Alpha-1-antitrypsin deficiency alleles are not associated with cervical artery dissections

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Abstract—The authors searched for the presence of α-1-antitrypsin (AAT) deficiency alleles *PiZ* and *PiS* in 74 patients with spontaneous cervical artery dissections (sCADs) and in 74 healthy control subjects. In both groups, the authors found four carriers of deficiency alleles. The connective tissue morphology of one additional patient with sCAD with *PiZM* genotype and her relatives was studied in skin biopsies. The *PiZ* allele did not segregate with morphologic alterations of the dermal connective tissue in the family. Therefore, *AAT* deficiency alleles may not play a role in the etiology of sCAD.

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α-1-Antitrypsin (AAT) deficiency is a relatively common inherited condition, mostly caused by two defective alleles of the AAT gene, called *PiZ* and *PiS*.[1] The *PiZZ* and *PiZS* genotypes are associated with an increased risk to develop pulmonary emphysema in adults and liver disease in children and adults.[2] Some case reports also suggest an association of *AAT* deficiency with neurovascular diseases.[3] Because connective tissue aberrations are found in most skin biopsies from patients with spontaneous cervical artery dissections (sCADs), genetic factors that are involved in degrading or remodeling the extracellular matrix are potential candidate genes for this disease.[4] However, a systematic search for *AAT* deficiency alleles in patients had not been performed previously. In the present study, we searched for *PiS* and *PiZ* alleles in patients with sCAD and in healthy control subjects.[1] Moreover, we studied the association between connective tissue morphology and *AAT* deficiency in a family with two patients.

Methods. Blood was sampled from a consecutive series of 74 German patients with sCAD (42 men; 32 women; mean age, 41.5 years). MRI of the neck (mural hematoma) confirmed the diagnosis of CAD in all patients. For all patients, the presence of known hereditary connective tissue disorders was excluded by careful clinical examination. Patients who reported a possible relevant trauma within 1 month before the onset of the clinical symptoms were not included. For 66 of the 74 patients with sCAD, a skin biopsy was analyzed by electron microscopy. Skin biopsies were taken and processed for electron microscopic and histologic analysis as described previously.[4] Mild morphologic alterations were found in skin biopsies from 32 patients. In the majority of them, flower-like composite collagen fibrils and irregular elastic fibers with fragmentation and calculous inclusions were the most salient aberrations in the dermal connective tissue. In three patients, the alterations resembled findings in skin biopsies from patients with vascular Ehlers–Danlos syndrome (EDS; variable diameter of collagen fibers with hardly any composite fibrils). A molecular analysis of the *COL3A1* gene did not lead to the identification of a mutation, suggesting that these patients did not have EDS type IV. For the sequence analysis of COL3A1 cDNA, cultured fibroblasts were analyzed after reverse transcriptase-PCR.

We sampled a skin biopsy and blood from one additional patient with sCAD from Switzerland whose brother had died from a stroke caused by carotid artery dissection 1 year before and from seven healthy relatives. A dermatologist and a neurologist clinically examined all eight family members for signs of a connective tissue disease and symptoms or signs of previous cerebrovascular diseases, respectively. Genomic DNA from healthy German students and staff members was used as control samples (36 men and 38 women; mean age, 36.6 years).

DNA fragments were amplified by PCR with primers for exon 3 (TCTTATTCTGCTACACTCTTCCAAACC/GTCCCAACATGGCTAAGAGGTGTG) and exon 5 (GTGTCCACGTGAGCCTTGCTG/TCAGAGAAAACATGGGAGGGATTTAC) of the *AAT* gene. Digestion with *Sex*AI restriction enzyme of the amplified sequences of exon 3 enabled the detection of the *PiS* mutation and the distinction between the *M1*(ala^{213}) and *M1*(val^{213}) normal variants. The sequence of exon 3 was additionally analyzed for all patients and control subjects by automatic sequence analysis. For the detection of the *PiZ* mutation, the PCR products of exon 5 were denatured in formamide and analyzed on single-strand conformational polymorphism (SSCP) gels as described previously.[5] All samples with a mobility shift in the SSCP gels, indicating the presence of the *PiS* mutation, were analyzed by direct cycle sequencing of exon 5.

The performance of skin biopsies and the sampling of blood were approved by the local ethical committees (University of Heidelberg and University of Basel) and required informed consent of each subject.

Results. We found the *M1*(ala^{213}) allele on 26 of 148 chromosomes from patients and on 38 of 148 chromosomes from healthy control subjects. These allele frequencies do not significantly differ from comparable values in white patients in the United States (OMIM, 20 to 23%). Because the *PiZ* mutation cannot be detected as restriction frag-

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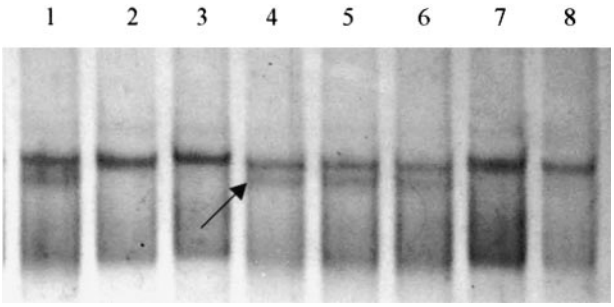

Figure 1. Identification of the PiZ *deficiency allele using single-strand conformational polymorphism (SSCP) analysis. Exon 5 of the* AAT *gene was amplified by PCR and analyzed after denaturation on a 10% polyacrylamide gel under SSCP conditions. Shifts (arrow) in lanes 1, 4, 5, and 6 indicated the presence of sequence variants. Subsequent sequence analysis revealed that these subjects carried the* PiMZ *genotype. The subjects analyzed on this gel are members of the Swiss family and numbered according to the pedigree shown in figure 2.*

ment length polymorphism, we tested for its presence by SSCP analysis and confirmed each observed mobility shift by sequence analysis. Figure 1 shows a typical SSCP gel with several heterozygous *PiZ* carriers. The results of the analyses of exon 3 and 5 are summarized in table 1. Among the 74 patients with sCAD, we found 4 carriers of an AAT deficiency allele (three patients with *PiMZ* genotypes and one patient with *PiMS*). Among the 74 healthy subjects, we detected 2 carriers of *PiMZ*, 1 carrier of *PiMS*, and 1 subject homozygous for *PiSS*. Other rare deficiency alleles were not detected during sequencing analysis.

Skin biopsies and DNA probes from seven first-degree relatives of a Swiss patient (not included in the association study because of her non-German nationality) were analyzed. An additional brother of the index patient died from ischemic stroke caused by carotid artery dissection and was not studied. The index patient had a transient hemiparesis on the right side together with a left-side Horner syndrome caused by spontaneous dissection of the extracranial left internal carotid artery at age 59 years. Digital subtraction angiography confirmed the diagnosis and further revealed an asymptomatic aneurysm of the right pericallosal artery. Neurologic examinations of the entire family were normal. Electron microscopic analysis of a skin biopsy showed mild aberrations that resembled the

Table *The distribution of the M1(ala) and M1(val) variants and of the* PiZ *and* PiS *alleles among patients and control subjects*

Genotypes	Patients	Control subjects
M1a/M1a	2	4
M1a/M1a + PiZ	0	1
M1v/M1a	19	26
M1v/M1a + PiZ	3	1
M1v/M1a + PiS	0	1
M1v/M1v	49	40
M1v/M1v + PiS	1	0
M1v/M1v + PiS/PiS	0	1

M1a = M1(ala^{213}); M1v = M1(val^{213}).

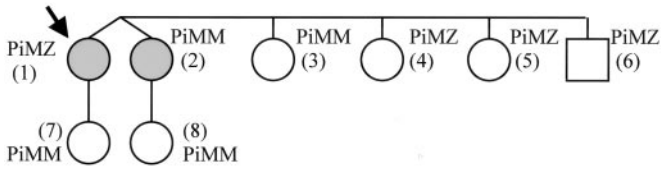

Figure 2. Segregation of a connective tissue phenotype and the PiZ *allele in the family of a patient with spontaneous cervical artery dissection (sCAD). Circle, female subject; square, male subject; open symbol, normal morphology of the connective tissue in a skin biopsy; filled symbol, aberrant morphology of the connective tissue in a skin biopsy; arrow, index patient with sCAD and PiMZ, heterozygous carriers of* PiZ *deficiency allele. An additional brother of the index patient died from ischemic stroke after a cervical artery dissection and was not included in this study.*

findings in patients with vascular EDS type IV. However, because sequence analysis of the *COL3A1* gene did not result in the identification of a mutation, the patient probably did not have a genuine vascular EDS. The index patient was heterozygous for the *PiZ* allele. Identical connective tissue aberrations were only observed in a skin biopsy from her (nonidentical) twin sister, who had a PiMM (normal) genotype. None of the other first-degree relatives revealed similar aberrations, but three of them carried the *PiZ* allele (figure 2).

Discussion. AAT is the most abundant plasma protease inhibitor, important for control of tissue proteolysis and the protection of blood vessels, especially arterial integrity. Inherited deficiencies are associated with an increased risk for tissue destruction in inflammatory diseases. The prevalence of the inherited deficiency alleles *PiZ* and *PiS* varies substantially between different white populations. In the German population, the major *PiZ* mutation has an estimated prevalence of 8 to 18 per 1000. The prevalence of *PiS* is estimated between 20 and 30 per 1000.[1] Our genotyping of the *AAT* deficiency alleles in a series of 74 control subjects is in agreement with these prevalence values. The finding of three heterozygous *PiZ* carriers and one *PiS* allele among our patients is not significantly different from the expected prevalence in the normal population.

Observations of the healthy relatives of a patient with sCAD with the *PiMZ* genotype and a connective tissue aberration enabled us to analyze a possible relationship between AAT deficiency and connective tissue alterations. The independent distribution of the *PiZ* allele and the connective tissue aberrations within the family indicate that there is no relationship between *AAT* deficiency and the connective tissue morphology.

Our observations confirm earlier observations of normal AAT activity in serum of 43 patients with acute sCAD.[6] However, low AAT plasma levels were recently found in a study of 22 Spanish patients with sCAD.[7] In a series of patients with renal fibromuscular dysplasia, a condition associated with arterial

dissections, the *AAT* phenotypes were normal.[8] Because *AAT* deficiency is a relatively frequent condition, the finding of deficiency alleles in some patients with sCAD may be accidental.[9,10] Therefore, our data do not suggest a causal relationship between *AAT* deficiency alleles and sCAD.

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