Protease Inhibitors in Spontaneous Cervical Artery Dissections

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Background and Purpose—Observations in patients with arterial aneurysms, fibromuscular dysplasia, and spontaneous cervical artery dissection (sCAD) indicate that protease inhibitor deficiency might boost the enzymatic destruction of arterial tissue and increase the risk of these arterial wall diseases. Here we present the first large investigation of the protease inhibitor hypothesis in patients with sCAD.

Methods—Eighty patients with sCAD were compared with 80 age- and sex-matched healthy individuals. α_1-antitrypsin (α_1-AT) and α_2-macroglobulin (α_2-MG) levels, and α_1-AT genotypes were assessed and compared between groups.

Results—α_1-AT and α_2-MG levels as well as α_1-AT genotypes did not differ significantly between patients and controls. The frequency of *Z* alleles in the patient group was higher than in the control group and than in other cohorts from Europe; however, the difference remained nonsignificant. All patients with *Z* alleles had internal carotid artery dissections.

Conclusions—Overall, this data does not support the hypothesis that protease inhibitor levels or α_1-AT genotypes play an important role in the etiology of sCAD. The present data does not exclude that the *Pi-Z* allele might have an influence on subgroups of sCAD, such as internal carotid artery dissections. **(*Stroke*. 2005;36:9-13.)**

Key Words: alpha 1-antitryptsin ■ alpha-macroglobulins ■ dissection ■ protease inhibitors ■ risk factors

With an estimated annual incidence of ≈2.6 per 100 000, spontaneous cervical artery dissection (sCAD) is a rare disease in neurological practice.[1] Nevertheless, it is an important cause of stroke: 13% to 15.5% of strokes in adults <45 years[2,3] and 30% to 40% of brain stem and cerebellar infarctions in this population occur on account of sCAD.[4,5] Pathophysiologically, rupture of either the arterial intimal layer or the medial/adventitial layer, including vasa vasorum, causes an intramural arterial hematoma often leading to stenosis with a high risk of embolic brain infarction.[6,7] Association of sCAD with heritable connective tissue diseases, such as Ehlers-Danlos syndrome type IV and Marfan syndrome,[8,9] and ultrastructural connective tissue abnormalities in skin biopsies of sCAD patients[6,10] suggest an important etiologic role of connective tissue aberrations in sCAD.

Recently, a number of case reports on patients with protease inhibitor deficiency and sCAD have been published, suggesting a possible etiologic role of protease inhibitor deficiency for sCAD.[11–13] In a small series of 22 patients with sCAD, 27.3% showed low levels of α_1-antitrypsin (α_1-AT),[14] whereas no effect of α_1-AT was observed in 35 patients with sCAD.[15] Just recently, an investigation of α_1-AT deficiency alleles in 74 patients did not suggest a causal relationship between α_1-AT alleles and sCAD.[16] For other arterial wall pathologies, an association of reduced antiproteolytic activity has repeatedly been observed, including patients with arterial aneurysms[17–19] or fibromuscular dysplasia.[20–22] On the basis of these observations, it has been postulated that an imbalance of proteolytic and antiproteolytic enzymatic activity might be a possible risk factor for sCAD.[11–13] However, larger studies on proteinases inhibitor levels and genotypes in patients with sCAD are missing so far.

The major inhibitors of human proteinases are α_1-AT and α_2-macroglobulin (α_2-MG). The 2 most important genetic variants leading α_1-AT deficiency are named *S* and *Z* alleles. The normal variant is called *M* allele. *S* and *Z* alleles are caused by point mutations leading to amino acid exchanges (*S* allele: glutamic acid 264 to valine; *Z* allele: glutamic acid 342 to lysine). Severe α_1-AT deficiency is in the majority of cases associated with the Pi-ZZ genotype and causes severe damage of connective tissues in lungs, liver, and skin.[23] In this study, we tested the hypothesis that reduced antiproteolytic enzyme activity may increase the risk of cervical arterial dissections by assessing α_1-AT serum levels beyond the acute phase, α_1-AT genotype, and α_2-MG serum levels in a large group of patients with sCAD and age- and sex-matched healthy controls population.[24–30]

Received April 22, 2004; final revision received August 10, 2004; accepted October 6, 2004.

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Stroke **is available at http://www.strokeaha.org** **DOI: 10.1161/01.STR.0000149631.52985.27**

Methods

Patient Population

All patients with the diagnosis of sCAD established in our department between 1992 and 2002 were contacted. The diagnosis of sCAD was based on clinical signs, that is either a local compressive syndrome or cerebral ischemia, and at least 1 sign confirming neuroradiological investigation (MRI with transversal sections through the neck or/and arterial digital subtraction angiography). Eighty patients, who gave written informed consent and had blood samples taken >1 month after sCAD to avoid the acute phase reaction, were included in the study. Excepting this, there were no other criteria for patients inclusion. An equal number of sex- and age-matched control subjects was drawn from the Prospective Cardiovascular Munster (PROCAM) study, a prospective population-based study on cardiovascular risk factors.[24–30] EDTA-anticoagulated blood samples for genetic analysis could be obtained from 75 patients and 73 controls.

Biochemical and Genetic Analysis

For biochemical and genetic analysis, venous blood samples were taken in the early morning after overnight fasting (12 hours). The parameters α_1-AT and α_2-MG were determined immunonephelometically with the Dade Behring BNII system using polyclonal antisera from rabbit against these 2 proteins. C-reactive protein (CRP) was measured with the Roche Hitachi 747 system using Roche high-sensitive CRP reagents. For our laboratory, normal values are 90 to 200 μmol/L for α_1-AT, 130 to 300 μmol/L for α_2-MG, and <0.5 mg/dL for CRP. DNA was extracted from EDTA-anticoagulated blood samples with magnetic beads by Tecan DNA sample preparation system and frozen until analysis at −20°C. The α_1-AT genotype was determined with the Light Cycler (Roche Diagnostics) according to a method from Aslanidis et al[31] using the following primers and probes from TIB MOLBIOL (Berlin, Germany): *Pi*S* allele: sense GGTGCCTATGATGAAGCGTTTAGGC; antisense AGGTGTGGGCAGCTTCTTGGTCA; probe TTCTTCCTGCCTGATGAGGGGAAACTA-fluorescein; reporter LC-red640-GCACCTGGAAα-ATGAAC-p and for the *Pi*Z* allele: sense TCCACGTGAGCCTTGCTCGAGGCCTG, antisense TTGGGTGGGα-ATCACCACTTTTC, probe CTCCAGGCCGTGCATAAGGCTGT-fluorescein; reporter LC-red640-GACCATCGACGAGAAAGGG-p.

Statistical Analysis

Data were analyzed using SPSS for Windows, release 11.5.1. Patients and control subjects were matched according to age and sex. Baseline demographic characteristics of the study population were compared using χ^2 test for categorical data (with Fisher exact correction for cell sums <5) or Wilcoxon signed rank test for continuous data. CRP was classified in steps of 1 mg/dL. α_1-AT and α_2-MG levels were compared between groups using Wilcoxon signed rank test. In addition, we analyzed whether significant level differences between both groups existed after taking age and gender into account. This was done in 2 ANOVA models with α_1-AT and log-10–transformed α_2-MG as dependent variables and case status, age (in years), and gender as independent variables. Assumptions of normality were checked before the use of the ANOVA models and not found to be violated. Colinearities between factors were examined using Spearman correlations and ANOVA models. α_1-AT genotypes were compared between groups using χ^2 test with Fisher exact correction. Post hoc dissections were stratified into internal carotid artery (ICA) and vertebral artery (VA) dissections. The risk to experience ICA or VA dissection associated with the *Pi-Z* allele was determined using a binary logistic regression model.

Results

Thirty women and 50 men were included in each group, with a median age of 43.1 years (Table 1). χ^2 test with Fisher exact correction showed no significant differences between patients and controls concerning smoking, diabetes, and hypertension

TABLE 1. Baseline Characteristics

	Patients With sCAD	Controls
No.	80	80
Gender (female: male)	30:50	30:50
Age, y	42.2±10.2	44.0±8.5
Smoker	31	23
Diabetes mellitus	1	2
Hypertension	22	13
Minor trauma before sCAD	16	NA
Chiropractic manipulation before sCAD	14	NA
sCAD of carotid:vertebral:basilary artery	60:41:1	NA
sCAD in 1:2:3:4 arteries	63:14:1:2	NA
Completed strokes	57	NA

(P=0.24, P=1.00, and P=0.09, respectively). More than 70% of CRP levels were <0.5 mg/dL in both groups and >90% below 1 mg/dL. CRP did not significantly differ between patients and controls (Wilcoxon P=0.129). Sixteen patients could remember a minor trauma before the symptoms of dissection, including working with hyperextension of the neck, rapid neck movements, etc. Fourteen patients had cervical chiropractic manipulation to alleviate neck pain before the diagnosis of sCAD. SCAD occurred in 4 vessels in 2 patients (both vertebral and both ICAs), 3 vessels in 1 patient (both vertebral and left ICA), 2 vessels in 14 patients (6 both vertebral arteries, 1 left vertebral and basilary artery, and 7 both ICAs), in 63 patients in 1 artery (22 vertebral and 41 ICAs). Fifty-seven patients experienced completed ischemic infarction, 23 had transient ischemic or local compressive symptoms. Median time between sCAD and blood withdrawal was 35.3 months (mean 40.7±29.0).

α_1-AT and α_2-MG

Median α_1-AT levels were 130.5 μmol/L in the patient group and 124.5 μmol/L in the control group (Wilcoxon P=0.384). Median α_2-MG levels were 159.5 μmol/L for patients and 146.5 μmol/L for controls (Wilcoxon P=0.359). All α_1-AT levels <90 μmol/L were associated with either *Pi-S* or *Pi-Z* alleles. α_1-AT correlated with α_2-MG and with CRP (Spearman correlation coefficient r_S=0.247, P=0.004 and r_S=0.235, P=0.006, respectively). The time between sCAD and blood withdrawal had no effect on α_1-AT or log-transformed α_2-MG levels (ANOVA P=0.939 and P=0.926, respectively). Gender had a significant influence on α_1-AT and log-transformed α_2-MG levels (ANOVA P=0.007 and P=0.001, respectively), with male subjects having lower median α_1-AT and α_2-MG levels than female subjects (124.0: 131.0 μmol/L and 142.0: 179.0 μmol/L, respectively). Age, smoking habits, diabetes, or hypertension had no influence on α_1-AT and log-transformed α_2-MG concentrations. α_1-AT serum levels were significantly influenced by the α_1-AT genotype: subjects with MM-genotype had a median α_1-AT level of 127.5 μmol/L, MS had 118.0 μmol/L, MZ had 80.6 μmol/L, and ZZ had 20.9 μmol/L (ANOVA P=0.001).

One of the main results is that α_1-AT and α_2-MG levels did not differ significantly between patients and controls (Table

TABLE 2. α_1-AT and α_2-MG Serum Levels

	Patients:Median, Mean, and SD	Controls:Median, Mean, and SD	Wilcoxon Test
α_1-AT, μmol/l	130.5	124.5	
	130.9±37.5	129.8±32.1	P=0.384
α_2-MG, μmol/l	159.5	146.5	
	165.3±36.2	160.8±44.8	P=0.359

2). After adjustment for age and gender, there was still no significant difference between cases and controls. Introduction of CRP as a covariate does not change this result. Pathological α_1-AT values occurred in 8.6% of patients and 6.3% of controls, which were not significant in the χ^2 test. Pathological α_2-MG values were measured in 17.9% and 27.5%, which were not significant in the χ^2 test either.

α_1-AT Genotypes

An equal number of Pi-MM genotypes was identified in patients and controls. The allele frequencies found in the control group are 0.9521 for *Pi-M*, 0.0342 for *Pi-S*, and 0.0137 for *Pi-Z*. The allele frequencies in the patients with sCAD were 0.9333 for *Pi-M*, 0.0267 for *Pi-S*, and 0.0400 for *Pi-Z*. There was an almost equal distribution of the Pi-MS genotype (Table 3). The overall distribution of α_1-AT genotypes did not differ statistically between patients and controls (χ^2 test with Fisher exact correction P=0.839). However, the *Z* allele tended to occur slightly more often in patients: four patients had Pi-MZ and 1 patient ZZ genotype, compared with 2 controls with MZ-genotype. All patients with *Z* allele had sCAD of the ICA, 1 of them of both ICAs, none of them had VA dissection (Table 4). Post hoc stratification according to ICA- and VA-dissection did not show a significant effect of the occurrence of a *Z* allele on the occurrence of ICA- or VA-dissections (binary logistic regression).

Discussion

Abnormal proteolytic activity and altered α_1-AT genotypes have so far been described in patients with arterial aneurysms and fibromuscular dysplasia. A number of case reports demonstrated an association α_1-AT deficiency with arterial aneurysms.[12,21,32,33] Protease-antiprotease imbalance was revealed by measurements of the elastase-α_1-AT balance in tissue of aortic aneurysms and in the serum of patients with ruptured cerebral aneurysms.[17,34] Genetic studies provided divergent results, some suspecting an association of α_1-AT variants with arterial aneurysms,[19] others questioning such an association.[35–37] For fibromuscular dysplasia, an association with α_1-AT deficiency is also suspected.[20–22]

TABLE 3. α_1-AT Polymorphism

	α_1-AT Genotype	Patients	Controls	Fisher Exact Test
α_1-AT gene	Pi-MM	66	66	P=0.839
	Pi-MS	4	5	
	Pi-MZ	4	2	
	Pi-ZZ	1	0	
No. of subjects with genetic analysis		75	73	

TABLE 4. Patients With *Z* Alleles

α_1-AT Genotype	α_1-AT Levels, μmol/l	Age	Gender	Smoking	Dissected Vessel
Pi-MZ	80.0	54	male	no	Left ICA
Pi-MZ	86.9	43	female	no	Left ICA
Pi-MZ	72.6	38	female	no	Left and right ICA
Pi-MZ	74.2	37	female	no	Right ICA
Pi-ZZ*	20.9	45	male	no	Left ICA

*A detailed description of the patient with *Pi-ZZ* is given elsewhere.[11]

For sCAD, 6 case reports and 1 case series suggest an etiologic role of α_1-AT for spontaneous arterial dissections: dissection of the internal and external iliac arteries occurred in a 34-year-old man with Pi-SZ genotype,[38] a dissecting hematoma of the left coronary trunk in an α_1-AT-deficient 46-year-old woman,[39] ICA dissections occurred in a 38-year-old woman and a 50-year-old male with Pi-MZ genotype,[12,40] and sCAD with multiple aneurismal dilatations in a man with M_1S genotype.[33] Our group reported a 45-year-old male patient with Pi-ZZ genotype who had spontaneous ICA dissection with embolic middle cerebral artery occlusion and was successfully treated by systemic thrombolysis.[11] Recently, an increased rate of lowered α_1-AT levels was observed in a series of 22 sCAD patients,[14] whereas no association of α_1-AT with sCAD was found in 35 patients (16 in the acute phase, 19 in the convalescent phase).[15] The reports cited above raise the possibility that reduced levels of protease inhibitors, such as α_1-AT and α_2-MG, might be a risk factor for sCAD. However, patient numbers studied so far are much too small to generalize for the results, and underlying genetics were only analyzed in single cases. One very recent investigation of α_1-AT deficiency alleles in 74 patients did not find a relationship between α_1-AT alleles and sCAD; however, α_1-AT levels and other protease inhibitors were not investigated.[16]

This study investigates the protease inhibitor hypothesis, including a systematic genetic analysis in a relatively large number of patients, considering the low annual incidence of this disease.[1] Because of this fact, a retrospective study design was chosen, accepting the limitation that only patients who were able and willing to give written informed consent and blood samples up to 10 years after sCAD could be included. A strength of this study is the well-matched control population originating from a large population-based study.[24–30] The allele frequencies found in the present control cohort are within the range estimations of 0.9272 to 0.9708 for *Pi-M*, 0.0176 to 0.0564 for *Pi-S*, and 0.0074 to 0.0153 for *Pi-Z* for different European regions.[41] Because the regional provenance plays an important role, it is advantageous that the present control cohort was recruited within the same region of Germany (Westfalia) than the patient group. Rare α_1-AT deficiency alleles and null alleles genotypes occurring with frequencies like $1.1\times10^{(-4)}$ for Pi*Mmalton, $2.5\times10^{(-5)}$ for Pi*Mcobalt, or $1.410^{(-4)}$ for all null alleles combined were not included in the genetic analysis. However, these variants

lead to a dramatic reduction of α_1-AT serum levels to 3% to 15% of their normal values or <1% for null alleles.[42] That all α_1-AT serum levels <90 μmol were associated with either *Pi-S* or *Pi-Z* alleles makes it unlikely that we overlooked one of the rare variants. Acute phase reactions leading to artificially increased α_1-AT levels were avoided by testing patients >1 month after dissection. Nevertheless, the statistic power of the present sample is insufficient to test whether a combination of genetically determined protease inhibitor deficiency with acquired risk factors, such as smoking,[43,44] recent infection,[15] or reduced vitamin levels[18] may increase the risk of sCAD.

In summary, this data does not support the hypothesis that protease inhibitor levels or α_1-AT genotypes play an important role in the etiology of sCAD. The frequency of *Z* alleles in the patient group was higher than in the control group and than in other cohorts from Europe; however, the difference remained nonsignificant. Surprisingly, all patients with *Z* alleles had ICA and not VA dissections. Differences in the pathobiology of VA and ICA dissections have so far not been described. However, our group has reported that VA dissections are more often preceded by minor trauma-like chiropractic manipulations than ICA dissections, pointing toward different causative mechanisms.[45] Future research might therefore consider VA and ICA dissections separately.

Acknowledgments

Studies on sCAD at the University of Münster are supported by a grant from the Deutsche Schlaganfallhilfe and the Bundesministerium für Bildung und Forschung (Kompetenznetz Schlaganfall, 01GI 2909/3).

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Stroke. 2005;36:9-13; originally published online November 18, 2004;
doi: 10.1161/01.STR.0000149631.52985.27
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628