Tissue Plasminogen Activator and Risk of Myocardial Infarction

The Rotterdam Study

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Background Impaired fibrinolytic capacity, as assessed by euglobulin clot lysis time or plasma concentration of fibrinolytic parameters, has been associated with an increased risk of myocardial infarction (MI). We studied the association of a polymorphism in the gene for TPA and of plasma concentrations of TPA (antigen and activity) with the prevalence of MI.

Methods and Results A case-control study was performed. Subjects with a history of MI (n=121) and controls (n=250) were drawn from the Rotterdam Study, a population-based cohort study of 7983 subjects ≥55 years old. We determined TPA antigen and activity in plasma and genotyped all subjects for the Alu repeat insertion/deletion polymorphism in intron h in the TPA gene. Homozygosity for the insertion was associated with twice as many cases of MI as was homozygosity for the deletion (odds ratio, 2.24; 95% CI, 1.11-4.50). TPA antigen was positively associated with the risk of MI; compared with that in the lowest quartile, the relative risks (odds ratio) in the second, third, and upper quartiles were 1.7 (CI, 0.9-3.3), 2.3 (1.2-4.4), and 2.0 (1.0-3.8), respectively. When adjusted for body mass index, HDL and total cholesterol, systolic and diastolic blood pressures, and current smoking, the risk associated with TPA antigen concentration was attenuated. Increased concentrations of TPA activity tended to be associated with an increased risk of MI.

Conclusions This study provides evidence for an independent association of the insertion allele of the insertion/deletion polymorphism in the TPA gene with nonfatal MI. Increased TPA antigen is associated with an increased risk of MI; however, this association was not independent of cardiovascular disease risk factors. (Circulation. 1997;95:2623-2627.)

Key Words • cardiovascular diseases • risk factors • fibrinolysis • plasminogen activators • thrombolysis

It is now widely accepted that blood clot formation in coronary arteries is the key event in MI.1 Intervention studies have demonstrated that in patients with MI, activation of the fibrinolytic system by infusion of TPA results in rapid clot dissolution and improved case fatality.2 It has been hypothesized that people with a decreased endogenous fibrinolytic capacity are at increased risk of an MI. However, to measure the fibrinolytic capacity is a major challenge. Some have suggested measuring plasma levels of TPA months or years before an MI. A decreased level of TPA activity in patients with a history of MI was directly associated with an increased risk of a recurrent MI.3,5 In contrast, in patients with angina pectoris, TPA activity level was not associated with subsequent MI.6 In the latter study, increased levels of TPA antigen were associated with an increased risk of MI. This finding had been reported earlier.7-9 Whether the increased TPA antigen levels represent decreased or increased fibrinolytic activity is still a subject of debate.10

A different approach to seek differences between subjects who do develop an MI and those who do not is to look at differences at the DNA level. Unlike plasma concentrations of proteins, DNA is not influenced by the event of an MI, by preclinical atherosclerosis, or by its risk factors. The gene for TPA has been sequenced11 and mapped to chromosome 8p12-p11.2.12 Recently, one polymorphism, an Alu repeat I/D polymorphism, was found in intron h of this gene.13

The present study was set up to further explore whether plasma levels of TPA antigen and activity are associated with MI and whether the Alu I/D polymorphism could serve as a genetic marker for coronary heart disease in survivors of an MI.

Methods

Population

A cross-sectional case-control study was performed in subjects selected from the Rotterdam Study, a prospective population-based study of 7983 subjects. Between March 1990 and July 1993, all subjects ≥55 years old living in Ommoord, a suburb of Rotterdam, Netherlands, were invited to participate. The overall response rate was 78%. The study has been 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.12

Case patients (n=150) were selected from the cohort on the basis of the presence of an infarction pattern on the ECG according to the diagnostic classification system of the

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Modular ECG Analysis System15,16 independent of a history of chest pain. Two control subjects per case patient were drawn from the same 5-year age strata in which the cases of MI arose and constituted a sample of study participants who had no history of cardiovascular disease, ie, no history of MI, angina pectoris, or stroke, a normal ECG, and no peripheral arterial disease (ankle/arm index >0.9). We excluded subjects using anticoagulant drugs.

Measurements

All subjects were first visited at their homes. Information on current health status, medical history (including MI and stroke), drug use, and smoking behavior was obtained by a computerized questionnaire, which included the Dutch version of the Rose cardiovascular questionnaire.17 The home interview was followed by two visits at the research center between 8 AM and 4 PM. Patients were not asked to fast or to refrain from smoking. During those visits, several cardiovascular risk indicators were determined. Height and weight were measured, and body mass index was calculated (in kilograms per meter squared). Sitting blood pressure was measured at the right upper arm with a random-zero sphygmomanometer. The average of two measurements obtained at one occasion was used. Systolic blood pressure at the ankles (posterior tibial artery) was measured with the patient in the supine position with an adult-size regular cuff just above the malleoli and an 8-MHz Doppler transducer.18 The ankle/arm index is the ratio of the systolic blood pressure at the ankle to the arm systolic pressure. Peripheral arterial disease was defined as a right or left ankle/arm index <0.9. Blood sampling and storage have been described elsewhere.19 Blood samples were collected in vacuum containers with CTAD (0.11 mol/L citrate, 15 mmol/L theophylline, 3.7 mmol/L adenine, and 0.198 mmol/L diprydramole; Diatube H, Diagnostica Stago) and Stabilyle vacuum containers (Biopool).20 Stabilyle plasma was collected after the subjects were visibly sitting using a biliner vacuum center in the first 2.5 years. TPA antigen was measured in CTAD plasma with the Biopool Immulys TPA enzyme immunoassay.21 TPA activity was measured in Stabilyle plasma with the Biopool ChromoTate TPA biotinimmunoassay. Serum total and HDL cholesterol were determined with an automated enzymatic procedure.

After the gene was isolated from blood cells, an I/D polymorphism resulting from the presence/absence of an AI repeat in the eighth intron of the TPA gene was identified in all subjects. Amplification of the 967/655-bp fragments of the TPA gene was performed essentially as previously described13 with the 5′ primer 5′-TCCGTAACAGGACAGCTA-3′ (PR-TPAO1; nt 25,216-25,234) and the 3′ primer 5′-ACCGTG CCTACGATCTAGGA-3′ (PR-TPAO2; nt 26,181-26,162). The following conditions applied: 50 μL of a mixture containing 20 mmol/L Tris-HCL (pH 8.4), 50 mmol/L KCl, 1 mmol/L MgCl2, 0.05% (vol/vol) detergent (polyoxyethylene ether), 0.05% (vol/vol) DMSO, 0.2 mmol/L each of nucleotide triphosphates, 100 ng of each primer, 100 μg DNA, and 1 U Taq polymerase was subjected to denaturation for 4 minutes at 94°C, followed by 32 cycles of 94°C (1 minute), 58°C (1 minute), 72°C (2 minutes), and finally followed by 4 minutes at 72°C. Some 25 μL of the PCR products was separated on a 2.5% agarose gel.

Statistical Analysis

Means and proportions for baseline cardiovascular risk factors were computed for the case and the control subjects. The relative risks of MI (estimated as the odds ratio) for those heterozygous and homozygous for the insertion compared with those homozygous for the deletion and for the quartiles of TPA antigen and TPA activity compared with the lowest quartile were calculated by logistic regression. Results are presented with a corresponding 95% CI. Means and proportions of potential confounders were calculated for the three genotypes, and differences between the genotypes were tested with ANOVA. Adjusted relative risks were calculated further by logistic regression models that controlled for age, sex, and systolic blood pressure. To assess the possible influence of other cardiovascular disease risk factors on the association between the plasma TPA antigen activity and MI, adjusted estimates of risk were obtained with logistic regression models that controlled for age and sex, total and HDL cholesterol, systolic and diastolic blood pressures, body mass index, and current smoking. The association between TPA antigen and TPA activity was evaluated by multivariate linear regression analyses. Separate analyses were performed for TPA activity in blood collected at baseline and that collected in the second phase of the study. No material differences were found (data not shown).

Results

Of the 450 subjects selected, blood cells were available for 421 subjects, CTAD plasma was available for 433 subjects, and Stabilyle plasma was available for 411 subjects. After exclusion of those with missing data, the study population consisted of 121 case and 250 control subjects. Characteristics of the study population are shown in Table 1. As expected, there were more men among the case than among the control subjects, and subjects with a history of MI had a higher prevalence of conventional cardiovascular risk factors than control subjects. Serum total cholesterol was higher in control than in case subjects.

I/D Polymorphism

Among the 250 control subjects, 48 (19%) were found to be homozygous for the deletion allele, 127 (51%) heterozygous for both the deletion and the insertion alleles, and 75 (30%) homozygous for the insertion allele. The observed distribution of genotypes was consistent with that predicted by the Hardy-Weinberg equilibrium.

Table 1. Baseline Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n=250)</th>
<th>Case Patients (n=121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>72 (9)</td>
<td>73 (10)</td>
</tr>
<tr>
<td>Women, %</td>
<td>60</td>
<td>43</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>139 (21)</td>
<td>143 (24)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73 (11)</td>
<td>76 (13)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26 (4)</td>
<td>27 (4)</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.5 (1.1)</td>
<td>6.2 (1.1)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4 (0.3)</td>
<td>1.2 (0.3)</td>
</tr>
<tr>
<td>TPA antigen, ng/mL</td>
<td>10.1 (5.5)</td>
<td>11.2 (5.6)</td>
</tr>
<tr>
<td>TPA activity, IU/mL</td>
<td>1.12 (0.64)</td>
<td>1.21 (0.75)</td>
</tr>
</tbody>
</table>

Values are unadjusted percentages or means and SDs. *This variable was used as a matching criterion.
The genotypes characterized by either one or two insertion alleles (genotypes I/D and I/I) were associated with an excess number of cases of MI compared with the D/D genotype. The “crude” relative risks (odds ratios) of non-fatal MI for the different genotypes are presented in Table 2. To examine whether the results of our study were confounded by other risk factors for MI, we evaluated whether these cardiovascular risk factors were associated with the different genotypes. Table 3 presents means and proportions of these risk factors for the different genotypes. No significant differences in cardiovascular risk factors between the genotypes were found. Only the systolic blood pressure was somewhat higher in the high-risk genotype. Additionally, we adjusted the relative risks of nonfatal MI for the different genotypes for age, sex, and systolic blood pressure (Table 2). There was no difference in mean plasma levels of TPA activity or antigen for the three genotypes (Table 3). Further adjustment for plasma levels of TPA antigen and activity did not alter the associations.

**Plasma Levels of TPA Antigen and Activity**

Case subjects had a higher mean concentration of TPA antigen than control subjects. The difference adjusted for age and sex was 1.08 ng/mL (SEM, 0.63; P=.09). The TPA activity levels were slightly and not significantly higher in case compared with control subjects; age- and sex-adjusted difference was 0.07 IU/mL (SEM, 0.07; P=.36). There was no difference in the levels of TPA antigen or activity between men and women.

TPA antigen was positively associated with the risk of MI; compared with subjects with a level in the lowest quartile of the TPA antigen distribution, the relative risk for MI increased in the second, third, and upper quartiles: 1.7 (95% CI, 0.9-3.3), 2.3 (1.2-4.4), and 2.0 (1.0-3.8), respectively. When adjusted for HDL and total cholesterol, body mass index, systolic and diastolic blood pressures, and current smoking, the risk was attenuated; compared with that in the lowest quartile, the relative risks in the second, third, and upper quartiles were 1.7 (95% CI, 0.8-3.6), 1.7 (0.8-3.5), and 1.3 (0.6-2.8), respectively.

The relative risks for MI in the second, third, and upper quartiles compared with the lowest quartile of the TPA activity distribution were 1.1 (95% CI, 0.6-2.2), 1.1 (0.6-2.2), and 1.3 (0.7-2.6), respectively. When adjusted for HDL and total cholesterol, body mass index, systolic and diastolic blood pressures, and current smoking, the risk for MI associated with TPA activity increased; compared with that in the lowest quartile, the relative risks in the second, third, and upper quartiles were 1.2 (95% CI, 0.6-2.3), 1.3 (0.6-2.5), and 1.8 (0.9-3.7), respectively.

There was an inverse association between TPA antigen and activity. After adjustment for age and sex, an increase in TPA antigen of 1 ng/mL was associated with a decrease in TPA activity of 0.014 IU/mL (SEM, 0.006; P=.02). However, after additional adjustment for body mass index, systolic blood pressure, and total and HDL cholesterol, the association was no longer present (regression coefficient, −0.001 IU/mL; SEM, 0.006; P=.86).

**Discussion**

The objective of the present study was to assess the association of the I/D polymorphism of the TPA gene and the plasma concentration of TPA antigen and activity with MI. The insertion allele of the TPA gene was associated with an increased risk of nonfatal MI independent of plasma concentrations of TPA and of other known risk factors. Consequently, this polymorphic marker offers an independent predictor for nonfatal MI. TPA antigen concentration was positively associated with the risk of MI, but after adjustment for other cardiovascular disease risk factors, this association was markedly attenuated. Increased TPA activity, usually interpreted as the main determinant of clot-dissolving capacity of the hemostatic system, showed a tendency to be associated with an increased risk for MI.

Concerning the design of the study, several aspects need to be discussed. First, it is unlikely that population heterogeneity can explain our results. Case and control subjects were drawn from one single-center population-based study of 7983 subjects. All subjects participating in our study were white, and allele frequencies did not differ from those

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**Table 3. Potential Confounders of the Association Between the Polymorphism and Myocardial Infarction Evaluated for the Different Genotypes (When Appropriate, Adjusted for Age, Sex, and Case)**

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Women, %</th>
<th>Systolic blood pressure, mm Hg</th>
<th>Diastolic blood pressure, mm Hg</th>
<th>Body mass index, kg/m²</th>
<th>Total cholesterol, mmol/L</th>
<th>HDL cholesterol, mmol/L</th>
<th>TPA antigen, ng/mL</th>
<th>TPA activity, IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>71</td>
<td>56</td>
<td>133</td>
<td>75</td>
<td>27</td>
<td>6.3</td>
<td>1.3</td>
<td>10.0</td>
<td>1.14</td>
</tr>
<tr>
<td>73</td>
<td>57</td>
<td>141</td>
<td>74</td>
<td>26</td>
<td>6.5</td>
<td>1.3</td>
<td>10.7</td>
<td>1.18</td>
</tr>
<tr>
<td>72</td>
<td>51</td>
<td>142</td>
<td>74</td>
<td>26</td>
<td>6.4</td>
<td>1.3</td>
<td>10.5</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 2. Values are percentages or means. *P for trend obtained from linear regression analysis.
observed by others. Second, by virtue of its design, a cross-sectional study is limited to cases of nonfatal MI. To investigate whether the I/D polymorphism is also a marker for fatal MI, a longitudinal study is required. Third, a source of bias in a cross-sectional study may be a changed risk profile after the MI. As for plasma levels of TPA antigen and activity, this type of bias cannot be excluded; hence, we hesitate to interpret the findings concerning plasma levels as causal. As for the association between the I/D polymorphism and the risk of MI, no such bias is expected. Fourth, blood samples were taken at various times during the day, and subjects were not asked to fast. Therefore, the between-subject variability of TPA levels in our study population may be larger than in some other studies. This may influence the precision of the estimated difference between case and control subjects. However, the point estimate of the difference in TPA antigen level between case and control subjects was 10%, which is similar to that reported by others.

Finally, in previous studies, TPA activity was measured without measures against complex formation with the main inhibitor of TPA, PAI-1, which led to relatively low levels of TPA activity. In our study, an acid blood collection and more adequate methodology were used to measure the circulating free TPA activity.

The theory underlying this research is that people with a decreased fibrinolytic capacity are at increased risk of an acute MI. Several methods to assess "fibrinolytic capacity" have been proposed. Plasma levels of the fibrinolytic factors have been associated with the risk for MI. We chose to assess the only polymorphism in the gene for PAI-1 identified thus far. Assessment of genetic parameters has the advantage that intersubject variability can be measured irrespective of where and when the fibrinolytic capacity should be at its highest, namely, in the coronary arteries at the moment of thrombus formation. The nature of the I/D polymorphism, an insertion of an Alu repeat in an intron, a nontranslated region, makes a direct functional effect of the I allele on the TPA protein unlikely but not impossible. In this respect, the Alu insertion in the TPA gene shows a similarity with the Alu I/D polymorphism present in the gene for ACE. Here, the deletion allele is strongly associated with plasma levels of ACE. Despite intensive sequence effort spanning the entire coding region of the ACE gene, no apparent functional polymorphism has been found. It was suggested that the Alu I/D event can alter mRNA stability and/or splicing. In contrast to the ACE I/D polymorphism, the Alu polymorphism in the TPA gene was not associated with TPA plasma levels. Furthermore, the basal endothelial TPA synthesis was reported not to be influenced by the TPA Alu polymorphism. However, these findings do not exclude the existence of an association between this polymorphism and the endogenous fibrinolytic capacity. As indicated above, the circulating levels of TPA as measured in an asymptomatic period might not reflect the fibrinolytic capacity at the moment and site of thrombus formation. In addition, the Alu repeat insertion may be closely linked to a mutation at or near the TPA gene that produces a functional effect (impaired fibrinolytic capacity?) and may cause an increased risk for MI.

The heterogeneous genotype seemed to have an intermediately increased risk for MI, which is suggestive of a gradually increased risk for MI across the genotypes. This suggests a dose-related response and supports a causal relation. It may be speculated that a relative risk of two is relatively small for a genetic disorder. However, cardiovascular disease is a multifactorial disease. The complex process of atherothrombosis is influenced by many, partly competitive determinants. Interaction of known and unknown cardiovascular disease risk factors may influence the impact of these factors on the course of the disease, resulting in a relatively low relative risk.

Interpretation of the finding that increased plasma levels of antigen concentrations of the activator of plasminogen, TPA, and possibly also increased activity of TPA were associated with increased risk for MI against a background of a supposedly decreased fibrinolytic activity is difficult. Increased TPA antigen concentration in subjects at higher risk for MI is in agreement with findings in other studies and has been suggested to reflect predominantly TPA/PAI-1 complex. Because PAI-1 concentration in plasma is much higher than TPA antigen concentration and the TPA antigen assay measures both free and complexed TPA, increased concentration of TPA antigen is supposed to indicate a reduced rather than enhanced fibrinolytic activity. In the present study, increased TPA antigen was indeed associated with decreased TPA activity. This supports the notion that the increased risk of MI in those with increased TPA antigen concentrations is due to decreased TPA activity. The observation that the association between TPA antigen and MI is attenuated when cardiovascular risk factors are taken into account indicates that TPA antigen is associated with these risk factors. Whether TPA antigen is associated with the risk for MI as an intermediate factor in the same causal chain or in concert with the established cardiovascular disease risk factors remains to be established. Our findings on TPA activity, however, introduce a novel difficulty. The present study confirms an inverse association between TPA antigen and TPA activity, but in contrast, an increased TPA activity tended to be associated with an increased risk for MI. Because we are the first to assess the association between MI and TPA activity as measured in Stable plasma and because the association was not statistically significant, it is difficult at this moment to judge the relevance of this finding.

In conclusion, the insertion allele of the Alu repeat I/D polymorphism of the TPA gene is independently associated with nonfatal MI. This polymorphism consequently appears to be an independent genetic indicator for increased risk of nonfatal MI. The increased risk associated with the I allele is not reflected in TPA plasma concentrations.

Acknowledgments

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References


