Plasminogen Activator Inhibitor-1 4G Allele in the 4G/5G Promoter Polymorphism Increases the Occurrence of Cerebral Ischemia After Aneurysmal Subarachnoid Hemorrhage

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Background and Purpose—In several acute life-threatening diseases, the 4G-allele in the 4G/5G-promotor polymorphism in the plasminogen activator inhibitor-1 (PAI-1) gene is associated with higher PAI-1 levels and increased poor outcome, probably by promoting the formation of microthrombi. The aim of the present study was to investigate whether the PAI-1 4G/5G polymorphism is associated with the occurrence of cerebral ischemia, rebleeding, and other events, and clinical outcome after aneurysmal subarachnoid hemorrhage.

Methods—DNA was collected and analyzed in 126 patients with aneurysmal subarachnoid hemorrhage admitted to the Academic Medical Centre Amsterdam and University Medical Centre Utrecht in the Netherlands. All episodes of deterioration were classified according to predefined criteria. Causes of poor outcome and functional outcome were assessed 3 months after the initial bleeding according to the 5-point Glasgow Outcome Scale (GOS).

Results—Secondary ischemia occurred more often in patients with the 4G genotype than in patients homozygous for the 5G allele (RR: 3.3; 95% CI: 1.1 to 10.0). No significant differences were found between the groups for rebleeding or other events. Patients with the 4G genotype tended to have a higher risk for poor outcome than patients with the 5G/5G genotype (RR 1.2; 95% CI 0.7 to 2.2).

Conclusion—The 4G allele in the PAI-1 gene increases the risk for cerebral ischemia after aneurysmal subarachnoid hemorrhage (SAH) and probably also the risk for poor outcome. After early aneurysm occlusion, treatment aimed at enhancing fibrinolysis might be effective to prevent and treat cerebral ischemia in patients with aneurysmal SAH. (Stroke. 2004;35:1280-1283.)

Key Words: plasminogen activator inhibitor-1 ■ subarachnoid hemorrhage ■ cerebral ischemia

Plasminogen activator inhibitor-1 (PAI-1) is the main inhibitor of tissue plasminogen activator (t-PA). It inhibits the conversion of plasminogen into fibrinolytically active plasmin. Plasma levels of PAI-1 are associated with the 4G/5G promoter polymorphism in the PAI-1-gene. The 4G allele is correlated with higher PAI-1 levels.1–3 Elevated PAI-1 levels are associated with poor outcome in several acute life-threatening diseases such as severe (head) trauma, meningococcal sepsis, meningitis, preeclampsia, malaria, and burns.1–6 These studies suggested that poor outcome is caused by elevated levels of PAI-1, which promote the formation of microthrombi. These microthrombi can lead to multiorgan failure.

Patients with aneurysmal subarachnoid hemorrhage (SAH) are threatened by rebleeding and cerebral ischemia. The fibrinolytic system appears to play an important role in these complications. Antifibrinolytic treatment reduces the occurrence of rebleeding but increases the risk of poor outcome from cerebral ischemia.7–9 Treatment with nimodipine, which decreases the occurrence of cerebral ischemia, increases fibrinolytic activity by decreasing PAI-1 levels in plasma.10 This increased fibrinolytic activity may add to the beneficial effects of nimodipine in patients with SAH.

The aim of the present study was to investigate whether the PAI-1 4G/5G polymorphism is associated with the occurrence of cerebral ischemia, rebleeding, and other events, and clinical outcome after aneurysmal subarachnoid hemorrhage.

Patients and Methods

Patients
Patients with signs and symptoms of aneurysmal SAH admitted to the Academic Medical Centre Amsterdam and University Medical Centre Utrecht were included if a computed tomography (CT) scan...
showed an aneurysmal bleeding pattern. Patients with a perimesencephalic bleeding pattern were included only when angiography showed the presence of an appropriate aneurysm. Patients without hemorrhage on CT, but with xanthochromia of cerebrospinal fluid confirmed by spectrophotometric analysis and presence of an aneurysm on angiography, were included as well. At both centers, patients were treated according to similar protocols, including nimodipine treatment.

**Definition of Events**

The clinical condition on admission was scored on the Glasgow Coma Scale (GOS). An event was defined as the occurrence of focal neurological impairment or a decreased level of consciousness of at least 1 point as recorded on the Glasgow Coma Scale, lasting for >1 hour, or both. Events were distinguished in definite or probable rebleeding and ischemia, hydrocephalus, operative complications (other than ischemia), and other events as follows: (1) definite rebleeding defined as sudden clinical deterioration with increased hemorrhage on CT scan compared with previous CT imaging or found at autopsy; (2) probable rebleeding defined as sudden deterioration or death suspect for rebleeding in which no CT scan or autopsy was obtained; (3) definite ischemia defined as gradual deterioration or death with infarction on CT scan compatible with clinical presentation or proven at autopsy; and (4) probable ischemia defined as gradual deterioration suspect for ischemia in which no CT scan or autopsy was obtained and other causes have been excluded by appropriate laboratory studies. In patients with deterioration directly after intervention, distinction was made between deterioration from ischemia or by other causes such as postoperative bleeding. Hydrocephalus was defined as an event with CT proven hydrocephalus. Other causes of clinical deterioration were also recorded (ie, pneumonia, meningitis).

**Outcome**

Functional outcome was assessed 3 months after the initial bleeding according to the 5-point GOS. Death, persistent vegetative state, and severe disability were defined as poor outcome, moderate disability, and good recovery as favorable outcome. In patients with poor outcome, causes of poor outcome were recorded. In patients with impaired consciousness or focal neurological deficits existing from the outset without further clinical deterioration, poor outcome was classified as a consequence of the initial hemorrhage. Other causes of poor outcome were defined as rebleeding, secondary ischemia, deterioration after intervention not caused by ischemia (ie, postoperative bleeding, epidural hematoma), hydrocephalus, and other causes (ie, adult respiratory distress syndrome, meningitis). Investigators (M.D.I.V., Y.B.W.E.M.R., and C.J.M.F.) were blinded for PAI-1 genotype during registration of events and determination of functional outcome.

**DNA Analysis**

PAI-1 genotype was determined using DNA derived from fibroblasts (Amsterdam patients) and white blood cells (Utrecht patients). A 189-basepair product, including the polymorphic site of the PAI-1 gene, was generated by polymerase chain reaction amplification with the primers 5'-CAACCTCATCCCAGACAGGT-3' and 5'-CACCGCTATGGTAGG-3'. After dNTP removal, a primer extension reaction with ddNTPs only was performed with the primer 5'-GAGAGTCCTGACACGGG-3'. Single base extension products reflecting the 4G or 5G genotype were purified and analyzed on a Biflex III MALDI TOF mass spectrometer (Bruker Daltonik) in linear mode.

**Statistical Analysis**

We used χ² and Fisher exact test when appropriate with corresponding 95% confidence intervals (95% CI) to compare differences between groups.

**Results**

The study group (n=126) consisted of patients admitted to the Academic Medical Centre, Amsterdam, the Netherlands (n=36) and the University Medical Centre Utrecht, the Netherlands (n=90).

Table 1 shows the baseline characteristics and the distribution of the 4G and 5G alleles. Mean age was similar in the groups with 4G/4G, 4G/5G, and 5G/5G genotypes. There were slightly more women in the 4G/5G group and less patients with an initial Glasgow coma score of 15 in the 5G/5G group.

In the 4G/4G and 4G/5G group, more patients had secondary ischemia than in the 5G/5G group (RR 3.3; 95% CI: 1.1 to 10.0) (Table 2). No statistically significant differences were found between the 3 groups for rebleeding or other events. More patients in the 4G/4G and 4G/5G groups had poor outcome (RR 1.2; 95% CI: 0.7 to 2.2).

**Discussion**

In the present study we demonstrate a significant association between the 4G allele in the PAI-1 gene and the occurrence of cerebral ischemia after aneurysmal SAH. The association of the 4G allele and cerebral ischemia could not be explained by differences in baseline characteristics. Patients with the 4G genotype tended to have a higher risk for poor outcome than patients with the 5G/5G genotype. The distribution of the PAI-1 4G/5G polymorphism in the present study was different from that in other studies. Several studies have shown that baseline PAI-1 levels are similar or just slightly higher in patients homozygous for the 4G allele than in patients homozygous for the 5G allele. Because bleeding in SAH lasts not more than a few seconds, it is not to be expected that the PAI-1 genotype has an effect on the extent of the initial hemorrhage, and hereby on the condition of the patient immedi-

**TABLE 1. Baseline Characteristics and 4G/5G Polymorphism**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>4G/4G</th>
<th>4G/5G</th>
<th>5G/5G</th>
</tr>
</thead>
<tbody>
<tr>
<td>N of patients (%)</td>
<td>126 (100)</td>
<td>26 (21)</td>
<td>72 (57)</td>
<td>28 (22)</td>
</tr>
<tr>
<td>Mean age, y (SD)</td>
<td>53 (13)</td>
<td>55 (11)</td>
<td>54 (13)</td>
<td>51 (12)</td>
</tr>
<tr>
<td>N of women (%)</td>
<td>86 (68)</td>
<td>15 (58)</td>
<td>54 (75)</td>
<td>17 (61)</td>
</tr>
<tr>
<td>N of patients with initial GCS score of 15 (%)</td>
<td>59 (47%)</td>
<td>12 (46%)</td>
<td>37 (51%)</td>
<td>10 (36%)</td>
</tr>
</tbody>
</table>

*No initial GCS score available for 1 patient from the 4G/4G group because of sedation and intubation at admission.
ately after the hemorrhage. Nevertheless, there was a slight difference in the number of patients with impaired consciousness between the groups in favor of patients with the 4G allele.

In acute disorders PAI-1 seems to act as an acute-phase reactant. Variation in PAI-1 levels between genotype groups is much more pronounced in the hours and days after the onset of an acute life-threatening disease compared with baseline concentrations. In these circumstances, concentrations in patients with the 4G/4G genotype are 2- to 4-times higher than in patients with the 5G/5G genotype. In acute life-threatening diseases, a genetic predisposition to produce high levels of PAI-1 is associated with poor outcome. This is explained by elevation of PAI-1 levels after stressful events in patients with the 4G allele, resulting in impaired fibrinolysis. The formation of microthrombi by disseminated intravascular coagulation during the acute illness is no longer counteracted by the fibrinolytic system, finally leading to multiorgan failure.

The exact mechanism of the development of cerebral ischemia in SAH is still unknown. Vasospasm seems to play an important role, but not all patients with vasospasm have cerebral ischemia. Probably the development of microthrombi in patients with vasospasm determines whether cerebral ischemia will occur. PAI-1 levels may be decisive in the development and dissolution of microthrombi.

Because more patients in the 5G/5G group had a GCS of 14 or less on arrival, an increase in poor outcome in this group could be expected. However, in our study, patients with the 4G allele had an increased risk of poor outcome compared with patients with the 5G/5G allele. This higher risk of poor outcome is likely a consequence of the higher risk of cerebral ischemia in patients with the 4G allele because the frequency of other complications was not different. That the statistically significant effect on cerebral ischemia did not result in a statistically significant effect in poor outcome can probably be explained by dilution, because cerebral ischemia is only one of the many complications that causes poor outcome in patients with SAH.

The effect on poor outcome may also be less obvious because the increase in PAI-1 levels is partially counteracted by the calcium-antagonist nimodipine, which all patients received. Nimodipine has been shown to reduce the risk of cerebral ischemia; however, the exact mechanism by which nimodipine reduces the occurrence of ischemia is not known. One of the actions of nimodipine is to increase fibrinolytic activity by reducing PAI-1 levels in plasma. By decreasing PAI-1 levels, nimodipine may antagonize an increase in poor outcome caused by cerebral ischemia. This might especially be true in patients carrying the 4G allele in whom higher PAI-1 levels can develop in the first few days after the initial hemorrhage.

Drugs that reduce fibrinolytic activity decrease the risk of rebleeding after SAH. Therefore, we expected to find a lower incidence of rebleeding in the 4G genotype group. This association was only found in the 4G/5G group and was not in the 4G/4G group, which is probably because of the small number of rebleeds in this study. Another explanation might be that the inhibition of fibrinolysis around the aneurysm by PAI-1 is insufficient to reduce the risk of rebleeding.

This study shows that patients with the 4G allele in the PAI-1 gene have an increased risk for cerebral ischemia after SAH. The results suggest that in these patients, after early occlusion of the aneurysm, treatment aimed at the enhancement of fibrinolysis might be effective to prevent and treat cerebral ischemia.

Acknowledgments
The authors thank M.E. Jakobs for expert technical assistance with the MALDI-TOF analysis.

References

### TABLE 2. Distribution of Complications and Poor Outcome

<table>
<thead>
<tr>
<th></th>
<th>All Patients (n=26)</th>
<th>4G/4G (n=26)</th>
<th>4G/5G (n=72)</th>
<th>5G/5G (n=28)</th>
<th>Relative Risk of Patients With the 4G Allele (95% CI*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rebleeding (%)</td>
<td>15 (12)</td>
<td>5 (19)</td>
<td>6 (8)</td>
<td>4 (14)</td>
<td>0.8 (0.3–2.3)</td>
</tr>
<tr>
<td>Ischemia (%)</td>
<td>38 (30)</td>
<td>9 (35)</td>
<td>26 (36)</td>
<td>3 (11)</td>
<td>3.3 (1.1–10.0†)</td>
</tr>
<tr>
<td>Other events (%)</td>
<td>40 (32)</td>
<td>7 (27)</td>
<td>23 (32)</td>
<td>10 (36)</td>
<td>0.9 (0.5–1.5)</td>
</tr>
<tr>
<td>Poor outcome (%)</td>
<td>47 (37)</td>
<td>10 (38)</td>
<td>28 (39)</td>
<td>9 (32)</td>
<td>1.2 (0.7–2.2)</td>
</tr>
</tbody>
</table>

*Relative risks are expressed as compared with patients homozygous for the 5G allele.
†Significant. P=0.01 (χ² analysis)
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