**α₁-Antichymotrypsin Polymorphism**

**A Risk Factor for Hemorrhagic Stroke in Normotensive Subjects**

Víctor Obach, MD; Marián Revilla, MD; Nicolás Vila, MD; Álvaro Cervera, MD; Ángel Chamorro, MD, PhD

**Background and Purpose**—Although genetic factors may be important in the pathogenesis of ischemic stroke (IS), little is known on the potential role of genes in most cases of hemorrhagic stroke (HS). Preliminary data showed that the TT genotype of the α₁-antichymotrypsin (ACT) gene polymorphism was associated with HS, although it remained unsettled whether prevalence of this polymorphism might differ between hypertensive and normotensive HS.

**Methods**—Ninety-nine patients with HS, 182 patients with IS (symptomatic control subjects), and 80 asymptomatic control subjects were genotyped for the ACT polymorphism using polymerase chain reaction amplification. Chronic hypertension was recorded in 66 patients with HS.

**Results**—The ACT-TT genotype was more prevalent in patients than in asymptomatic or symptomatic control subjects: 26%, 15%, and 16%, respectively. The ACT-TT genotype was obtained in 33% of HS who lacked arterial hypertension (P<0.05). After adjustment for age, gender, and vascular risk factors, the ACT-TT genotype remained independently associated with HS (OR 2.80, 95% CI 1.19 to 6.58, compared with asymptomatic control subjects; OR 1.79, 95% CI 0.95 to 3.40, compared with symptomatic control subjects). In analyses restricted to HS in normotensive patients, the ORs were 3.10 (95% CI 1.10 to 8.68) and 2.53 (95% CI 1.04 to 6.18), respectively.

**Conclusions**—These findings confirm in a larger series of patients the association between the ACT-TT genotype and HS. This polymorphism is more prevalent in normotensive bleedings. Pathological studies will be required to establish whether the ACT-TT genotype facilitates proteolytic rupture of vessels that harbor amyloidotic changes or another form of nonhypertensive cerebral angiopathy. (Stroke. 2001;32:2588-2591.)

**Key Words:** α₁-antichymotrypsin ■ cerebral hemorrhage ■ cerebral amyloid angiopathy ■ genetics ■ stroke

Hemorrhagic stroke (HS) represents 10% of all strokes, including a large proportion of fatal or severe cases.1,2 Advancing age and hypertension are the most important risk factors for HS.3,4 Although hypertension is the principal modifiable factor, abnormal blood pressure is only found in 55% to 80% of patients with HS.5,6 Therefore, additional nonhemodynamic factors intervene in the process of arterial rupture. Of those, cerebral amyloid angiopathy (CAA) has been increasingly recognized as a major cause of HS in the elderly.7,8 Cranial trauma, neoplasms, hematological disorders, drugs, and vascular malformations account for most identifiable causes of HS.9 However, due to a lack of pathological confirmation, in many cases of HS, the cause remains unknown.

Evidence has accumulated to suggest the role of genetic factors in the pathogenesis of ischemic stroke (IS), but much less is known of the role of genetic predisposition to HS. This statement is particularly pertinent in patients with HS who lack vascular risk factors such as arterial hypertension.11,13 In a preliminary report,14 we found that the TT genotype polymorphism of the α₁-antichymotrypsin (ACT) gene was associated with HS. However, insufficient number of patients precluded a more detailed analysis of the association between the ACT gene and HS and whether the relationship varied between hypertensive and normotensive individuals. Because markers of disease are especially needed in human conditions in which no modifiable factors are encountered, this study was aimed at investigating whether the increased prevalence of the ACT-TT genotype persisted in a larger series of patients with HS. Furthermore, we sought to assess whether the ACT-TT genotype could be more prevalent in normotensive HS.

**Subjects and Methods**

**Subjects** We studied 99 consecutive patients with HS who were admitted to our stroke unit between September 1998 and November 2000, including 38 patients previously reported.14 Sixty-six patients (67%) had a history of chronic hypertension; they received hypertensive medications at the time of the qualifying event or disclosed blood pressure recordings of >160 mm Hg systolic or >90 mm Hg diastolic on repeated measures. Patients were excluded from the study if the HS was associated with trauma, neoplasm, coagulation...
disorders, thrombolytic therapy, aneurysms, arteriovenous malformations, or alcohol ingestion of $>100$ g/d. In addition, 182 consecutive patients with IS admitted to our stroke unit and 80 subjects without a prior history of cerebrovascular symptoms, who were selected through random-digit dialing of the same geographic area of residence, were included in the study as a control group. In addition to hypertension, the following baseline characteristics and vascular risk factors were recorded in patients and control subjects: age, gender, current smoking, diabetes (treated or fasting glucose $\geq110$ mg/dL or $\geq2$ separate analyses), hypercholesterolemia (treated or $\geq240$ mg/dL), angina, and myocardial infarction, as previously described. All symptomatic subjects had at least a brain CT scan performed at hospital admission. A brain MRI was also performed in 9 patients and in 44 control subjects with IS. HS was defined on CT scan as a homogenous and well-defined area of increased density in the brain parenchyma. Patients with mixed areas of hypodense and hyperdense signals consistent with hemorrhagic transformation of an ischemic infarction were not included in the study because this condition is on occasion difficult to differentiate from HS. All CT scans were reviewed by investigators blinded to clinical and genetic data. Informed consent was requested to patients and control subjects, and the study was approved by the local ethics committee.

**Genotype Determinations**

To avoid the bias of early mortality, genotype determinations were obtained from blood samples drawn 1 day after admission. Genomic DNA was isolated from venous blood through erythrocyte lysis, proteinase K digestion, chloroform extraction, and ethanol precipitation. The $ACT^*$ polymorphism in the signal peptide (−15 Ala→Thr) was determined by polymerase chain reaction (PCR) amplification of a 124-bp fragment with the primers 5′-CAG TGA GAA TGG AGA-3′ and 5′-TTC TCC TGG GTC AGA TT-3′ as previously described with minor modifications. DNA amplification was performed with 120 ng of each patient’s DNA in a 25-μL PCR volume containing 1.5 mmol/L MgCl$_2$, 200 μmol/L concentration of each dNTP, 50 mmol/L KCl, 10 mmol/L Tris (pH 8.3), 400 μmol/L concentration of each primer, and 1 U of Taq polymerase (Boehringer-Mannheim). The amplification reaction consisted of an initial denaturation for 7 minutes at 94°C followed by 35 cycles of 30 seconds of annealing at 55°C, 45 seconds of extension at 72°C, 30 seconds of denaturation at 94°C, and a final extension step of 7 minutes at 72°C. The 124-bp PCR products were then digested with 5 U of the enzyme MvaI (MBI Fermentas) for 3 hours at 37°C and electrophoresed on a 8.9% polyacrylamide gel. After electrophoresis, the DNA was detected by silver staining. Two alleles were detected: $ACT^*$A (2 fragments of 84 and 33 bp) and $ACT^*$T (fragment of 117 bp).

**Statistical Methods**

Categorical variables were compared using the $\chi^2$ test. Age was expressed as mean±SD and was compared using unpaired Student’s $t$ test. Logistic regression models were used to determine the independent association of $ACT$ genotype and stroke subtype adjusted for confounders. Covariates included age, gender, current smoking, hypertension, diabetes, ischemic heart disease, and hypercholesterolemia as defined in Subjects and Methods. Moreover, separate logistic regression models were built for patients without a history of hypertension or for whom the hospital blood pressure recording was within the normal range. ORs and 95% CIs were calculated from β coefficients and SE values. $P<0.05$ was established as statistically significant. Hardy-Weinberg equilibrium was assessed using the $\chi^2$ test. All statistical analyses were performed with SPSS software version 9.0.

**Results**

As expected, the prevalence of vascular risk factors differed between patients and symptomatic and asymptomatic control subjects, as shown in Table 1. Thus, patients and symptomatic control subjects had a higher prevalence of hypertension and diabetes than did asymptomatic control subjects, and hypercholesterolemia, diabetes, and ischemic heart disease were more prevalent in symptomatic control subjects.

Genotype frequencies for the $ACT$ polymorphism are described in Table 2. We confirmed that the genotype frequency distributions were in Hardy-Weinberg equilibrium in the subgroup of normotensive patients ($\chi^2=1.61$) and in symptomatic ($\chi^2=0.68$) and asymptomatic ($\chi^2=0.45$) control subjects with $X^2_{df}<3.84$ ($df=1$, $P=0.95$). As reflected in Table 2, the $ACT$-$TT$ genotype was more prevalent in patients than in symptomatic and asymptomatic control subjects. Moreover, genotypic differences increased when patients were assessed according to blood pressure records. As also shown in Table 2, the $ACT$-$TT$ genotype was most prevalent: 33% of the patients, if they did not have a history of arterial hypertension on repeated blood measurements during hospitalization, revealed normal values. As illustrated in Table 3, logistic regression models adjusted for age, gender, current smoking, history of hypertension, diabetes, ischemic heart disease, and hypercholesterolemia showed that the $ACT$-$TT$ genotype was independently associated with HS compared with asymptomatic control subjects. To further assess the role of hypertension, separate models were built in normotensive and hypertensive patients. When the model was restricted to normotensive patients, the $TT$ genotype was an independent factor associated with HS compared with symptomatic and asymptomatic control subjects. Nevertheless, these analyses provided relative large CIs as the likely result of the sample size.

**TABLE 1. Main Characteristics of Study Population**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Asymptomatic Control Subjects (n=80)</th>
<th>Ischemic Control Subjects (n=182)</th>
<th>HS Patients (n=99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>68.1 (6)</td>
<td>68.8 (11)</td>
<td>71.5 (11)</td>
</tr>
<tr>
<td>Female gender, %</td>
<td>31 (38.8)</td>
<td>61 (33.5)$^\dagger$</td>
<td>45 (45.5)</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>12 (15.0)</td>
<td>46 (25.3)</td>
<td>21 (21.2)</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>21 (26.3)$^*$</td>
<td>124 (68.1)</td>
<td>66 (66.6)</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>7 (8.8)$^*$</td>
<td>64 (35.7)$^\dagger$</td>
<td>22 (22.2)</td>
</tr>
<tr>
<td>Ischemic heart disease, %</td>
<td>4 (5.0)</td>
<td>34 (18.7)$^\dagger$</td>
<td>8 (8.1)</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>18 (22.8)</td>
<td>62 (34.1)$^\dagger$</td>
<td>14 (14.1)</td>
</tr>
</tbody>
</table>

Values are given as mean (SD).

$^*$Significant differences between asymptomatic control subjects and HS patients.

$^\dagger$Significant differences between ischemic control subjects and HS patients.

**TABLE 2. ACT Genotype Distribution According to Blood Pressure Measurements**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>TT, n (%)</th>
<th>AT, n (%)</th>
<th>AA, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic control subjects</td>
<td>80</td>
<td>12 (15)</td>
<td>42 (53)</td>
<td>26 (32)</td>
</tr>
<tr>
<td>Ischemic control subjects</td>
<td>182</td>
<td>29 (16)</td>
<td>89 (49)</td>
<td>64 (35)</td>
</tr>
<tr>
<td>HS patients</td>
<td>All</td>
<td>99</td>
<td>26 (26)$^*$</td>
<td>37 (37)</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>66</td>
<td>15 (23)</td>
<td>24 (36)</td>
</tr>
<tr>
<td></td>
<td>Normotensive</td>
<td>33</td>
<td>11 (33)$^\dagger$</td>
<td>13 (40)</td>
</tr>
</tbody>
</table>

$^*$P<0.05, HS patients vs ischemic control subjects.

$^\dagger$P<0.05, HS patients vs control subjects.
A calcium-activated serine protease similar to cathepsin G was not mentioned whether premortem HS had occurred. Small pathological series of patients with CAA in whom it was found an association between the ACT-TT genotype and increased prevalence of the ACT-TT genotype in patients with HS. Interestingly, the strongest association was encountered in patients whose bleeds occurred despite normal blood pressure values and who had a history of previous trauma, neoplasm, coagulation disorder, thrombolytic or antithrombotic therapy, aneurysm, arteriovenous malformation, or excessive alcohol drinking. Because the strongest association of the ACT-TT genotype with HS was found in normotensive patients after adjustment for potential confounders, it could be suggested that this genetic trait is a marker of CAA. However, lacking pathological data to confirm the diagnosis of CAA, we cannot exclude that these patients had another form of nonhypertensive cerebral angiopathy in which vascular damage and subsequent bleeding were mediated by the intervention of proteolytic enzymes.

The role of proteolytic enzymes in the pathophysiology of cerebral bleeding has been recently suggested. ACT is a protease inhibitor that regulates the activity of serine proteases such as neutrophil cathepsin G. Release of this enzyme may lead to degradation of vascular matrix proteins and coagulation factors. The ACT gene is located on the long arm of chromosome 14 and belongs to a cluster of structurally related serine protease inhibitor genes. The polymorphism in the signal peptide sequence (−15 Ala→Thr) of the ACT gene has been evaluated in patients with Alzheimer’s disease and Parkinson’s disease. To the best of our knowledge, there are only 2 previous reports that evaluated ACT gene polymorphism in patients with amyloid deposits in the vessel wall of the central nervous system. Yamada et al found an association between the A allele and CAA in patients with Alzheimer’s disease. More recently, Durany et al found an increased prevalence of the TT genotype in a small pathological series of patients with CAA in whom it was not mentioned whether premortem HS had occurred.

Additional data link amyloid formation to serine proteases. A calcium-activated serine protease similar to cathepsin G was found involved in the generation of β-amyloid. Moreover, ACT is able to bind β-amyloid peptide in vitro in the absence of other proteins. ACT and APOE are also considered components of the chaperon protein system, that is, proteins that regulate the spatial conformation of other proteins such as the β-amyloid. Alternatively, the relation between the TT genotype with HS could indicate that the ACT polymorphism may be in linkage disequilibrium with another mutation of this gene or in another gene of the 1q4 region, perhaps pointing to other serine proteases or additional gene products.

The clinical diagnosis of CAA is controversial without pathological confirmation. According to the Boston criteria, the diagnosis of probable CAA includes MRI or CT demonstration of multiple cortical or corticosubcortical hemorrhages in individuals aged ≥55 years if other causes of hemorrhage are appropriately excluded. Brain MRI was performed in only 9 of our patients, and the most sensitive gradient-echo T2-weighted technique were performed in only 3 patients. None of the patients in our series had experienced previous hemorrhagic events, which is another characteristic of CAA, and all showed single instead of multiple lesions on CT or MRI. Therefore, it is uncertain whether we assessed patients with incipient CAA or whether they harbored some form of nonhypertensive HS. Moreover, because arterial hypertension has been documented in 32% of patients with CAA and because some patients with CAA may experience IS, we cannot exclude an stronger influence of the ACT-TT genotype on the bleeding risk associated with CAA if some of patients included in the IS control group and some of patients with hypertension and HS also had CAA.

In conclusion, the TT genotype of the ACT gene is found in patients with HS, most notably in patients with nonhypertensive HS in whom identification of disease markers is increasingly needed, because therapeutic strategies aimed at preventing or diminishing the bleeding risk are lacking. Whether the TT genotype of the ACT gene is a genetic predisposing factor to cerebral bleeding in patients with incipient CAA or whether it facilitates proteolytic damage of nonhypertensive cerebral vessels awaits appropriate pathological confirmation in future studies.

**Discussion**

We confirm in this larger study the results of our previous report indicating that the prevalence of the ACT-TT genotype is increased in patients with HS. Interestingly, the strongest association was encountered in patients whose bleeds occurred despite normal blood pressure values and who had a history of previous trauma, neoplasm, coagulation disorder, thrombolytic or antithrombotic therapy, aneurysm, arteriovenous malformation, or excessive alcohol drinking. Because the strongest association of the ACT-TT genotype with HS was found in normotensive patients after adjustment for potential confounders, it could be suggested that this genetic trait is a marker of CAA. However, lacking pathological data to confirm the diagnosis of CAA, we cannot exclude that these patients had another form of nonhypertensive cerebral angiopathy in which vascular damage and subsequent bleeding were mediated by the intervention of proteolytic enzymes.

ACT-TT genotype of the ACT gene is a genetic predisposing factor to cerebral bleeding in patients with incipient CAA or whether it facilitates proteolytic damage of nonhypertensive cerebral vessels awaits appropriate pathological confirmation in future studies.

**Table 3. OR of TT Genotype in Prediction of HS Dichotomized According to History of Hypertension**

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>TT Prevalence</th>
<th>Adjusted OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All HS vs ischemic control subjects</td>
<td>25% vs 16%</td>
<td>1.79 (0.95 to 3.40)</td>
</tr>
<tr>
<td>All HS vs asymptomatic control subjects</td>
<td>25% vs 15%</td>
<td>2.80 (1.19 to 6.58)</td>
</tr>
<tr>
<td>Normotensive HS vs ischemic control subjects</td>
<td>33% vs 16%</td>
<td>2.53 (1.04 to 6.18)</td>
</tr>
<tr>
<td>Normotensive HS vs asymptomatic control subjects</td>
<td>33% vs 15%</td>
<td>3.10 (1.10 to 8.68)</td>
</tr>
<tr>
<td>Hypertensive HS vs ischemic control subjects</td>
<td>23% vs 16%</td>
<td>1.56 (0.73 to 3.32)</td>
</tr>
<tr>
<td>Hypertensive HS vs asymptomatic control subjects</td>
<td>23% vs 15%</td>
<td>2.20 (0.88 to 5.47)</td>
</tr>
</tbody>
</table>

*Logistic regression model adjusted for age, gender, current smoking, history of hypertension, diabetes, ischemic heart disease, and hypercholesterolemia.

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