Tissue Plasminogen Activator, Fibrin D-Dimer, and Insulin Resistance in the Relatives of Patients With Premature Coronary Artery Disease

Joseph D. Mills, Michael W. Mansfield, Peter J. Grant

Abstract—Elevated levels of tissue-type plasminogen activator antigen (tPA), fibrinogen, and fibrin D-dimer predict coronary artery disease (CAD) events and stroke in healthy subjects as well as recurrent coronary events and cardiovascular death in patients with established CAD. In addition, increased fibrin D-dimer (a marker of fibrinolytic activity) is associated with myocardial infarction (MI) in healthy, middle-aged men and stroke in men and women. Impaired fibrinolysis, as determined by increased levels of plasminogen activator inhibitor 1 (PAI-1), elevated tPA and insulin resistance are known to cluster with other cardiovascular risk factors, however, the status of the fibrinolytic system and insulin resistance has yet to be determined in subjects with a strong family history of premature CAD. Elevated fibrinogen concentrations have been reported in the healthy members of families in which coronary disease has occurred prematurely. Given the association between fibrinogen and insulin resistance, increased levels of fibrinogen may act as a potential confounder in studies of the insulin resistance syndrome in such families.

First-degree relatives of patients with premature CAD are themselves at increased risk of premature coronary disease. The principle aim of this study was to investigate tPA and PAI-1 antigen levels, fibrin D-dimer, fibrinogen, insulin resistance, and physical fitness (which is known to affect insulin resistance and fibrinolysis) in the healthy, male, first-degree relatives of patients with premature CAD. This association is independent of potential confounding factors. (Arterioscler Thromb Vasc Biol. 2002;22:704-709.)

Key Words: coronary artery disease ■ family history ■ hemostatic factors ■ insulin ■ exercise

Elevated levels of tissue-type plasminogen activator (tPA) predict the occurrence of coronary artery disease (CAD) events and stroke in healthy subjects as well as recurrent coronary events and cardiovascular death in patients with established CAD. In addition, increased fibrin D-dimer (a marker of fibrinolytic activity) is associated with myocardial infarction (MI) in healthy, middle-aged men and stroke in men and women. Impaired fibrinolysis, as determined by increased levels of plasminogen activator inhibitor 1 (PAI-1), elevated tPA and insulin resistance are known to cluster with other cardiovascular risk factors, however, the status of the fibrinolytic system and insulin resistance has yet to be determined in subjects with a strong family history of premature CAD. Elevated fibrinogen concentrations have been reported in the healthy members of families in which coronary disease has occurred prematurely. Given the association between fibrinogen and insulin resistance, increased levels of fibrinogen may act as a potential confounder in studies of the insulin resistance syndrome in such families.

First-degree relatives of patients with premature CAD are themselves at increased risk of premature coronary disease. The principle aim of this study was to investigate tPA and PAI-1 antigen levels, fibrin D-dimer, fibrinogen, insulin resistance, and physical fitness (which is known to affect insulin resistance and fibrinolysis) in the healthy, male relatives of CAD patients.

Methods

One hundred twenty-five male patients (probands) ≤65 years old at the time of diagnostic coronary angiography and with confirmed 2- or 3-vessel CAD (World Health Organization criteria of ≥50% stenosis in a major epicardial vessel) were identified via the surgical revascularization waiting list at the Yorkshire Heart Center, Leeds. From the 125 male patients, 185 of their male, first-degree relatives ≤65 years old at the time of recruitment and free from a personal history of CAD were contacted and interviewed. An equal number of male, community control subjects ≤65 years old and without a personal or family history of CAD or diabetes mellitus were recruited via the Leeds Health Authority Family Health Service register. Subjects with a personal history of hypertension and/or taking cardiovascular medication (including aspirin and lipid-lowering drugs) were subsequently excluded from the study. All remaining subjects (175 in each group) were white, North European, and they gave informed consent according to a protocol approved by the United Leeds Teaching Hospitals (NHS) Trust Research Ethics Committee.

At the time of recruitment, all subjects had fasted for a minimum of 10 hours overnight. Venous blood, 50 mL, was taken from an antecubital vein with a 19-gauge needle without venous stasis with the subject in a supine position. Blood was collected in lithium
heparin for lipid fraction analysis, a 10-ml tube containing 1 mL 0.9% citrate (pH 8.8) at 4°C for assay of insulin, PAI-1, tPA, and fibrin D-Dimer and a similar citrate tube at room temperature for assay of fibrinogen. The citrate samples were centrifuged at 2560g at 4°C or room temperature for 30 minutes, and 0.5-ml aliquots of plasma supernatant snap-frozen in liquid nitrogen for storage at −40°C until assay. All subjects underwent 75-g oral glucose tolerance testing with blood collected in lithium fluoride for fasting and 2-hour glucose estimation. DNA was extracted from blood collected in EDTA for subsequent genotyping at the PAI-1 4G/5G promoter polymorphism by using a previously described method.22 Standard 12-lead electrocardiographs were recorded for all subjects, performed manually to the nearest 2 mm Hg with subjects supine, and intra-assay coefficients of variation were 3.8% and 2.5% for fibrinogen. The citrate samples were centrifuged at 2560g at 4°C for assay of insulin, 9.8% and 4.4% for PAI-1, 10.4% and 7.0% for tPA, 5.3% and 3.5% for D-dimer, and 3.5% and 2.0% for fibrinogen. A glucose oxidase method was used for measurement of plasma glucose and a Hitachi 747 autoanalyzer (Boehringer Mannheim) for estimation of triglyceride and total cholesterol. HDL cholesterol was measured by a Hitachi 717 autoanalyzer after removal of LDL, chylomicrons, and VLDL by precipitation with phosphotungstic acid and magnesium chloride. LDL cholesterol was calculated by the Friedewald equation.

Values for age for the 3 study groups did not conform to a normal distribution and are presented as medians with 25th and 75th percentiles. Differences in age between groups were assessed by the Kruskal-Wallis test. To achieve a normal distribution for insulin, PAI-1, tPA, and D-dimer. Fibrinogen was measured by using the Clauss method.23 Inter-assay and intra-assay coefficients of variation were 3.8% and 2.5% for insulin, 9.8% and 4.4% for PAI-1, 10.4% and 7.0% for tPA, 5.3% and 3.5% for D-dimer, and 3.5% and 2.0% for fibrinogen. A glucose oxidase method was used for measurement of plasma glucose and a Hitachi 747 autoanalyzer (Boehringer Mannheim) for estimation of triglyceride and total cholesterol. HDL cholesterol was measured by a Hitachi 717 autoanalyzer after removal of LDL, chylomicrons, and VLDL by precipitation with phosphotungstic acid and magnesium chloride. LDL cholesterol was calculated by the Friedewald equation.

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TABLE 2. Bivariate Correlation Coefficients for Fibrin D-Dimer in Relatives and Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Relatives</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.32</td>
<td>0.40</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>−0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI</td>
<td>0.13</td>
<td>0.23</td>
</tr>
<tr>
<td>WHR</td>
<td>0.18</td>
<td>0.31</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.14</td>
<td>0.25</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.06</td>
<td>0.16</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−0.06</td>
<td>−0.04</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.24</td>
<td>0.29</td>
</tr>
<tr>
<td>tPA antigen</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>PAI-1 antigen</td>
<td>−0.10</td>
<td>−0.06</td>
</tr>
</tbody>
</table>

P<0.05 where r>0.16.

Results
Clinical, biochemical, and hemostatic assay data are shown in Table 1. D-dimer, tPA, and fibrinogen levels were significantly higher in relatives compared with controls, 55 (52 to 58) ng/mL versus 49 (45 to 53) ng/mL, P<0.01, for D-dimer, 8.0 (7.5 to 8.6) ng/mL versus 5.6 (5.2 to 6.1) ng/mL, P<0.001, for tPA, and 3.0 (2.9 to 3.1) g/L versus 2.8 (2.7 to 2.9) g/L, P<0.05, for fibrinogen. There was no difference in PAI-1 levels between the two groups. Apart from fibrinogen, |r|<0.19 in relatives and controls. Correlation data for fibrin D-dimer is shown in Table 2. There was a strong correlation with age, r=0.32 and 0.40 (P<0.01), and fibrinogen, r=0.24 and 0.29 (P<0.01) in relatives and control subjects, respectively. The subgroup of relatives and controls who underwent the cardiological exercise test were similar in terms of age (median, 42 years) and all conventional CAD risk factors. Relatives had increased levels of tPA, D-dimer, and fibrinogen compared with controls. Significant correlation data for VO2 max is shown in Table 3.

TABLE 3. Bivariate Correlation Coefficients for VO2max

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Relatives</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−0.60*</td>
<td>−0.71*</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>−0.34*</td>
<td>−0.41*</td>
</tr>
<tr>
<td>tPA</td>
<td>−0.32*</td>
<td>−0.56*</td>
</tr>
<tr>
<td>PAI-1</td>
<td>−0.31*</td>
<td>−0.34*</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>−0.39*</td>
<td>−0.36*</td>
</tr>
<tr>
<td>D-dimer</td>
<td>−0.21†</td>
<td>−0.20†</td>
</tr>
</tbody>
</table>

*P<0.01; †P<0.05.

Multiple linear regression models were used to calculate adjusted tPA and D-dimer levels using the significant covariates identified from bivariate correlation analysis. Only those covariates which either independently predicted tPA or D-dimer levels or contributed >1% variance were included in the final models (Tables 4 and 5). Exclusion of the remaining correlates did not affect the R^2 values or the adjusted levels of tPA or D-dimer. The final models accounted for 53.7% and 21.6% of the variance in tPA and D-dimer, respectively. Relative/control status and PAI-1 levels were the most influential contributors to tPA variance, and relative/control status, age, and fibrinogen were the only significant factors in

TABLE 4. Multiple Linear Regression Model for Log_{10} tPA Antigen: Parameter Coefficient, Effect Size, and Contribution to Variance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient, 95% CI</th>
<th>Effect Size,* on tPA level</th>
<th>Contribution to Variance, %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative</td>
<td>0.142 (0.107 to 0.177)</td>
<td>1.4-fold increase</td>
<td>15.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log PAI-1 antigen</td>
<td>0.233 (0.183 to 0.283)</td>
<td>1.2-fold increase</td>
<td>19.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.005 (0.003 to 0.006)</td>
<td>1.3-fold increase</td>
<td>10.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log triglyceride</td>
<td>0.144 (0.058 to 0.231)</td>
<td>1.2-fold increase</td>
<td>3.1</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Log fibrinogen</td>
<td>0.304 (0.079 to 0.520)</td>
<td>1.1-fold increase</td>
<td>1.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>0.005 (0.001 to 0.010)</td>
<td>1.1-fold increase</td>
<td>1.1</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Log_{10} tPA antigen used as the dependent variable. Effect size is calculated from antilogged coefficient and based on relative versus control, 2-fold increase in PAI-1, 20-year increase in age, 2-fold increase in triglyceride, 2-fold increase in fibrinogen, and 10-kg/m^2 increase in BMI.
TABLE 5. Multiple Linear Regression Model for Log₁₀ Fibrin D-Dimer: Parameter Coefficient, Effect Size, and Contribution to Variance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient, 95% CI</th>
<th>Effect Size,* on D-dimer level</th>
<th>Contribution to Variance, %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.006 (0.004 to 0.007)</td>
<td>1.3-fold increase</td>
<td>13.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Relative</td>
<td>0.052 (0.015 to 0.090)</td>
<td>1.1-fold increase</td>
<td>2.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Log fibrinogen</td>
<td>0.314 (0.077 to 0.550)</td>
<td>1.1-fold increase</td>
<td>1.9</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Log₁₀ fibrin D-dimer used as the dependent variable. Effect size is calculated from antilogged coefficient and based on 20-year increase in age, relatives versus control and 2-fold increase in fibrinogen.

...the D-dimer model. From the calculated effect size, relatives had a 1.4-fold increase in tPA and a 1.1-fold increase in D-dimer levels. An increase in age by 20 years was associated with a 1.3-fold rise in both tPA and D-dimer levels. Adjusted mean tPA levels were 8.1 (7.7 to 8.6) ng/mL for relatives versus 5.8 (5.5 to 6.1) ng/mL for controls, P<0.001, and adjusted D-dimer levels were 55 (52 to 59) ng/mL for relatives and 49 (46 to 52) ng/mL for controls, P=0.006.

Data concerning the probands (patients with premature, multi-vessel CAD) are included for completeness (Table 1). As expected, probands were older than the relatives and controls with higher BMI and WHR and elevated levels of tPA, PAI-1, D-dimer, fibrinogen, and estimated insulin resistance. BP and lipid profiles were affected by concurrent cardiovascular medication, which the majority of these patients were receiving at the time of recruitment.

Discussion

In this study, we have found elevated levels of tPA antigen, fibrin D-dimer, and fibrinogen in the healthy, male, first-degree relatives of patients with severe, premature CAD. tPA antigen and D-dimer remained higher in relatives compared with controls after adjustment for all significant correlates, including fibrinogen. Confounding factors cannot be ignored, but the principle strength of this study lies in the quality of subject recruitment, thereby avoiding many of the confounders that undermine studies of similar design. We have successfully enrolled more than 90% of the suitable relatives identified from our CAD patient group and simultaneously recruited a well characterized and highly comparable group of community control subjects. The lack of any significant differences between the groups in terms of history of smoking, alcohol consumption, clinical and biochemical parameters, and physical fitness would all mitigate against any significant confounding factors.

tPA: CAD Risk Factor

In prospective studies involving healthy, middle-aged subjects, increased levels of tPA antigen independently predict the development of coronary\textsuperscript{1,6} and cerebrovascular\textsuperscript{5} events. In some studies, however, the identified association between tPA levels and CAD has been weakened by adjustment for other confounding risk factors,\textsuperscript{2–4} principally, features of the insulin resistance syndrome (BMI, BP, and HDL cholesterol). These findings may simply reflect differences in age, sex, and geographical distribution of subjects in these studies or, given the close association between tPA and the insulin resistance syndrome,\textsuperscript{8,25} may represent the effect of multivariate modeling if BMI, BP, and HDL cholesterol levels differ significantly between cases and controls.

There are several plausible mechanisms that may explain the observed relationship between tPA and atherothrombotic vascular disease. In the context of atherosclerotic CAD, increased circulating levels of tPA may reflect increased endothelial tPA content and expression\textsuperscript{26} and enhanced plasmin-mediated breakdown of the extracellular matrix, resulting in plaque instability.\textsuperscript{27} In addition, tPA levels may reflect the acute phase response given the association between CAD and markers of chronic infection or inflammation.\textsuperscript{8,28} Alternatively, increased tPA antigen may represent increased tPA/PAI-1 complex (because the majority of tPA circulates in this inactive, bound form) and therefore a net reduction in fibrinolytic capacity.\textsuperscript{29} Previous studies have identified strong correlations of tPA/PAI-1 complexes with both tPA and PAI-1 antigens.\textsuperscript{9,25} In the present study, PAI-1 antigen levels were not elevated in relatives whereas tPA antigen remained higher in relatives compared with controls after multivariate analysis that included PAI-1. Data from the Northwick Park Heart Study suggests that impaired fibrinolysis (as measured by dilute blood clot lysis time) predicts CAD events in healthy subjects;\textsuperscript{30} however, this finding may represent increased levels of PAI-1 in subjects older than those found in the present study. Therefore, elevated levels of tPA (which are independent of PAI-1) may reflect enhanced rather than impaired fibrinolysis in the healthy relatives of CAD patients.

Fibrin D-Dimer

Fibrin D-dimer is a product of the action of plasmin on cross-linked fibrin and therefore reflects fibrinolytic activity and fibrin turnover.\textsuperscript{31} Fibrin D-dimer levels are elevated in patients with established atherothrombotic vascular disease\textsuperscript{32,33} and predict arterial thrombotic events in prospective studies involving healthy, middle-aged subjects.\textsuperscript{2,5,10–12} It is possible that D-dimer levels merely reflect the underlying fibrinogen concentration; however, in the Caerphilly Study, adjustment for other CAD risk factors including fibrinogen did not affect the independent relationship between elevated D-dimer levels and the relative risk of CAD events.\textsuperscript{2} In this study, there was a significant correlation between fibrinogen and D-dimer levels, but the increased levels of D-dimer observed in relatives compared with controls remained inde-
dependent of adjustment for other covariates, including fibrinogen. Tobacco smoking and leisure time activity are known to affect D-dimer levels and are potential confounding factors; however, history of smoking and cardiorespiratory fitness did not differ between relatives and controls in the present study. Increased fibrin D-dimer, in conjunction with elevated levels of tPA, supports the hypothesis that fibrinolytic activity is increased in the healthy relatives of patients with premature CAD.

**Insulin Resistance Syndrome**

The insulin resistance syndrome, which includes elevated levels of tPA antigen and PAI-1, is associated with the development and progression of atherothrombotic vascular disease. The healthy, first-degree relatives of patients with type II diabetes mellitus exhibit many of the features of the insulin resistance syndrome, and in the present study there was a nonsignificant trend toward increased BMI, systolic BP, and estimated insulin resistance in relatives compared with controls. Adjustment for these factors in multivariate analyses did not affect the observed differences in tPA and D-dimer levels between relatives and control subjects. Increased PAI-1 activity has also been found in the relatives of diabetic probands; however, in agreement with other studies which have examined PAI-1 in the offspring of patients with premature MI, we did not find any difference in PAI-1 levels between relatives and controls. One small study has reported increased PAI-1 activity but not tPA antigen in children with a family history of premature MI. However, this finding was based on univariate analysis alone and no correlation was found between PAI-1 and tPA. In the present study, we have identified the well reported associations between the 4G allele of the PAI-1 gene 4G/5G polymorphism and increased PAI-1 levels but found no difference in genotype frequency between relatives and controls. This is in contrast to a study by Margaglione et al which found the 4G/4G genotype to be independently associated with family history of MI. It is possible that the relative contribution of environmental and genetic factors to the development of coronary disease is different between an Italian and a UK population, and this, coupled with the much larger number of subjects in the Italian study, would make the identification of a link between a polymorphism and disease (or family history of disease) more likely. However, there are numerous studies reporting both positive and negative associations between the PAI-1 4G/5G polymorphism and atherothrombotic disease, and given this lack of consistency, it seems unlikely that genotyping for this polymorphism will improve the risk stratification of relatives of affected patients.

Increased cardiorespiratory fitness (as estimated by VO₂max during treadmill exercise testing) has been shown to correlate negatively with features of the insulin resistance syndrome, including insulin-mediated glucose uptake and tPA and PAI-1 levels. In the present study, we have also identified a significant association between insulin resistance, associated hemostatic factors, and VO₂max, but we find no difference in VO₂max or anaerobic threshold between relatives and controls. Therefore, given the similarity between the groups in terms of cardiorespiratory fitness and other lifestyle factors, it seems unlikely that our findings concerning estimated insulin resistance and tPA and PAI-1 levels were significantly influenced by such potential confounders.

We have found increased levels of tPA antigen and D-dimer in a group of healthy, male subjects who are at increased risk of developing clinically apparent, premature CAD by virtue of their deleterious family history. These findings concur with data from prospective studies which have identified tPA antigen and fibrin D-dimer as independent predictors of CAD events. The measurement of circulating levels of tPA and fibrin D-dimer may improve the risk stratification of individuals who appear to be at low risk in terms of routinely measured cardiovascular risk factors.

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**References**


Tissue Plasminogen Activator, Fibrin D-Dimer, and Insulin Resistance in the Relatives of Patients With Premature Coronary Artery Disease
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