1c and for the categorical variables city; sex; and history of stroke, arrhythmia, asthma, or artherosclerosis. SNPs were coded by applying an additive genetic model (ordinal variable coded 0, 1, or 2 representing the number of copies of the minor allele), which assumes that two alleles have twice the effect of one allele. We tested for evidence against the additive genetic model (14) by additionally including an indicator variable for heterozygous subjects (1 = heterozygous, 0 = else). This model has the advantage that it simultaneously allows one to estimate the additive genetic effect and to test whether there is evidence of departure from the additive effect model. We also performed a likelihood ratio test comparing the model including the indicator with the model with only the additive effect to derive the better fitting model.

The main effect of particulate matter on fibrinogen was estimated in city-specific additive mixed models adjusting for patient characteristics, time trend, and weather to accommodate the differences in panel characteristics and meteorologic conditions across Europe as previously reported (8) (see Table E1 in the online supplement).

Gene–environment interactions were estimated for each SNP in models including the main effect of the SNP, the main air pollution effect, the interaction term between air pollution and SNP, and selected city-specific time-invariant risk factors and time-varying factors as independent variables (Table E1). The 5-day period before blood withdrawal was selected as the primary exposure window for all gene–environment interaction analyses as in earlier analyses (8). As secondary analyses we explored shorter time windows of exposure.

For the gene–environment interactions, air pollution effect estimates are presented by genotype. These estimates and 95% confidence intervals were calculated on the basis of the estimated PM main effects, the PM–SNP interactions, and their respective estimated variances and covariances. Estimates are presented for an increase in the pollutants corresponding to the interquartile range and are expressed as percent change of the overall mean in fibrinogen. For overall results, city-specific effect estimates were pooled by meta-analysis methodology (15). Heterogeneity of the effect estimates between cities was assessed with a χ² test with 4 degrees of freedom. If the χ² test suggested heterogeneity between the city-specific effect estimates (P < 0.1), city-specific estimates were combined using random effect models (15). Otherwise, fixed effect models calculating variance-weighted averages of the city-specific estimates were applied.

As sensitivity analysis, we calculated haplotypes for the four correlated SNPs in FGB, using the expectation–maximization algorithm presented by Schaid and colleagues (16) with SNPs entered in gene-specific reading directions. We found two haplotypes with a frequency of or exceeding 5% (Table E2). We collapsed the three rare haplotypes with a frequency less than 5% into one group. For assessing the PM–haplotype interactions, we used the same model as for the single SNP analysis to test single haplotypes (assuming an additive genetic effect model) against all other haplotypes while including indicators for the haplotypes and rare haplotypes as main effects.

RESULTS

Males (predominantly middle aged) who had survived their myocardial infarction on average for more than 2 years were recruited from five European cities (Table 1). Overall, 5.95 repeated fibrinogen measurements per person were obtained in 854 subjects (99% of 6 scheduled samples). Mean fibrinogen levels showed some variation across cities (analysis of variance [ANOVA], P < 0.001). PM10, PM2.5, and coarse particles (defined as PM10 minus PM2.5) were moderately to highly correlated (r² > 0.70), whereas correlations between PM and particle number concentration were low to moderate in the various cities (−0.15 < r² < 0.60) (8). We had hypothesized that elevated ambient particulate matter concentrations over the past 5 days were associated with fibrinogen levels and observed a 0.6% increase per 13.5 μg/m³ PM10 (95% confidence interval [CI], 0.1 to 1.1%) as reported previously (8).

Less than 5% of the population was homozygous with respect to the minor alleles of the FGB SNPs, but more than 15% was homozygous with respect to the minor alleles of the FGA SNPs (Table 2). The SNPs comprised three clusters: (1) the two FGA SNPs (r² = 0.97), (2) the FGB SNPs rs1800790, rs2223799, rs6056, and rs4220 (r² > 0.88), and (3) the FGB SNP rs1800791 (Figure E1). Between the three clusters no strong correlation was observed (r² < 0.12). Genotype frequencies differed between the cities for FGA rs2070011 and rs2070006 (ANOVA, P < 0.02) and for FGB rs1800790, rs1800791, and rs2223799 (ANOVA, P < 0.03) (Figure 1).

The minor alleles of the SNPs within the FGA and FGB genes were associated with increased fibrinogen levels, with the exception of rs1800791, where the minor allele was associated with decreased fibrinogen levels (Table 2). There was no evidence of deviation from the additive genetic model for any of the SNPs.
neither by testing the indicator variable for significance, nor by applying the likelihood ratio test (P value of the additional indicator ranged from 0.12 to 0.77 and P values of the likelihood ratio test ranged from 0.11 to 0.75).

The homozygous genotypes of the major allele of FGA rs2070006 and rs2070011 (cluster 1) showed augmented fibrinogen response to increased PM10, although the interaction was not statistically significant (Table 3). In contrast, the four correlated FGB SNPs (cluster 2) indicated larger fibrinogen response to increased PM10 for subjects who were homozygous for the minor allele (P < 0.04 for all interaction terms). An 8- to 11-fold difference in the fibrinogen response to increased PM10

TABLE 2. SINGLE-NUCLEOTIDE POLYMORPHISMS (SNPs) AND SNP–FIBRINOGEN ASSOCIATION: CHARACTERISTICS OF ANALYZED SNPS OF FIBRINOGEN GENES FGA AND FGB AND THEIR ASSOCIATION WITH PLASMA FIBRINOGEN CONCENTRATIONS

<table>
<thead>
<tr>
<th>SNP</th>
<th>Functional Region</th>
<th>Alleles (Major [1]/Minor [2])</th>
<th>Minor Allele Frequency [% (n)]</th>
<th>Genotype (1 1)</th>
<th>Heterozygote (1 2)</th>
<th>Homozygote (2 2)</th>
<th>Change in Fibrinogen* (% of Overall Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1 FGA</td>
<td>rs2070006</td>
<td>Promoter</td>
<td>C/T</td>
<td>40 (677)</td>
<td>37 (319)</td>
<td>46 (393)</td>
<td>17 (142)</td>
</tr>
<tr>
<td></td>
<td>rs2070011</td>
<td>Promoter</td>
<td>C/T</td>
<td>39 (672)</td>
<td>38 (321)</td>
<td>46 (394)</td>
<td>16 (139)</td>
</tr>
<tr>
<td>Cluster 2 FGB</td>
<td>rs1800790</td>
<td>Promoter</td>
<td>G/A</td>
<td>19 (330)</td>
<td>65 (557)</td>
<td>31 (264)</td>
<td>4 (33)</td>
</tr>
<tr>
<td></td>
<td>rs2227399</td>
<td>Intron</td>
<td>T/G</td>
<td>19 (329)</td>
<td>65 (557)</td>
<td>31 (265)</td>
<td>4 (32)</td>
</tr>
<tr>
<td></td>
<td>rs6056</td>
<td>5er1895er</td>
<td>C/T</td>
<td>18 (300)</td>
<td>68 (579)</td>
<td>29 (250)</td>
<td>3 (25)</td>
</tr>
<tr>
<td></td>
<td>rs4220</td>
<td>Lys478Arg</td>
<td>G/A</td>
<td>18 (299)</td>
<td>68 (579)</td>
<td>29 (251)</td>
<td>3 (24)</td>
</tr>
<tr>
<td>Cluster 3 FGB</td>
<td>rs1800791</td>
<td>Promoter</td>
<td>C/T</td>
<td>16 (266)</td>
<td>72 (612)</td>
<td>26 (218)</td>
<td>3 (24)</td>
</tr>
</tbody>
</table>

* Effect estimates and 95% confidence intervals from linear regression with fibrinogen measurements as outcome (100% = 3.58 g/L) and an additive genetic model, with a random effect for subject to account for repeated measurements, using an additive model for the genotypes, and adjusted for age, sex, city, BMI, HDL/total cholesterol, pack-years of smoking, glycosylated hemoglobin (HbA1c), N-terminal–pro-B–type natriuretic peptide (NT-proBNP), and medical history of arrhythmia, asthma, arthrosis, or stroke.
was observed for the homozygous minor alleles compared with the homozygous major alleles in this cluster. These results were confirmed by applying a haplotype analysis to these four SNPs. Effects of similar magnitude were found for the presence of all four minor alleles. Only weak evidence of effect modification by the genotypes of the SNP representing cluster 3 was found.

For the minor allele of FGB rs1800790, the observed increase in fibrinogen level in association with 5-day averages of particulate matter was quite consistent across cities, with no evidence of significant heterogeneity across cities (Figure 2A). The analyses considering various induction times between ambient particulate matter concentrations and fibrinogen levels suggested that the association with particulate matter concentrations in subjects with the minor allele was delayed and cumulative over the 5 days before blood withdrawal (Figure 2B). Fibrinogen responses to PM2.5 and coarse particles were generally consistent with the observed PM10 effect, whereas no effect was observed for particle number concentration (Figure 3).

Additional adjustments for statin therapy slightly weakened the modification by FGB rs1800790 (2.05% increase in fibrinogen [95% CI, 0.61 to 3.50%] for the 2 2 genotype, 1.18% [95% CI, 0.43 to 1.92%] for the 1 2 genotype, and 0.18% [95% CI, −0.46 to 0.83%] for the 1 1 genotype for a 13.5-μg/m³ increase in PM10; interaction term P = 0.039). All models were adjusted for pack-years of smoking; additional adjusting for current smoking in the past 24 hours slightly strengthened the interaction (2.16% increase in fibrinogen [95% CI, 0.67 to 3.85%] for the 2 2 genotype, 1.20% [95% CI, 0.44 to 1.96%] for the 1 2 genotype, and 0.09% [95% CI, −0.57 to 0.75%] for the 1 1 genotype for a 13.5-μg/m³ increase in PM10; interaction term P = 0.026). Excluding current smokers left the associations unaltered (Figure E2). However, excluding current smokers and days on which participants reported environmental tobacco smoke exposure from the analyses increased the estimates of the PM–fibrinogen response (Figure E2).

As sensitivity analysis we also applied a dominant genetic model, that is, set 0 = homozygous major (1 1 genotype) and 1 = heterozygous/homozygous minor (1 2 genotype or 2 2 genotype). For a 13.5-μg/m³ increase in PM10, we estimated a 1.31% increase in fibrinogen (95% CI, 0.51 to 2.10%) for the 1 1 genotype of FGA rs2070011 and a 0.33% increase in fibrinogen (95% CI, −0.61 to 1.28%) for the 1 2 or 2 2 genotype; the interaction term was not significant (P = 0.25). We estimated a 1.41% increase in fibrinogen associated with a 13.5-μg/m³ increase in PM10 (95% CI, 0.60 to 2.23%) for the 1 2 or 2 2 genotype of FGB rs1800790 and a 0.24% increase (95% CI, −0.40 to 0.89%) for the 1 1 genotype; the interaction term was statistically significant (P = 0.029).

### DISCUSSION

In this study, promoter polymorphisms within FGA and FGB were associated with modifications of the fibrinogen response to ambient particulate matter. The synthesis of the FGB chain is the rate-limiting factor for the production of fibrinogen (17). Therefore, interest has focused on the potential role of genetic variability within FGB in the regulation of fibrinogen levels (18). Indeed, SNPs in FGB have been associated with fibrinogen levels (2, 18–20), with G455A (FGB rs1800790) being among the most widely studied (21, 22). The rs1800790 SNP locus on the FGB gene is considered the physiologically most relevant, because it has distinct nuclear protein-binding properties (21). Although most clinical studies have suggested that G455A is associated with plasma fibrinogen levels (22), this is not a uniform finding throughout the literature (23), suggesting that other factors such as complex gene–environment interactions (22) or the underlying inflammatory status or disease state could be important modifiers of this relationship. The PM–fibrinogen relationship is eightfold stronger in subjects with the homozygous minor allele genotype of FGB rs1800790 than for subjects with the homozygous major allele genotype. Marginally stronger modification of the PM–fibrinogen relationship was observed for the other three studied FGB SNPs that are highly correlated with rs1800790 and with each other, namely rs2227399, rs6056, and in particular rs4220, the latter resulting in an amino acid

### TABLE 3. SINGLE-NUCLEOTIDE POLYMORPHISM–ENVIRONMENT INTERACTION: ASSOCIATION BETWEEN 5-DAY AVERAGE OF AMBIENT PARTICULATE MATTER AND PLASMA FIBRINOGEN LEVELS BY GENOTYPE FOR THE ANALYZED POLYMORPHISMS IN GENES FGA AND FGB

<table>
<thead>
<tr>
<th>SNP†</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Genotype</th>
<th>P Value (SNP–Environment Interaction)</th>
<th>Relative Size of the PM–Fibrinogen Response‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 1</td>
<td>1 2</td>
<td>2 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster 1 FGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2070006</td>
<td>1.22 (0.47, 1.96)</td>
<td>0.31 (−0.19, 2.15)</td>
<td>0.11 (−1.94, 2.15)</td>
<td>0.43</td>
<td>0.09</td>
</tr>
<tr>
<td>rs2070011</td>
<td>1.16 (0.41, 1.90)</td>
<td>0.42 (−0.28, 1.13)</td>
<td>0.08 (−2.08, 2.24)</td>
<td>0.47</td>
<td>0.07</td>
</tr>
<tr>
<td>Cluster 2 FGB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1800790</td>
<td>0.27 (−0.36, 0.91)</td>
<td>1.28 (0.54, 2.01)</td>
<td>2.15 (0.71, 3.60)</td>
<td>0.037</td>
<td>8.0</td>
</tr>
<tr>
<td>rs2227399</td>
<td>0.27 (−0.36, 0.91)</td>
<td>1.28 (0.55, 2.02)</td>
<td>2.18 (0.73, 3.63)</td>
<td>0.034</td>
<td>8.1</td>
</tr>
<tr>
<td>rs6056</td>
<td>0.19 (−0.45, 0.83)</td>
<td>1.26 (0.49, 2.04)</td>
<td>2.24 (0.72, 3.77)</td>
<td>0.027</td>
<td>11.3</td>
</tr>
<tr>
<td>rs4220</td>
<td>0.19 (−0.45, 0.83)</td>
<td>1.27 (0.49, 2.04)</td>
<td>2.25 (0.73, 3.78)</td>
<td>0.026</td>
<td>11.3</td>
</tr>
<tr>
<td>Haplotype in cluster 2  †</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1800790</td>
<td>0.81 (0.21, 1.40)</td>
<td>0.40 (−0.48, 1.28)</td>
<td>−0.13 (−1.84, 1.58)</td>
<td>0.25</td>
<td>0.16</td>
</tr>
</tbody>
</table>

* Inverse variance–weighted sum of city-specific estimates and 95% confidence intervals from linear regression using fibrinogen concentrations as outcome (100% = 3.58 g/L) and an additive genetic model, with a random effect by subject, using the 5-day average of PM10 fitting to the time of fibrinogen measurement, applying an additive model for the genotype, and including a genotype–PM interaction adjusted for age, sex, BMI, HDL/total cholesterol, pack-years of smoking, glycosylated hemoglobin (HbA1c), NT-proBNP, history of arrhythmia, asthma, arthrosis, stroke, bronchitis, season, apparent temperature, relative humidity, weekday, and hour of visit in city-specific models.

† 1, major allele; 2, minor allele.

‡ Ratio between the estimate of the change in fibrinogen due to PM10 exposure in the 2 2 genotype (column 4) and the estimate of the change in fibrinogen due to PM10 exposure in the 1 1 genotype (column 2).

Test of heterogeneity between centers, P < 0.1, pooling based on random effect models.

Haplotypes were defined as follows: 1 1 homozygote for the major haplotype GTCG, 1 2 heterozygote and 2 2 homozygote for the minor haplotype AGTA; descriptive data are provided in Table E2 in the online supplement.
exchange. However, for a potential environmental stimulus such as ambient particulate matter, modified plasma fibrinogen levels based on a promoter polymorphism may be considered more plausible, although feedback mechanisms depending on protein variants could produce similar effects. Also, a haplotype analysis within this cluster suggested that these highly correlated SNPs form a "yin yang haplotype" (24), so that a clear attribution to one of the SNPs seems to be impossible. For **FGA** rs2070011, the PM–fibrinogen response was sevenfold higher for subjects with the homozygous major allele genotype than for subjects with the homozygous minor allele genotype, but the interaction was not statistically significant. Previous analyses of the biochemical properties of fibrin clot structures have suggested that clots are more porous in the presence of the minor allele of rs2070011 (25). In addition, in our data (2), we have previously shown that **FGA** rs2070011 modified the associations between IL-6 and fibrinogen levels, leaving room for the speculation that inflammatory stimulation by time-varying factors such as air pollution might be relevant.

The overall estimate for the PM10–fibrinogen response was small. We found that an increase of 25 \( \mu g/m^3 \) in PM10 (5-d average) in those homozygous in the minor allele of **FGB** rs1800790 has a similar effect as an increase of 5 kg/m2 in body mass index (3.9%; 95% CI, 2.6 to 5.3%) when estimated in the same model. Increases of this magnitude occur repeatedly in southern Europe and were also observed in the Nordic cities. The results of this study on short-term effects of ambient particulate matter suggest that increases in fibrinogen levels can be observed repeatedly in urban areas in susceptible subpopulations. The study presented here is a large multicenter study using repeated measurements to assess gene–environment interactions, purposely designed for this research question. However, the multicenter design might also have contributed to an increase in study heterogeneity, which may not have been entirely removed by conducting city-specific analyses and subsequent pooling of the effect estimates. The application of a two-step approach allowed the specification of the genetic model and the selection of confounder variables and of induction times for the PM–fibrinogen analyses independently of the gene–environment analyses. Although considerable variation in the effect estimates was observed between the cities, the test for heterogeneity, although of low power, suggested that these variations were within the range of the statistical variability to be expected. Nevertheless, the pooled estimates for the effect modification by **FGB** rs1800790 and **FGA** rs2070011 are dominated by the data from southern Europe. The minor alleles were even more frequent in the Nordic samples than in the southern European samples. One must note that although the number of available blood samples was among the highest in the Nordic countries, analyses have somewhat less power in the Nordic countries than in southern Europe because of lower average exposure and lower absolute variation in ambient particulate matter. Also, particulate air pollution composition is considered to be more toxic in southern Europe (26), while at the same time dietary habits might provide protection (27). For the present study we hypothesized that the associations between ambient particulate matter and fibrinogen would be observed for cumulative air pollution exposure over several days (8) because of the half-life of fibrinogen of 2 to 3 days. We consistently observed delayed responses in the study. For the subjects who had one or two minor alleles of rs1800790, the pattern observed with respect to induction time was consistent.

### Table 1: Frequencies of genotypes of **FGA** rs2070011 (left) and **FGB** rs1800790 (right) (1, major allele; 2, minor allele) by city in a total of 854 survivors of myocardial infarction.

<table>
<thead>
<tr>
<th>City</th>
<th>Genotype</th>
<th>Frequency [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helsinki</td>
<td>11</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>35</td>
</tr>
<tr>
<td>Augsburg</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>40</td>
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<td>Barcelona</td>
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<td></td>
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<tr>
<td>Rome</td>
<td>11</td>
<td>20</td>
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<tr>
<td></td>
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<td>30</td>
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<tr>
<td></td>
<td>22</td>
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<tr>
<td>Overall</td>
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<tr>
<td></td>
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<td></td>
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### Figure 1. Frequencies of genotypes of **FGA** rs2070011 (left) and **FGB** rs1800790 (right) (1, major allele; 2, minor allele) by city in a total of 854 survivors of myocardial infarction.

![Figure 1](image1.png)

### Figure 2. Modification of fibrinogen response to increased ambient PM10 (particulate matter with an aerodynamic diameter below 10 \( \mu m \)) by **FGB** rs1800790 genotypes (1, major allele; 2, minor allele). (A) City-specific and pooled estimates for 5-day averages of PM10. (B) Pooled estimates using different induction times between PM10 exposure and fibrinogen levels. Interquartile ranges (IQRs) for PM10: Helsinki and Stockholm, 10 \( \mu g/m^3 \); Augsburg 20 \( \mu g/m^3 \); Barcelona, 24 \( \mu g/m^3 \); Rome, 19 \( \mu g/m^3 \); overall, 13.5 \( \mu g/m^3 \); for pooled estimates, *heterogeneity of the city-specific effect estimates with \( P < 0.1 \).
with a delayed and cumulative response over several days, and the pattern was also similar for FGB rs1800790 genotypes (1, major allele; 2, minor allele). *Heterogeneity of the city-specific effect estimates with \( P < 0.1 \). Interquartile ranges of 5-day moving averages: 13.5 \( \mu g/m^3 \), PM10; 8.1 \( \mu g/m^3 \), coarse PM; 8.8 \( \mu g/m^3 \), PM2.5; 11,000/ cm\(^3\), PNC.

Figure 3. Modification of fibrinogen response to increased ambient 5-day averages of PM\(_{10}\), coarse PM, PM\(_{2.5}\), and ultrafine particles (particle number concentration, PNC) by FGB rs1800790 genotypes (1, major allele; 2, minor allele). *Heterogeneity of the city-specific effect estimates with \( P < 0.1 \). Interquartile ranges of 5-day moving averages: 13.5 \( \mu g/m^3 \), PM10; 8.1 \( \mu g/m^3 \), coarse PM; 8.8 \( \mu g/m^3 \), PM2.5; 11,000/ cm\(^3\), PNC.

As fibrinogen is a substrate for coagulation and an acute-phase protein, subjects with variants of these frequent SNPs may experience increased risks not only due to constitutionally higher fibrinogen concentrations, but also due to an attenuated response to environmental inflammatory stimuli such as ambient particulate matter. A meta-analysis of FGB rs1800787, highly correlated with FGB rs1800790, did not show a direct overall association with an elevated risk of myocardial infarction (44). In addition, no association was found between a number of coagulation factor polymorphisms previously associated with plasma levels, and the risk of recurrent cardiovascular events in survivors of myocardial infarction (3). Nevertheless, the gene–environment interaction seen here for rs1800790 may in part contribute to an elevated risk of myocardial infarction observed for elevated plasma levels of fibrinogen (1) for those with higher exposures to particulate matter because the exposures, although variable in space and time, are ubiquitous. However, this is speculation until follow-up data on the patient sample become available.

In conclusion, the gene–environment interaction demonstrated here may contribute to the overall burden of disease observed for particulate matter (45, 46) and render a relatively large subgroup of the white population more susceptible than currently estimated.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript. Fredrik Nyberg, employed by AstraZeneca, is also Lecturer in Epidemiology at the Karolinska Institute; AstraZeneca did not contribute any direct financing to this study.

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References


