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. Advancing age and hypertension are the most important risk factors for HS. Although hypertension is the principal modifiable factor, abnormal blood pressure is only found in 55% to 80% of patients with HS. 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. Cranial trauma, neoplasms, hematological disorders, drugs, and vascular malformations account for most identifiable causes of HS. 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. In a preliminary report, 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. Sixty-six patients (67%) had a history of chronic hypertension; they received hypotensive 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 ≥110 mg/dL or ≥2 separate analyses), hypercholesterolemia (treated or ≥240 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′-CATG TGA GAA TGG AGA-3′ and 5′-TCT CTC TGA GTC AGA TTC-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 MgCl2, 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 an 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 χ² 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 χ² 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 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](http://stroke.ahajournals.org/)

<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)</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)†</td>
<td>22 (22.2)</td>
</tr>
<tr>
<td>Ischemic heart disease, %</td>
<td>4 (5.0)</td>
<td>34 (18.7)†</td>
<td>8 (8.1)</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>18 (22.8)</td>
<td>62 (34.1)†</td>
<td>14 (14.1)</td>
</tr>
</tbody>
</table>

*Values are given as mean (SD).
†Significant differences between asymptomatic control subjects and HS patients.
‡Significant differences between ischemic control subjects and HS patients.

![Table 2. ACT Genotype Distribution According to Blood Pressure Measurements](http://stroke.ahajournals.org/)

<table>
<thead>
<tr>
<th>ACT Genotype Distribution According to Blood Pressure Measurements</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></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>99</td>
<td>26 (26)*</td>
<td>37 (37)</td>
<td>36 (36)</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>66</td>
<td>15 (23)</td>
<td>24 (36)</td>
<td>27 (41)</td>
</tr>
<tr>
<td>Normotensive</td>
<td>33</td>
<td>11 (33)†</td>
<td>13 (40)</td>
<td>9 (27)</td>
</tr>
</tbody>
</table>

*P<0.05, HS patients vs ischemic control subjects.
†P<0.05, HS patients vs control subjects.
A calcium-activated serine protease similar to cathepsin G was found involved in the generation of \( \beta \)-amyloid. Moreover, \( ACT \) is able to bind \( \beta \)-amyloid peptide in vitro in the absence of other proteins. \(^{26} \) \( ACT \) 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 \( \beta \)-amyloid. \(^{27,28} \) 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. \(^{22} \)

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 \( \geq 55 \) years if other causes of hemorrhage are appropriately excluded. \(^{8} \) Brain MRI was performed in only 9 of our patients, and the most sensitive gradient-echo T2-weighted technique\(^{29} \) were performed in only 3 patients. None of the patients in our series had experienced previous hemorrhagic events, which is another characteristic of CAA. \(^{30} \) 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\(^{31} \) and because some patients with CAA may experience IS, \(^{32} \) 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.

### References


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