α₁-Antichymotrypsin Gene Polymorphism in Patients With Stroke

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Background and Purpose—A role for proteolytic enzymes has been suggested in the pathogenesis of stroke. In a search for new genetic factors, we investigated the gene polymorphism of the serine protease inhibitor α₁-antichymotrypsin (ACT) in patients with stroke.

Methods—Two hundred twenty patients with acute ischemic stroke (n=182) and primary intracerebral hemorrhage (n=38) and 70 control subjects without clinical cerebrovascular disease were genotyped for the ACT polymorphism.

Results—The ACT-TT genotype was more frequent in patients with primary intracerebral hemorrhage than in patients with ischemic stroke (31.6% versus16.4%, P<0.05) or in control subjects (21.4%, P=0.1). After adjusting for age, gender, and vascular risk factors, the ACT-TT genotype was associated with primary intracerebral hemorrhage, with an OR of 2.3 (95% CI 1.0 to 5.2) compared with ischemic stroke and an OR of 1.8 (95% CI 0.85 to 9.65) compared with controls.

Conclusions—Pending confirmation in a larger study, our results suggest that the ACT-TT genotype might be a risk factor for primary cerebral hemorrhage. (Stroke. 2000;31:2103-2105.)

Key Words: α₁-antichymotrypsin ■ cerebral hemorrhage ■ genetics ■ polymorphism ■ stroke, ischemic

There is accumulating evidence from twin and family studies that stroke is a complex disease with a strong interaction between environmental and genetic factors. Recently, several gene polymorphisms have been associated with an increased risk of ischemic and hemorrhagic stroke. Identification of new potential genes that may increase the risk of stroke independently or by modulating the effect of known vascular risk factors is a matter of great interest. A role for proteolytic enzymes has been suggested in the pathophysiology of ischemic and hemorrhagic stroke. Neutrophil cathepsin G, a proteolytic enzyme, may lead to vascular matrix degradation, platelet aggregation, and coagulation disorders. These effects are generally prevented by α₁-antichymotrypsin (ACT), a serine protease inhibitor that regulates the activity of neutrophil cathepsin G. The ACT gene is located on the long arm of the chromosome 14 and belongs to a cluster of structurally related serine protease inhibitor genes. A gene polymorphism for ACT has been evaluated in patients with Alzheimer’s disease, Parkinson’s disease, and cerebral amyloid angiopathy (CAA). However, there are no previous reports evaluating the ACT gene polymorphism in a stroke population.

Subjects and Methods
Study Population
We recruited 220 patients (145 men and 75 women; median age 68.5 years, range 27 to 88 years) with acute stroke admitted consecutively to our Stroke Unit. The following baseline characteristics and vascular risk factors were recorded: age, gender, current smoking, ischemic heart disease, diabetes (treated or fasting glucose ≥110 mg/dL), hypercholesterolemia (treated or ≥240 mg/dL), and hypertension (treated or >160 mm Hg systolic or >90 mm Hg diastolic). All patients had an emergency cranial CT scan to determine the stroke phenotype: ischemic infarct (IS, n=182) or parenchymal intracranial hemorrhage (PICH, n=38). The following additional diagnostic tests were performed as appropriate to document the stroke etiology in IS according to criteria used by the Stroke Data Bank: MR angiography (24%), carotid ultrasound (52%), angiography (6%), transcranial Doppler (10%), and echocardiography (24%). IS were classified as lacunar (n=24), atherothrombotic (n=33), cardioembolic (n=47), and undetermined (n=78). Patients with PICH related to trauma, neoplasms, coagulation disorders or thrombolytic therapy, aneurysms, or other vascular malformations were excluded. There were 25 patients with deep PICH and 13 patients with lobar PICH. To determine the distribution of ACT genotypes in the general population, we recruited 70 control subjects with no clinically detectable cerebrovascular disease (51 men and 19 women; median age 65.7 years, range 41 to 82 years), who were identified by random-digit dialing of the same geographic area of residence. Patients and controls gave their informed consent to participate in the study according to a protocol approved by local Ethics Committee.

Genotype Determinations
Blood samples were drawn the day after admission in all patients to avoid the influence of different early mortality rates between stroke phenotypes. 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 by using the primers 5'-CAG AGT TGA GAA TGG AGA-3' and 5'-TTC TCC TGG GTC AGA TTC-3' as described, with minor modifications. DNA amplifica-
Results

Table 1 shows demographics and vascular risk factors in patients and controls. As expected, patients with PICH and IS had a higher rate of hypertension and diabetes than did control subjects. However, current smoking was the only risk factor different between IS and PICH, whereas in the second model the dependent variable was PICH versus control. Independent covariates included age (<65 years versus control subjects). However, current smoking was the only risk factor different between IS and PICH. The distribution of ACT genotypes in patients and controls is reflected in Table 2. A similar distribution of ACT genotypes was found in patients with IS compared with controls and also among IS subtypes. Within patients with PICH, there was no association between TT genotype and the presence of advanced age (>75 years), hypertension, or hemorrhage topography (deep versus lobar).

Discussion

Although we found no evidence that ACT polymorphism was associated with any subtype of IS, the slightly higher prevalence of the ACT-TT genotype that was observed in patients with PICH might suggest that this genetic trait represented a susceptibility factor for this condition. Unfortunately, because of an insufficient number of patients, we were unable to perform reliable comparisons between patients with suspected different causes of PICH.

The increased risk of cerebral hemorrhage derived from this genotype could indicate that the ACT polymorphism itself was functionally involved by modifying the plasma levels or the enzymatic activity of the ACT. Alternatively, the relationship between the TT genotype and PICH could indicate that the ACT polymorphism may be in linkage disequilibrium with another mutation of this gene or in another gene of the 14q region, perhaps pointing to other gene products or additional gene products that could also be implicated.

The main limitation of the study is that the results were gathered in the setting of a case-control study with a relatively small number of PICH and controls. Another shortcoming is derived from the proper nature of PICH, which can result from manifold conditions and pathomechanisms, such as chronic hypertension or CAA. As a result, we cannot exclude a stronger association between TT genotype and PICH had a larger series of patients with hypertensive hemorrhage or CAA hemorrhage been genotyped.

There is some recent evidence linking amyloid formation to serine proteases. Thus, a calcium-activated serine protease...
similar to cathepsin G was found to be involved in the generation of β-amyloid, and this protease is the substrate for the ACT in the brain. Moreover, ACT binds with high affinity to β-amyloid peptide in cerebral vessels. Accordingly, TT genotype might be related to hypertensive PICH, given that ACT can inhibit angiotensin-converting enzyme protases that transform angiotensinogen I to the biologically active vasoconstrictor angiotensinogen II in vivo.

In conclusion, further investigations are required to confirm the contribution of ACT polymorphisms to the risk of cerebral hemorrhage and to assess the relationship between ACT genotype and ACT activity. The relative weight of genetic factors should also be evaluated in larger populations of patients with different sources of cerebral bleeding; a better understanding of the factors that determine cerebral bleeding susceptibility could certainly result in more effective prophylactic strategies.

References
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