Familial Cervical Artery Dissections: Clinical, Morphologic, and Genetic Studies
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Familial Cervical Artery Dissections
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Background and Purpose—Genetic risk factors are thought to play a role in the etiology of spontaneous cervical artery dissections (CAD). However, familial CAD is extremely rare. In this study we analyzed patients with familial CAD and asked the question whether familial CAD has particular features.

Methods—Seven families with 15 CAD patients were recruited. All patients were carefully investigated by a neurologist, a neuroradiologist, and a dermatologist for clinical characteristics. From 11 patients a skin biopsy was performed to study the morphology of the connective tissue and to analyze the coding sequences of COL3A1, COL5A1, COL5A2, and part of COL1A1.

Results—The mean age of the patients (n=15, 9 women) at their first dissection was 36.2 years (median age 32 years, range 18 to 59). Two patients had bilateral CAD. One patient had a right and a left internal carotid artery dissection in successive weeks, another patient had 5 dissections over a period of 8 years. A high intrafamilial correlation was found between the affected vessels (ie, the carotid and the vertebral arteries) and between ages at the first dissection. In 1 patient we found clear and reproducible ultrastructural abnormalities in the skin biopsy, but the second patient from the family was not studied, because he died as a result of CAD before this study. The dermal connective tissue aberrations in the examined patient were similar to mild findings in patients with vascular Ehlers-Danlos syndrome (EDS type IV), but might be iatrogenic and related to long-term corticosteroid inhalation therapy. All other analyzed patients showed normal connective tissue morphology. In patients from 6 families we analyzed the whole coding sequence of COL3A1, COL5A1, and COL5A2, and from part of COL1A1. A missense mutation in the COL3A1 gene (leading to a G157S substitution in type III procollagen) was detected in both patients from 1 family. Two patients from another family carried a rare nonsynonymous coding polymorphism in COL5A1 (D192N); 1 of them carried also a rare variant in COL5A2 (T12337).

Conclusions—Familial CAD patients are young and probably are at high risk for recurrent or multiple CAD. Ultrastructural alterations of the dermal connective tissue might not be an important risk factor for familial CAD. However, the finding of a COL3A1 mutation revealed the presence of an inherited connective tissue disorder in 1 family.

Key Words: cervical artery dissection familial genetics

Spontaneous cervical artery dissection (CAD) is an important cause of ischemic stroke in young patients (<50 years). The pathogenesis of spontaneous CAD (sCAD) is not yet clear. The classical and vascular types of Ehlers-Danlos syndrome (EDS), hereditary connective tissue diseases, predispose to sCAD, which may be the result of structural defects of the arterial wall and the surrounding extracellular matrix. Only a minority of the patients with sCAD show clinical signs of a defined connective tissue disorder. Very few cases of familial CAD have been reported, some of them in families with known inherited connective tissue diseases.

Despite the absence of clinical signs of a connective tissue disease electron microscopic investigation of skin biopsies revealed the presence of abnormalities in the connective tissue morphology of 50% to 60% of patients with CAD. Disease-causing mutations in CAD patients without further signs of a known connective tissue disease have not yet been found, but positive genetic associations point to the existence...
of genetic risk factors for CAD (under the assumption that these associations are not spurious). A candidate locus on chromosome 15q2 was recently found by genetic linkage analysis in 1 large family with connective tissue alterations associated with CAD. That study of 3 families with sporadic CAD patients analyzed the segregation of the connective tissue phenotype as an intermediate marker. A segregation analysis of subjects affected with CAD was virtually impossible because familial cases of CAD were too rare.

For this study we collected 7 families with familial CAD. The index patients were referred to the Neurology and Dermatology Departments of the Heidelberg Medical School for an ultrastructural connective tissue diagnosis between 1997 and 2004. In the patients with familial CAD we searched for distinctive clinical features and studied the morphology of a skin biopsy by light and electron microscopy. The sequence of 3 candidate genes for vascular or classical EDS (COL3A1, COL5A1, and COL5A2) of 1 candidate gene for osteogenesis imperfecta (OI) (COL1A1) was analyzed. We also tested for 2 genetic variants that are considered as risk factors for CAD (MTHFR C677T and ICAM-1 E496K).

Materials and Methods

Patients and Clinical Diagnosis

We present 7 CAD patients with a familial history of CAD who were referred to the Neurology and Dermatology Departments of the Heidelberg University for morphological analysis of a skin biopsy between 1997 and 2004. We studied these patients (here referred to as “index patients”) as well as their relatives who have also had CAD (here referred to as “related patients”). Family I, IV, VI, and VII have not been included before in any study of this nature. Family II was included in a study of the association of COL3A1 and CAD. Family III was included in a sequence analysis of COL5A1 in 19 patients with CAD. The segregation of α-antitrypsin deficiency mutation PiZ was studied before in family V. Family VI are whites from Argentina, family V is German-Swiss, and the other 5 families are from Germany. None of the families was related with each other. All patients except patient II-2 were seen and investigated by one of the authors. All morphological studies and genetic analyses were performed in the Departments of Dermatology and Neurology of the University Heidelberg.

Patients were carefully examined by a stroke neurologist (for diagnosis of sCAD and an assessment of the clinical syndrome and the risk factor profile). A dermatologist examined all patients (except patients II-2, IV-2, and V-2) for the following signs of a known hereditary connective tissue disorder: marfanoid habitus, joint hypermobility, skin hyperextensibility, poor wound healing, easy bruising, hernias, thin translucent skin, striae, and varices. Migraine was diagnosed according to the International Headache Society Criteria.

Diagnosis of CAD was based on clinical signs at presentation, categorized in local syndromes (ie, Horner syndrome, cranial nerve palsy, etc) or focal cerebral ischemia (transient ischemic attack or stroke using the Oxfordshire Community Stroke Project Classification), as performed previously, in combination with at least 1 confirmatory neurovascular examination. The latter included magnetic resonance imaging of the neck with magnetic resonance angiography, extracranial Doppler ultrasonography, computed tomography angiography, and digital subtraction angiography. The diagnosis of CAD was additionally confirmed by autopsy in one patient (V-2). Functional outcome was assessed with the dichotomized modified Rankin Scale (mRS) by each patient’s neurologist. The criteria used was mRS 0 to 2 for “no or minor disability” versus mRS 3 to 5 for “major disability.” One patient who had died was assessed with mRS 6.

Laboratory Tests

Skin biopsies from the outer aspect of the upper arm were processed for microscopy and fibroblast culture as described. The morphological findings were compared with those of the control subjects from the previous published series. The performance of skin biopsies was approved by local ethical committees (University of Heidelberg, University of Basel) and required informed consent from each patient.

DNA was prepared from EDTA blood samples following standard procedures. For the analysis of the COL1A1, COL3A1, COL5A1, and COL5A2 genes, we prepared RNA from fibroblast, amplified the whole coding region of the genes in overlapping reverse-transcription polymerase chain reaction fragments, and analyzed these fragments by direct cycle sequencing as described in detail elsewhere. A fragment of the COL1A1 cDNA sequence encoding the N-terminal α-helical part of the procollagen was amplified with forward primer GAGTCACCCACCGACCAAGAAGAC and reverse primer CTGAAACCTCTGTGTCCTTCATCACT. The variants COL3A1 (G157S), COL5A1 (D192N), and COL5A2 (T1227S) were confirmed in genomic DNA samples from the patients by polymerase chain reaction and DNA sequencing analysis. DNA samples from 203 healthy subjects from the Heidelberg region were studied as control samples. For the detection of the COL5A1 D192N mutation in control subjects we amplified genomic DNA with primers ACCTTGATCCTCGACTGTAAA and AACACGATGAGCATTTGATG and analyzed the polymerase chain reaction products after TaqI digestion by 4% agarose gel electrophoresis. The MTHFR C677T single nucleotide polymorphism was assessed by polymerase chain reaction with primers CGTGCTGGTGAGAGTGAAGG and CCAAGCACCCCTGTGCAAGT and analyzed the polymerase chain reaction products after TaqI digestion with enzyme TaqI. Genotyping of ICAM-1 single nucleotide polymorphism E469K was performed as published previously.

Results

We analyzed 15 patients (9 women, median age 32 years, range 18 to 59) from 7 families (Table 1). One patient had 5 cervical artery dissections, spread over 8 years. Another patient had a contralateral internal carotid artery (ICA) dissection 1 week after her first ICA dissection. Two patients had bilateral cervical artery dissection. Within the families the same vessels (ie, either the carotid or the vertebral arteries) were affected. Ten patients had ischemic stroke as a result of the dissection; 13 out of the 15 patients recovered well (mRS <2).

Table 2 summarizes risk factors for CAD in the patients. Diabetes mellitus, dyslipidemia, fibromuscular dysplasia, or tortuosity of the carotid arteries did not occur among our patients with familial CAD. Trauma was associated with 7 out of the 23 analyzed events. Five patients had migraine before the onset of the CAD. Striae (unspecific signs of connective tissue weakness) were found in patients III-1, III-2, and V-1. Most risk factors were present in families II and IV, in which most dissections occurred.

The laboratory results are summarized in Table 3. The electron microscopic inspection of skin biopsies from the patients considered the morphology of the collagen fibrils and the elastic fibers. Semi-thin sections were also studied by light microscopy to evaluate the overall morphology of the skin. Unequivocal ultrastructural tissue alterations were present only in index patient V-1. Patient V-1 had a thin skin, and light microscopy showed low collagen content. Electron microscopic examination revealed small bundles of loosely spaced collagen fibrils with reduced diameters. Flower-like composite fibrils were rare. Her brother, patient V-2, died 2 years before and a skin biopsy could not be studied. The
findings in patient III-1 were very mild and might be in the range of the normal variability found in healthy control subjects. The morphology in patient III-2 was classified as normal. The findings in patient IV-2 were examined 3 decades after his dissection and his advanced age made a reliable diagnosis of his minor connective tissue aberrations particularly difficult.

TABLE 2. Possible Risk Factors for CAD

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AA indicates aorta aneurysm; AC, oral contraception; clinical CT signs, clinical signs of connective tissue weakness (joint hypermobility, skin hyperextensibility, poor wound healing, easy bruising, hernias, thin translucent skin, striae, varices); HRT, hormone replacement therapy; IA, intracranial aneurysm.

1. Chiropractic treatment of the cervical region (three days after the onset of initial spontaneous neck pain) triggered the subsequent development of local symptoms; 2. Three of the 5 cervical artery dissections in this patient were preceded by falls (2 of them during skiing) within 1 week before the onset of the symptoms; 3. Motor bike accident 6 months before dissection; 4. Initial symptoms developed after painting of ceiling; 5. Whiplash injury caused by roller coaster trip at parish fair.

*Migraine was diagnosed according to the International Headache Society Criteria.
The genetic studies included a sequence analysis of the whole coding regions of COL3A1, COL5A1, and COL5A2 in those patients who underwent skin biopsy. Genomic DNA was used to confirm genetic variants that were found in mRNA. A COL3A1 missense mutation was detected in family I. The G157S substitution in exon 14 was found in both patients as well as in their mothers (who are sisters) and in 2 unaffected sibs of patient I-2, too. The finding of this mutation suggests that the carriers suffer from genuine vascular EDS. However, neither the clinical appearance of the patients, nor the histology and electron microscopy of their skin biopsies revealed typical signs of vascular EDS. A missense mutation (D192N) was previously detected in the skin biopsies from patients III-1, IV-1, and IV-2 were classified according to their similarity to findings in patients with EDS.11 The alterations were less clear and pronounced than the typical findings in patients with sCAD as reported elsewhere.9-11

The evaluation of the dermal connective tissue morphology was based on electron microscopic and light microscopic analysis of skin biopsy material as described.3,4 The whole coding regions of COL3A1, COL5A1, and COL5A2 genes and the N-terminal part of the alpha-helical region of COL1A1 were analyzed by sequencing analysis of polymerase chain reaction-amplified cDNA. In family II the COL3A1/COL5A2 locus was excluded by linkage analysis as reported in an earlier study.17 The very mild morphologic alterations in the skin biopsies from patients III-1, IV-1, and IV-2 were classified according to their similarity to findings in patients with EDS.11 The T1227S nonsynonymous coding variant was genotyped as by RFLP analysis after polymerase chain reaction amplification as described.21,31

NS indicates not studied. *Patient was 69 years old at performance of the biopsy (38 years after the occurrence of CAD). †The alterations in patient 10 were mild, but repetitive and reproducible. However, long-term corticosteroid inhalation treatment might have induced structural alterations in the skin of the patient.

**Discussion**

In this study we presented findings from 15 patients with a familial history of CAD. In 1 family the father and both his daughters had dissections of the internal carotid arteries. Two first-degree relatives had CAD in 5 other families. A seventh family was investigated with 2 cousins who had ICA dissections at the ages of 18 and 19. Family V was the only large family (1 affected sister, 1 affected brother, and 8 healthy siblings), the other families were small (≤3 children). The median age of our patients at their first CAD was 32 years (mean age 36, range 18 to 59). Within the families the ages at which the patients had their dissections are correlated. In 6 families the patients suffered exclusively from dissections of either ICA or vertebral artery. Only in family IV were dissections in different cervical arteries diagnosed, because patient IV-1 developed vertebral artery (like his father at the age of 31 years) and subsequently ICA dissections.

Some earlier reports presented few cases of familial CAD and included relatives either with dissections in other arteries or with very remote relatives with dissections.6 Apparently, familial dissections are very rare, even thought CAD might be an underdiagnosed condition.22,23 Perhaps an earlier report of familiar fibromuscular dysplasia described in fact a case of familial CAD. Again a father and his daughter were affected at strikingly similar ages (32 and 33 years), and again the same vessel (ICA) was involved in both patients.24

The present series is the largest series of familial CAD patients reported so far. It is nevertheless too small to permit statistically significant generalizations. The data might suggest that familial CAD patients are younger than patients with sporadic CAD (mean age 42 to 44).25,26 The intrafamilial concordance of the CAD location is noteworthy because it suggests that the patients of a same family share specific familial risk factors. The classical vascular risk factors in our series of patients are apparently not different from those found in studies of sporadic CAD patients. A larger series of
families, however, has to be recruited in the future in order to validate these propositions.

We had expected to find known inherited connective tissue disorders among the patients with familial dissections, as suggested by 2 recent reports of familial dissections in relatives with OI and vascular EDS. In both published families the diagnosis was phenomenological and based on clinical and morphological observations, but not confirmed by the identification of a disease-causing mutation. However, light and electron microscopy of skin biopsy material did not permit the diagnosis of EDS or OI in any of the families in our study. We did not even find the mild but reproducible morphological aberrations that were described in 50% to 60% of sporadic CAD patients.

Previously, the connective tissue aberrations found in CAD patients have been classified according to a quantitative scale (from 0 to +++) that focused above all on the morphology of the collagen fibrils. We currently prefer a qualitative classification of the connective tissue alterations, with EDS-III like alterations most often found in CAD patients. In the analyzed patients from families I, II, VI and VII, the morphology of the dermal connective tissue was normal. In patients III-1, we found very mild aberrations, but could not find such aberrations in his sister, patient III-2. Because of this discordance we might question whether there is a causal relationship between CAD and the connective tissue morphology in this family. Because the aberrations in patient III-1 are extremely mild, we must be careful not to draw far-reaching conclusion. Minor EDS-IV–like alterations were found in patient IV-1, a patient that had 5 CADs over a period of 8 years. It is difficult to decide whether his father (IV-2) has similar (again very mild) alterations, because of a difference of 30 years of age between the subjects at the time of biopsy. Clear morphological aberrations (type EDS-IV like) were only found in patient V-1, but we could not reproduce these findings in here affected brother, who had died. Examination of skin biopsies from other siblings from this family revealed similar findings in one sister, whereas 4 other siblings and 2 children (1 of her and 1 of here affected sister) displayed a normal morphology. Both the index patient V-1 and the sister with positive electron microscopic findings had asthma and were treated with cortisone inhalation therapy for many years, which might have profound effects on the structure of the connective tissue.

In 4 of the 7 families, we did not find any irregularities in the morphology of the dermal connective tissue. In 2 other families the aberrations were very minor. In the only family with clear findings (family V), cortisone therapy might have interfered. In summary, we did not find any pair of patients with similar connective tissue aberrations in this series of seven families.

We analyzed the sequences of COL1A1, COL3A1, COL5A1, and COL5A2 after reverse-transcription polymerase chain reaction of RNA isolated from cultured skin fibroblasts of all patients except patients VI-1 and VI-6. We furthermore analyzed the sequences of exons 4 to 10 of COL1A1, because in a singular CAD patient with minor signs of OI, a mutation had been found in exon 6. A COL3A1 missense mutation was detected in both affected patients from family 1, as well as in the 2 patients' mothers (who are sisters). Clinical signs of connective tissue weakness were absent and skin biopsy findings were normal. This particular amino acid substitution at position 157 was not yet described, but substitutions in the adjacent glycines (at position 154 and 160) were found in EDS-IV patients with arterial complications. This type of mutation (glycine substitutions in triple helical region of COL3A1) is typical for EDS-IV, but was not found in CAD patients without clinical signs of vascular EDS. Our findings in family I suggested that a patient with familial CAD might be affected with mild and otherwise unrecognized forms of vascular EDS.

The relevance of the nonsynonymous COL5A1 and COL5A2 variants in family III is unclear, as discussed before. Both variants occurred at a low prevalence in the healthy control population. We found no exceptional distribution of alleles MTHFR or ICAM-1 variants among the patients with familial CAD.

This observational study of 15 patients with familial dissections suggested that familial CAD occurs early in life and is associated with an increased risk for either recurrent CAD or multivessel CAD. Familial CAD seems to strike preferentially the same arteries (either the internal carotid or the vertebral arteries) within a family. Microscopic analyses of skin biopsies in familial CAD patients were mostly unrevealing. In 1 of the families, a vascular EDS was diagnosed in both affected subjects after molecular genetic analysis.

Acknowledgments
We are indebted to Professor W. Hacke for his continuous support and to Inge Werner for excellent technical support. We also thank anonymous reviewers for the valuable suggestion to screen for the COL5A1 D192N variant in a larger series of healthy control subjects and for the hint to read the early article by Petit et al (ref. 24).

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Disclosures
None.

References


