Segregation Analysis of Plasminogen Activator Inhibitor-1 and Fibrinogen Levels in the NHLBI Family Heart Study

James S. Pankow, Aaron R. Folsom, Michael A. Province, D.C. Rao, Roger R. Williams, John Eckfeldt, Thomas A. Sellers

Abstract—Elevated plasminogen activator inhibitor-1 (PAI-1) and fibrinogen concentrations are risk factors for coronary heart disease. We investigated environmental, familial, and genetic influences on PAI-1 antigen and fibrinogen concentrations in 2029 adults from 512 randomly ascertained families in 4 US communities. We used maximum-likelihood segregation analysis to fit several genetic and nongenetic modes of inheritance to the data to determine whether mendelian inheritance of a major gene could best explain the familial distributions of these 2 hemostatic factors. Age- and gender-adjusted familial correlations for PAI-1 antigen level averaged 0.16 in first-degree relatives (95% CI=0.11 to 0.21); the spouse correlation was positive but not statistically significant (r=0.10, 95% CI=−0.02 to 0.23). Complex segregation analysis indicated a major gene associated with higher PAI-1 concentrations in 65% of individuals from these families. Demographic, anthropometric, lifestyle, and metabolic characteristics together explained 37% to 47% of the variation in PAI-1 antigen levels, and the inferred major gene explained an additional 17% of the variance. Positive and statistically significant age- and gender-adjusted familial correlations in first-degree relatives indicated a possible heritable component influencing plasma fibrinogen concentration (r=0.17, 95% CI=0.13 to 0.22); however, segregation analysis did not provide statistical evidence of a major gene controlling fibrinogen level. These family data suggest that there are modest familial and genetic effects on the concentration of PAI-1. (Arterioscler Thromb Vasc Biol. 1998;18:1559-1567.)

Key Words: plasminogen activator inhibitor-1 ■ fibrinogen ■ heritability ■ segregation analysis

Thrombosis after atherosclerotic plaque rupture plays a major role in acute myocardial infarction and sudden cardiac death. The balance of systemic thrombotic and fibrinolytic forces at the time of plaque disruption may determine the size, stability, and persistence of developing thrombi. Consistent with this hypothesis, prospective epidemiological studies have reported that elevated plasminogen activator inhibitor-1 (PAI-1) levels or elevated fibrinogen concentrations measured in middle-aged adults predict subsequent incident or recurrent coronary events.1–4 Elevated PAI-1 and fibrinogen may be causes of coronary heart disease (CHD), intermediates in the etiologic pathway linking traditional risk factors with CHD, or simple markers of subclinical atherosclerosis and chronic, low-grade inflammation.5

Although the demographic, anthropometric, lifestyle, and metabolic correlates of PAI-1 and fibrinogen have been well characterized, few population-based studies have examined the familial and genetic determinants of these hemostatic factors. We investigated familial, polygenic, and major gene effects on plasma concentrations of PAI-1 and fibrinogen in 512 randomly ascertained families using 2 statistical methods, familial correlation analysis and segregation analysis. Our data provide evidence of a major gene influencing plasma PAI-1 levels.

Methods

The National Heart, Lung, and Blood Institute Family Heart Study (FHS) is an investigation of genetic and nongenetic determinants of CHD, preclinical atherosclerosis, and cardiovascular risk factors.6 Unrelated individuals (probands) were selected from ongoing population-based cohort studies in 4 US communities. In 2 of the communities (Forsyth County, NC, and suburban Minneapolis, Minn), probands were participants in the Atherosclerosis Risk in Communities Study. In Salt Lake City, Utah, probands were participants in the Utah Health Family Tree Study, and in Framingham, Mass, probands were offspring of members of the original Framingham Cohort Study. Because the Utah and Framingham studies had already recruited some biological relatives, 1 individual was selected randomly from each eligible sibship to ensure that probands for FHS were unrelated. A total of 14 592 probands were identified in the 4 communities. From lists of eligible probands, a random sample of ~500 families and a nonrandom sample of ~500 high-risk families were selected at each center. We included only members of randomly ascertained families in this analysis.
Family History Questionnaires (Phase I)
Probands were mailed a family history questionnaire (FH1) and asked to provide information about their parents, siblings, spouses, and children, including demographic information and history of myocardial infarction, coronary procedures, angina, stroke, diabetes, and other diseases. Information on deceased relatives was also collected. The proband and each living family member named by the proband on the FH1 questionnaire were then mailed a personal health history questionnaire (FH3). Participants were asked to provide a detailed medical history, including diagnosis of or hospitalization for myocardial infarction, coronary procedures, angina, stroke, diabetes, and other diseases. The participation rate for probands was 67%; response rates varied from 63% to 82% across centers. Approximately 86% of eligible relatives completed the FH3 questionnaire; response rates varied from 78% to 94% across centers.

Physical Examination (Phase II)
Selected probands 45 years and older and their immediate family members (parents, siblings, children, current or former spouses) 25 years and older were invited for a comprehensive physical examination at a local clinic if their family satisfied minimum participation requirements in phase I of the study. A total of 2673 individuals from 541 random families completed either a full or abbreviated examination. The study was approved by an institutional review committee at each site, and subjects gave informed consent.

Participants were asked to fast at least 12 hours before arrival at the clinic. Blood was drawn from an antecubital vein with free blood flow and minimal trauma while patients were seated. Specimens for PAI-1 and fibrinogen assays were collected in vacuum tubes containing sodium citrate. Immediately after venipuncture, samples were placed in an ice-water bath, then centrifuged at 3000g for 10 minutes at 4°C. Plasma was removed, with special care taken to withdraw only the plasma and not the buffy coat containing platelets or the lipid layer, which would adversely affect the assays on specimens.

Samples were placed in a −70°C freezer no more than 90 minutes after venipuncture. Frozen samples were packaged in dry ice, shipped to the FHS central laboratory at the Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, and stored at −70°C until fibrinogen and PAI-1 assays could be completed.

Measurement of Hemostatic Variables
PAI-1 antigen was measured in plasma using an enzyme-linked immunosorbent assay. Mouse monoclonal anti-human PAI-1 antibodies coupled with peroxidase were directed against PAI-1 from plasma, and the amount of orthophenylenediamine substrate cleaved by peroxidase was used to estimate PAI-1 concentration. Reagents were from the Diagnostica Stago Asserachrom PAI-1 kit 00577 (American Bioproducts). The color intensity of the sample was measured using a MR700 Microplate Reader (Dynatech Laboratories). The coefficient of variation for PAI-1 antigen at 8 and 152 ng/mL was 21.2% and 8.8%, respectively.

Fibrinogen concentration was measured in plasma using the Clauss method. The rate of conversion of fibrinogen to a fibrin clot (clotting time) was measured using excess bovine thrombin (Parke Davis) and an MLA Electra 800 Automatic Coagulation Timer (Medical Laboratory Automation, Inc.). Clotting times of samples were compared with clotting times measured at various dilutions of standard plasma (Dade Data-Fi Fibrinogen Calibration Reference, Dade Baxter Scientific Products). Results using standard dilutions were plotted, and fibrinogen concentrations were estimated from the graph. The coefficient of variation for fibrinogen at 1.5 and 2.6 g/L was 6.7% and 3.8%, respectively.

Other Measurements
Current and former cigarette smoking habits were ascertained by questionnaire. Usual consumption of alcohol was estimated from self-reported weekly intake of wine, beer, and liquor. Intake in grams per week was computed by multiplying the average alcohol content of each source (wine, 10.8 g; beer, 13.2 g; liquor, 15.1 g) by the number of drinks consumed in a typical week. Average weekly physical activity during the previous year was estimated from self-reported frequency and duration of light, moderate, and strenuous exercise. In women, a reproductive history questionnaire was used to determine menopausal status and a medication inventory was used to ascertain current use of oral contraceptives and replacement hormones.

Standing height, rounded down to the nearest centimeter, was measured using a wall-mounted vertical metal ruler. Body weight was recorded to the nearest pound using a balance scale. Waist and hip circumferences, rounded to the nearest centimeter, were measured at the level of the umbilicus and at the maximum protrusion of the gluteal muscles, respectively. Body mass index (kg/m²) and waist-to-hip ratio were computed. Serum insulin was measured by using a radioimmunoassay (Coat-A-Count, Diagnostic Products Corp). Serum glucose was measured on the Kodak EKTACHEM Clinical Chemistry Slide.

The medical history of each participant was updated during the physical examination to include recent diagnoses of myocardial infarction, angina, stroke, diabetes, or other diseases. An electrocardiogram was obtained to assess known and silent Q-wave myocardial infarction, ventricular hypertrophy, ischemia, and other indicators of cardiac function. We defined prevalent cardiovascular disease as self-reported personal history of myocardial infarction, coronary angioplasty, coronary artery bypass surgery, stroke, angina, or electrocardiographic evidence of myocardial infarction (major Q-wave elevation [Minnesota codes 1.1 or 1.2] or minor Q-wave and ST elevation [Minnesota code 1.3 and codes 5.1 or 5.2]). We defined diabetes as self-reported history of diabetes, nonfasting glucose level of ≥200 mg/dL, fasting glucose level of ≥140 mg/dL, or current pharmacological treatment for diabetes.

Statistical Methods
Familial Correlations
We used the SEGPATH program to estimate familial correlations for PAI-1 antigen level and fibrinogen concentration. SEGPATH is a general purpose program, based on linear path models, that can estimate gender-specific familial correlations using maximum-likelihood methods. Before correlation analysis, PAI-1 and fibrinogen levels were standardized to a mean of 0 and an SD of 1.

In SEGPATH, we fitted a general model to the data to simultaneously estimate all 8 nuclear family correlations (mother-father, father-son, father-daughter, mother-son, mother-daughter, son-son, daughter-daughter, and son-daughter). We also fitted more restrictive models to the data (eg, assuming equal correlations for all parent-offspring pairs and all sibling pairs). We estimated maximal heritability by doubling the age- and gender-adjusted correlation estimate for first-degree relatives.

Segregation Analysis
We conducted complex segregation analysis of PAI-1 antigen level and fibrinogen concentration using class D regressive models as implemented in the REGC program. We fitted a series of explicit genetic and arbitrary nongenetic models of inheritance to the data and determined whether they provided an adequate fit to the data compared with a general model that was not constrained by mendelian patterns of inheritance. To compare the relative fit of each restricted model to the general model, we performed likelihood ratio tests by computing twice the difference in log likelihood values between the 2 models and comparing the result to a χ² distribution, with the number of degrees of freedom equal to the difference in the number of estimated parameters between models. Under this approach, more restrictive genetic or nongenetic models that provide a relatively poor fit to the data will have a high χ² value and will be rejected in favor of the general model. We compared nonnested models using Akaike’s Information Criterion (AIC). The AIC [−2 log likelihood+2(no. of estimated parameters)] incorporates a penalty for models fitting extra parameters; the more parsimonious model will have a lower AIC value.
We assumed that a major gene effect on the 2 phenotypes (PAI-1 or fibrinogen), if present, was due to an unmeasured autosomal locus with 2 alleles (designated H and L) associated with higher (H) and lower (L) values of the phenotype. Three genotypes are possible under this single-locus model (HH, HL, and LL). When mendelian inheritance is not established, a more general term, “ousiotype,” may be used to describe individuals belonging to a distinct phenotypic subgroup or type.

For mendelian modes of inheritance, basic parameters included the frequency of the allele associated with the higher value of the phenotype (\(q_H\)), the mean of the phenotype for each specific type (\(\mu_{HH}, \mu_{HL},\) and \(\mu_{LL}\)), and the residual variance of the phenotype within each type (\(\sigma^2\)). The transmission parameters (\(r_{HH}, r_{HL},\) and \(r_{LL}\)) define the probability that a parent of a specific type transmits an “H” allele to his or her offspring. For mendelian modes of inheritance, these probabilities are fixed at 1.0, 0.5, and 0.0 for the HH, HL, and LL types, respectively. For the general or unrestricted model, these transmission probabilities are estimated rather than fixed; deviations of \(r_{HH}, r_{HL},\) or \(r_{LL}\) from mendelian expectations may be due to a number of factors, including the presence of several major genes or genotype-environment interactions. We included residual spouse (\(\rho_{HS}\)), mother-offspring (\(\rho_{MS}\)), father-offspring (\(\rho_{FS}\)), and sibling (\(\rho_{SS}\)) correlation parameters in several of our models to account for sources of familial resemblance other than a major gene, such as polygenic effects, shared environmental effects, or cultural transmission. We assumed that the distribution of types was in Hardy-Weinberg equilibrium and that the residual variance of the phenotype was the same for all types (ie, \(\sigma^2_{HH}=\sigma^2_{HL}=\sigma^2_{LL}\)).

We inferred that a phenotype (PAI-1 or fibrinogen) was under the control of a major gene if the results of segregation analysis satisfied 3 widely accepted statistical criteria:

1. Rejection of the hypothesis of no major effect (\(q_H=1\)),
2. Rejection of the hypothesis of no transmission of the major effect (\(r_{HH}=r_{HL}=r_{LL}\)), and
3. Nonrejection of the hypothesis of mendelian transmission (\(r_{HH}=1, r_{HL}=0.5,\) and \(r_{LL}=0\)).

**Covariate Adjustments**

In preliminary analyses, we found that the statistical association between PAI-1 or fibrinogen and several covariates was modified by gender. Because interaction effects (eg, the interaction of gender and age) cannot be accommodated in segregation analysis using the REGC program, we used a 2-stage approach to account for covariates.

In the first stage, we used multiple linear regression to adjust the phenotype (PAI-1 or fibrinogen) for the effect of covariates. In the second stage, we fitted selected modes of inheritance to the data using the adjusted phenotype values obtained in the first stage.

In the regression analysis (first stage), we adjusted the phenotypes on groups of related variables. These groups of variables included age (age and age squared), anthropometric characteristics (height, weight, and waist-to-hip ratio), lifestyle characteristics (smoking status, current cigarettes smoked per day, drinking status, current alcohol intake, physical activity, oral contraceptive use, and hormone replacement therapy), and metabolic characteristics (diabetes and fasting serum insulin). We fitted regression models separately for women and men and included covariates in these models regardless of their statistical significance. Before conducting segregation analysis (second stage), we added a constant reflecting the sample mean [\(\ln\text{PAI-1}, 2.58; \text{fibrinogen}, 3.08 \text{g/L}\)] to each individual’s residual phenotype value.

**Exclusions**

Although families were independently ascertained, some participants were members of more than 1 extended pedigree (n = 61). We duplicated phenotype and covariate data for these participants to retain individuals in multiple pedigrees and to maximize the amount of information available for genetic analyses. We excluded members of 20 families because of insufficient information about the family structure. We randomly excluded 1 member of each identical twin pair (n = 7) because the genetic epidemiological programs used here do not appropriately model identical twin relationships. Only 2381 of the 2706 remaining participants (88%) had PAI-1 or fibrinogen

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**TABLE 1. Gender-Specific Characteristics of Participants**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women (n=1126)</th>
<th>Men (n=902)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>51.1 (13.5)</td>
<td>49.4 (13.9)</td>
</tr>
<tr>
<td>College graduate, %</td>
<td>27.3</td>
<td>43.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>71.5 (16.0)</td>
<td>87.5 (14.7)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163 (7)</td>
<td>178 (7)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8 (5.7)</td>
<td>27.6 (4.2)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.87 (0.08)</td>
<td>0.95 (0.06)</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>11.4</td>
<td>14.4</td>
</tr>
<tr>
<td>Former smoking, %</td>
<td>23.2</td>
<td>31.4</td>
</tr>
<tr>
<td>Cigarettes/d*</td>
<td>15.0 (10.3)</td>
<td>20.7 (12.9)</td>
</tr>
<tr>
<td>Current drinking, %</td>
<td>28.1</td>
<td>44.4</td>
</tr>
<tr>
<td>Alcohol intake, g/wk‡</td>
<td>79 (80)</td>
<td>141 (124)</td>
</tr>
<tr>
<td>Physical activity, met-min/wk</td>
<td>523 (661)</td>
<td>857 (1117)</td>
</tr>
<tr>
<td>Postmenopause, %</td>
<td>58.5</td>
<td></td>
</tr>
<tr>
<td>Current oral contraceptive use, %</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Current hormone replacement therapy, %</td>
<td>29.5</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>4.9</td>
<td>5.4</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L‡</td>
<td>64.6 (43.8)</td>
<td>75.3 (48.1)</td>
</tr>
<tr>
<td>PAI-1, ng/mL</td>
<td>24.2 (24.8)</td>
<td>33.2 (38.9)</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>3.18 (0.69)</td>
<td>2.97 (0.67)</td>
</tr>
</tbody>
</table>

*Among current smokers.
†Among current drinkers.
‡Among nondiabetic individuals.

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**Results**

**Participant Characteristics**

Participants ranged in age from 25 to 91 years; the median age was 52 years. Nearly 60% of the women were postmenopausal, and 30% were currently taking replacement hormones (Table 1). The prevalence of smoking (11% to 14%) was about 50% lower than estimates from surveys of the US
The prevalence of diabetes was 5% in both women and men. Plasma PAI-1 antigen levels ranged from 0.2 to 300 ng/mL, with a median of 13 ng/mL. The distribution of PAI-1 values exhibited extreme positive skewness (coefficient of skewness, 2.59). Because skewness can lead to false acceptance of a major gene in segregation analysis, we normalized the PAI-1 distribution using the natural logarithmic transformation (coefficient of skewness after transformation, 0.02). Geometric mean values of PAI-1 were higher in men than women (17.8 versus 10.8 ng/mL). Fibrinogen concentrations ranged from 1.5 to 6.1 g/L in the entire sample, and the distribution was slightly positively skewed. Mean levels of fibrinogen were 3.18 g/L in women and 2.97 g/L in men (Table 1).

**Correlates of PAI-1**

In women, PAI-1 antigen level was positively associated with age, weight, waist-to-hip ratio, diabetes, and fasting serum insulin level and negatively associated with height and physical activity. PAI-1 was lower in women currently using oral contraceptives or replacement hormones. In men, PAI-1 level was negatively associated with age and positively associated with weight, waist-to-hip ratio, alcohol intake, diabetes, and fasting serum insulin level. Demographic, anthropometric, lifestyle, and metabolic characteristics together explained 47% and 37% of the variance of PAI-1 concentration in women and men, respectively (Table 2). Among both women and men, the largest incremental change in the model $R^2$ was attributable to anthropometric variables (weight, height, and waist-to-hip ratio).

**Familial Correlations for PAI-1**

Age- and gender-adjusted familial correlations for PAI-1 antigen level ranged from 0.09 (mother-son) to 0.29 (sisters), with an average of 0.16 (95% CI = 0.11 to 0.21) among all first-degree relatives (Table 3). In contrast, the age- and gender-adjusted spouse correlation was not statistically significantly different from 0 ($r = 0.10, 95\% \text{ CI} = -0.02 \text{ to } 0.23$). Excluding spouse pairs, the correlation estimate for same-gender relatives ($r = 0.22$) was significantly different from that for opposite-gender relatives ($r = 0.12$) ($P < 0.005$).
TABLE 4. Segregation Analysis of Adjusted PAI-1 Antigen (ln ng/mL), NHLBI FHS*

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter Estimate†‡</th>
<th>Model Fit and Test Statistics‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( q_H )</td>
<td>( \mu_{HH} )</td>
</tr>
<tr>
<td>1. Sporadic</td>
<td>[1]</td>
<td>2.58</td>
</tr>
<tr>
<td>2. Familial correlations</td>
<td>[1]</td>
<td>2.59</td>
</tr>
<tr>
<td>3a. Mendelian dominant</td>
<td>0.41</td>
<td>2.98</td>
</tr>
<tr>
<td>3b. Mendelian dominant</td>
<td>0.41</td>
<td>2.98</td>
</tr>
<tr>
<td>3c. Mendelian recessive</td>
<td>0.81</td>
<td>2.94</td>
</tr>
<tr>
<td>4. No transmission of the major effect ( (q_H = \bar{q}) )</td>
<td>1.0</td>
<td>2.59</td>
</tr>
<tr>
<td>5. General ( (r ) values free)</td>
<td>0.45</td>
<td>2.59</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter Estimate†‡</th>
<th>Model Fit and Test Statistics‡</th>
</tr>
</thead>
</table>
| \( q_H \) indicates gene \( (type) \) frequency; \( \mu_{HH} \), \( \mu_{HL} \), and \( \mu_{LL} \), type-specific means; \( \sigma^2 \), residual variance; \( \tau_{HH} \), \( \tau_{HL} \), and \( \tau_{LL} \), transmission parameters; \( \rho_{SP} \), spouse correlation; \( \rho_{OS} \), mother-offspring correlation; \( \rho_{OF} \), father-offspring correlation; \( \rho_{SS} \), sibling correlation; \( \chi^2 \), likelihood ratio test compared with the general model; and \(-2 \ln L\), \(-2 \log \) likelihood. 

§AIC was scaled by subtracting the lowest value from among the competing models. 

†Parameter estimates in brackets were either dependent or fixed at the level shown. 

‡See Methods for further description of models, parameters, and test statistics. 

*Sample size was 1989 members of 512 families. PAI-1 was adjusted for age, age squared, gender, weight, height, and waist-to-hip ratio. 

Segregation Analysis of PAI-1

We first conducted segregation analysis on PAI-1 values adjusted for age and gender; the results did not provide statistical evidence of a major gene influencing plasma PAI-1 concentration (results not shown). We then repeated segregation analysis after further adjusting PAI-1 values for anthropometric characteristics (weight, height, and waist-to-hip ratio). The results are summarized in Table 4. We provide parameter estimates, model fit, and test statistics for 4 hypothetical modes of inheritance: (1) sporadic, which allows random environmental effects but no genetic transmission of the phenotype; (2) familial correlations, which allows familial resemblance for the phenotype but no major gene effect; (3a–3c) mendelian, which allows a major gene effect as well as other sources of familial resemblance; and (4) no transmission, which allows a major effect (3 distinct phenotypic subgroups or “types”) but no transmission of the major effect from parents to offspring.

We assessed the goodness of fit for each of the restricted models (1 to 4) by comparing them to the general or unrestricted model (5). The sporadic and familial correlations models were both rejected \( (P<0.001) \), as was the model of no transmission of the major effect \( (P<0.001) \). In contrast, neither the mendelian-codominant nor the mendelian-dominant models with familial correlations were rejected \( (P\geq0.08) \), suggesting that both of these models provide an adequate fit to the family data. The mendelian-dominant model had the lowest AIC value and was the most parsimonious of all the models tested. The maximum-likelihood estimates for the transmission parameters under the general model \( (\tau_{HH}=1, \tau_{HL}=0.45, \text{ and } \tau_{LL}=0) \) closely resembled the probabilities expected if there is mendelian transmission of a major gene effect. When we fitted similar genetic and nongenetic modes of inheritance to the family data after further adjusting PAI-1 values for lifestyle and metabolic variables, parameter estimates were slightly different than those presented in Table 4, but the main findings of segregation analysis were qualitatively unchanged. Together these findings meet the statistical criteria required to infer the presence of a major gene influencing PAI-1 level.

We used maximum-likelihood estimates from the best-fitting model (mendelian dominant) to estimate the proportion of the variance in PAI-1 antigen level that can be explained by the putative major gene. Regression analyses indicated that the variance of \( (\ln) \) PAI-1 level was reduced from 1.71 to 1.07 (37%) when we controlled for age, weight, height, and waist-to-hip ratio. The variance of adjusted \( (\ln) \) PAI-1 was further reduced to 0.78 when a major gene effect was included (model 3b, Table 4). These results suggest that the putative major gene explains 17% of the total variance of \( (\ln) \) PAI-1, ie, \( (1.07-0.78)/1.71 \), and 27% of the remaining variance if PAI-1 is first adjusted for age and anthropometric variables, ie, \( (1.07-0.78)/1.07 \).

Figure 1 shows predicted genotype-specific distributions for \( (\ln) \) PAI-1 antigen level based on maximum-likelihood parameter estimates from the mendelian-dominant model with familial correlations (Table 4). Genotype-specific curves are superimposed on the actual distribution of adjusted \( (\ln) \) PAI-1 (shown as a histogram). According to the allele frequencies obtained under the mendelian-dominant model \( (q_H) \), we estimate that 65% of the population carry 1 or 2 alleles associated with higher levels of PAI-1 (HH or HL). The remaining 35% are homozygous for the allele associated with lower values (LL). Geometric means for these putative high and low genotypes are 19.7 and 6.8 ng/mL, respectively.

Correlates of Fibrinogen

In women, plasma fibrinogen concentration was positively associated with age, weight, and number of cigarettes smoked.
TABLE 5. Segregation Analysis of Adjusted Fibrinogen (g/L), NHLBI FHS*

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter Estimates†‡</th>
<th>Model Fit and Test Statistics§</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>q_M</td>
<td>μ_M = μ_H = μ_L</td>
</tr>
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<td>1. Sporadic</td>
<td>[1]</td>
<td>3.09</td>
</tr>
<tr>
<td>2. Familial correlations</td>
<td>[1]</td>
<td>3.09</td>
</tr>
<tr>
<td>3. Mendelian codominant</td>
<td>0.29</td>
<td>4.26</td>
</tr>
<tr>
<td>4. No transmission of major effect (q_M = ρ)</td>
<td>0.07</td>
<td>5.29</td>
</tr>
<tr>
<td>5. General (ρ values free)</td>
<td>0.09</td>
<td>5.37</td>
</tr>
</tbody>
</table>

*Sample size was 2009 individuals from 512 families; fibrinogen was adjusted for age, gender, weight, height, and waist-to-hip ratio.
†Parameter estimates in brackets were either dependent or fixed at the level shown.
‡See Table 4 and Methods for definitions and further description of models, parameters, and test statistics.
§AIC was scaled by subtracting the lowest value from among the competing models.
gender-adjusted correlation for first-degree relative pairs suggests a maximum heritability of 34% for fibrinogen concentration. The age- and gender-adjusted spouse correlation (0.12) was not statistically significantly different from 0. Nevertheless, one might expect members of spouse pairs to be mutually exposed to acute determinants of fibrinogen, such as bacterial infections or psychological stress, often shared by members of the same household but not necessarily shared by adult family members living apart (ie, parents and offspring or siblings).

In a segregation analysis of 204 3-generation families, Livshits et al found evidence for a major codominant gene effect on fibrinogen level. Our results are more in line with those of Friedlander et al, who found that their data on 82 pedigrees were not consistent with mendelian transmission of a major gene regulating fibrinogen concentration. The no transmission model may have provided the best fit to the family data in our study because of residual skewness in the fibrinogen distribution, a real mixture of heterogeneous phenotypic subgroups in the study population, or a common, unmeasured environmental factor with large effects on plasma fibrinogen concentration. Genotype-environment interactions are also known to distort transmission patterns from their mendelian expectations. Several observational studies have reported that the association between plasma fibrinogen concentration and cigarette smoking or strenuous physical activity is influenced by a polymorphism in the promoter of the β-fibrinogen gene. If similar genotype-environment interactions were influential in our study population, then the parameter estimates from segregation analysis may have been biased and power to infer a major gene effect may have been reduced.

The 3 fibrinogen structural genes (FGA, FGB, and FGG) on chromosome 4 may account for some interindividual differences in plasma fibrinogen concentration. Some, but not all, studies have reported statistically significant associations between fibrinogen level and 1 or more polymorphisms in these 3 genes. One large-scale population-based study that enrolled >1200 adult subjects from Ireland and France found that fibrinogen gene haplotypes explained only 1% to 2% of the variance of fibrinogen concentration. These equivocal data may indicate that other genes play a role in the regulation of plasma fibrinogen level.

With >2000 individuals from 512 random families, the present study had excellent power to estimate familial correlations and to investigate major gene influences on PAI-1 and fibrinogen level. However, several factors may have effectively reduced the power to detect a major gene. Although we excluded participants with prevalent cardiovascular disease to reduce the possible confounding effects of disease on plasma levels of the hemostatic factors, some individuals in our sample may have had elevated PAI-1 and fibrinogen levels due to chronic, low-grade inflammation associated with subclinical atherosclerosis.

Furthermore, average family sizes were small and many families included only 2-generation nuclear families, which are less informative than larger pedigrees with 3 or more generations. Finally, there is considerable error associated with single measurements of these hemostatic factors; the within-person (biological) and
method variability for either trait is as high as 28\% at 57.68. Because residual environmental variance inflates the total variance of these 2 hemostatic factors, true genetic effects may have been underestimated in familial correlation analysis and segregation analysis because only 1 blood sample was available to estimate the habitual level for each participant. Segregation analysis is practically constrained by the limited number of genetic and nongenetic modes of inheritance that can be fitted to the data. In our analysis, we assumed a single major gene with 2 alleles. Our relatively simple mendelian single-locus models may have been inadequate if more complex patterns of inheritance, such as oligogenic effects, multiple alleles, epistasis, and gene-enviroment interactions explain the distribution of hemostatic factors within families. Although we found statistical evidence of a major gene influencing plasma PAI-1 concentration, our evidence remains circumstantial until it is confirmed by linkage analysis or other molecular genetic studies. In conclusion, our results are consistent with modest familial and genetic influences on the plasma concentrations of PAI-1 antigen and fibrinogen in randomly ascertained families from 4 US communities. We detected a putative major gene regulating PAI-1 levels and explaining 17% of the overall variance of the trait. Additional studies are needed to identify the genetic factors, environmental factors, and gene-environment interactions regulating PAI-1 and fibrinogen concentrations.

Acknowledgments

We thank the FHS study participants and staff. Support was provided by National Heart, Lung, and Blood Institute cooperative agreement grants N01-HC-25104, N01-HC-25105, N01-HC-25106, N01-HC-25107, N01-HC-25108, and N01-HC-25109 and institutional training grant T32 HL07036. Some of the results presented in this report were obtained by using the program S.A.G.E., which is supported by a US Public Health Service Resource Grant (1 P41 RR03655) from the National Center for Research Resources.

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doi: 10.1161/01.ATV.18.10.1559
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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