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# Pathogenesis of cervical artery dissections

## Association with connective tissue abnormalities

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**Article abstract—***Background:* The etiology of spontaneous cervical artery dissection (CAD) is largely unknown. An underlying connective tissue disorder has often been postulated. *Objective:* To further assess the association of CAD with ultrastructural abnormalities of the dermal connective tissue. *Methods:* In a multicenter study, skin biopsies of 65 patients with proven nontraumatic CAD and 10 control subjects were evaluated. The ultrastructural morphology of the dermal connective tissue components was assessed by transmission electron microscopy. *Results:* Only three patients (5%) had clinical manifestations of skin, joint, or skeletal abnormalities. Ultrastructural aberrations were seen in 36 of 65 patients (55%), consisting of the regular occurrence of composite fibrils within collagen bundles that in some cases resembled the aberrations found in Ehlers–Danlos syndrome type II or III and elastic fiber abnormalities with minicalcifications and fragmentation. A grading scale according to the severity of the findings is introduced. Intraindividual variability over time was excluded by a second biopsy of the skin in eight patients with pronounced aberrations. Recurrent CAD correlated with connective tissue aberrations. In addition, similar connective tissue abnormalities were detected in four first-degree relatives with familial CAD. *Conclusion:* CAD is associated with ultrastructural connective tissue abnormalities, mostly without other clinical manifestations of a connective tissue disease. A structural defect in the extracellular matrix of the arterial wall leading to a genetic predisposition is suggested. The dermal connective tissue abnormalities detected can serve as a phenotypic marker for further genetic studies in patients with CAD and large families to possibly identify the underlying basic molecular defect(s).

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Dissection of carotid and vertebral arteries is being diagnosed more frequently since Doppler sonography has become widely available as a sensitive tool for primary vascular assessment and mural hematoma of the vessel can be identified using MRI.[1-3] Among young and middle-aged patients, cervicocerebral artery dissection (CAD) is now recognized as an important cause of stroke.[1,2,4,5] Dissection often occurs in otherwise healthy individuals without known risk factors for stroke and frequently develops spontaneously without relevant trauma.[1] Occasionally, mild mechanical stress such as a sudden head movement, infection, coughing, or sport activities before dissection has been reported.[1,6-9]

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Although the clinical and diagnostic criteria for CAD are well established, the pathogenesis is largely unknown.[1,10] An underlying arteriopathy leading to a so-called "weakness of the vessel wall" and predisposing to dissection has often been postulated in spontaneous CAD, particularly because frequently more than one cervicocerebral vessel is involved and patients are often of a younger age.[1,10-12] The high prevalence of spontaneous CAD in patients with fibromuscular dysplasia (FMD) and hereditary connective tissue disorders such as the Ehlers–Danlos syndrome (EDS) also suggests the involvement of vessel wall abnormalities.[1,13-16] As endarterectomy is usually not used to treat CAD and postmortem examinations are rare, histomorphologic examination of the dissected vessel wall is seldom performed.

Recently, in a first small series, connective tissue aberrations were shown to correlate with acute CAD in otherwise healthy individuals.[8] In the current multicenter study, we further analyzed the association of CAD with ultrastructural abnormalities of the dermal connective tissue in another larger series of patients from different centers. Follow-up biopsies in some patients addressed the question of intraindividual variability of the findings over time. We hypothesized that a major part of CAD cases represent a manifestation of a genetic predisposition with a vascular phenotype. Ultrastructural dermal connective tissue abnormalities could be a further phenotypic marker in those patients and be used for familial genetic studies in the future.

**Methods.** *Patients.* Between June 1997 and June 2000, we studied a new series of 65 patients with spontaneous CAD (37 men and 28 women; mean age 41 years, range 21 to 63 years). Patients were recruited at the following hospitals: Department of Neurology of the University of Heidelberg (n = 37), Department of Neurology, City Hospital of Minden (n = 13), Department of Vascular Surgery of the University of Düsseldorf (n = 6), and the Departments of Neurology of the Universities of Lübeck (n = 2), Münster (n = 1), Gießen (n = 1), Kiel (n = 1), Hamburg (n = 1), Perugia (Italy) (n = 2), and Boston (New England Medical Center, MA) (n = 1). Whereas in an earlier pilot series (n = 25), we prospectively included only acute CAD,[8] here we also included patients with a history of CAD. All patients had symptomatic CAD with sudden onset of neck pain, Horner syndrome, cranial nerve deficits, or brain ischemia. Primary vascular screening of clinically suspected CAD was performed by Doppler sonography study (continuous wave, 4-MHz probe) so as to identify the typical high-resistance pattern with "to-and-fro" flow or absence of flow.[2] Diagnosis of CAD was confirmed in all patients by MRI of the neck (mural hematoma), spiral CT angiography (mural hematoma, string sign, or tapered extracranial occlusion), or digital subtraction angiography (string sign, long-segment pseudo-occlusion, or tapered extracranial occlusion). All patients were carefully examined for signs of a known hereditary connective tissue disorder such as a marfanoid habitus, hyperextensible joints, skin abnormalities, unusual scars, or abnormalities on funduscopy. Skin biopsies were obtained in all patients. In eight patients of the pilot series[8] with pronounced ultrastructural abnormalities, a second skin biopsy was performed on the other elbow, 9 to 22 months (mean 13 months) later for follow-up of the findings. The performance of skin biopsies was approved by the local ethical committee (University of Heidelberg) and required informed consent from each patient.

As a control group, 10 consecutive patients younger than 50 years (six men and four women; mean age 41 years, range 29 to 48 years) with acute cerebral ischemia of other etiologies (mostly cardioembolic) were studied, whose findings from the Doppler studies of the cervical arteries were normal and who showed no signs of a connective tissue disorder. The examiners of the skin biopsies were blinded to the control status of these patients.

Biopsies were obtained by open, deep knife biopsy from the outer aspect of the upper arm close to the elbow according to a standardized protocol used for diagnostic transmission electron microscopy of connective tissue disorders.[17] The specimens were divided and processed for histopathologic study and electron microscopic investigations. For light microscopy, specimens were fixed in formaldehyde, embedded in paraffin, and stained with hematoxylin and eosin and van Gieson elastica stain. All skin layers were examined to detect histomorphologic abnormalities in the connective tissue and the small vessels, such as hyperplasia, fibrosis, microaneurysms, elastic fiber disruption (lamina elastica), interstitial edema, lipid or fibrin deposition, and cellular infiltration. Specimens for transmission electron microscopy were prepared as described in detail elsewhere.[17,18] They were fixed in glutaraldehyde and $OsO_4$, embedded in glycidether 100, and cut into semithin and ultrathin sections. Semithin sections were stained with methylene blue. Ultrathin sections were contrasted by uranyl acetate and lead citrate and examined with an electron microscope (Philips EM 400, Best, the Netherlands). The ultrastructural aspect of connective tissue components, namely, collagen fibrils and elastic fibers, was investigated in all regions of the skin of each patient. The findings were compared with those of the control subjects and a large number of other control samples available from the skin biopsy bank of the Institute for Ultrastructure Research of the Skin (more than 4,000 patients included so far). For morphologic differences, we focused on the reticular dermal region, where connective tissue structures are usually very regular and not affected by environmental factors such as, for example, actinic elastosis.

The severity of the connective tissue aberrations was semiquantitatively classified as follows: regular irregularities of the contours and calibers of the collagen fibrils with only few single composite collagen fibrils within collagen bundles were defined as mild aberrations, more frequent irregularities of the contours and calibers of the collagen fibrils with more frequent but still single composite collagen fibrils were considered as pronounced aberrations, and anomalies with frequent composite flower-like fibrils in many collagen bundles and a degree of severity of collagen fiber aberrations comparable with EDS III as severe collagen aberrations.

Elastic fiber abnormalities were defined as mild by regular but mild irregularities of the contours of the elastic fibers and only few electron-dense inclusions (microcalcifi-

***Table*** *Distribution of CAD and percentage of connective tissue abnormalities*

| Type of dissection | Total no. of patients | No. of patients with connective tissue abnormalities (%) |
|---|---|---|
| Single-vessel CAD | 36 | 19 (53) |
| ICA | 29 | 15 (52) |
| VA | 7 | 4 (57) |
| Multiple-vessel CAD | 22 | 10 (45) |
| Including other vessels* | 3 | 3 (100) |
| Recurrent dissection | 7 | 7 (100)‡ |
| Familial dissection† | 6 | 4 (67) |
| All CAD | 65 | 36 (55)§ |

* Aortic (2) and renal artery (1) dissection.
† Numbers included in other subgroups.
‡ $p = 0.01$ compared with other CAD patients.
§ $p = 0.001$ compared with controls.

CAD = cervicocerebral artery dissection; ICA = internal carotid artery; VA = vertebral artery.

cations). Pronounced aberrations requested frequent irregularities of the contours of the elastic fibers, reaching in some fibers a fragmented "moth-eaten" aspect and several electron-dense inclusions (microcalcifications). Frequent fragmented elastic fibers reaching a degree of severity with a "moth-eaten" porous aspect resembling the findings of the marfanoid hypermobility syndrome in combination with frequent electron-dense inclusions (microcalcifications) were defined as severe elastic fiber aberrations.

Others, including borderline findings not definitely meeting these criteria, were considered as "normal." Two skin specimens could not be evaluated owing to insufficient conservation during shipping. Significance of the findings was calculated by $\chi^2$ or Fisher's exact test.

**Results.** Single-vessel CAD occurred in a total of 36 patients (55%), with internal carotid artery dissection in 29 and vertebral artery dissection in seven patients (table). Multivessel CAD was present in 22 patients (34%). Another seven patients (11%) had had recurrent CAD. In six patients (9%), CAD was familial, with one other first-degree relative with CAD also included in the study. Other vascular diseases were found in six patients (9%): intracranial aneurysms in two, aortic artery dissections in three, and renal artery dissection in one patient. Angiographic signs of FMD were seen in four patients (6%). In all, 52 patients (80%) showed symptoms of cerebral ischemia (infarcts 39, TIA 13), and the remaining patients had isolated cranial nerve palsies or neck pain.

Only three patients (5%) presented with signs of a connective tissue disorder with hyperextensible skin and joints in one, a marfanoid appearance in another, and hypertrophic pseudo-molluscoid scars at the elbows in a third patient. Another patient had a history of four spontaneous abortions. None of the other patients had skin, joint, or skeletal abnormalities characteristic of a known hereditary connective tissue disease. No patient reported any severe trauma recently (e.g., car accident or local injury) or

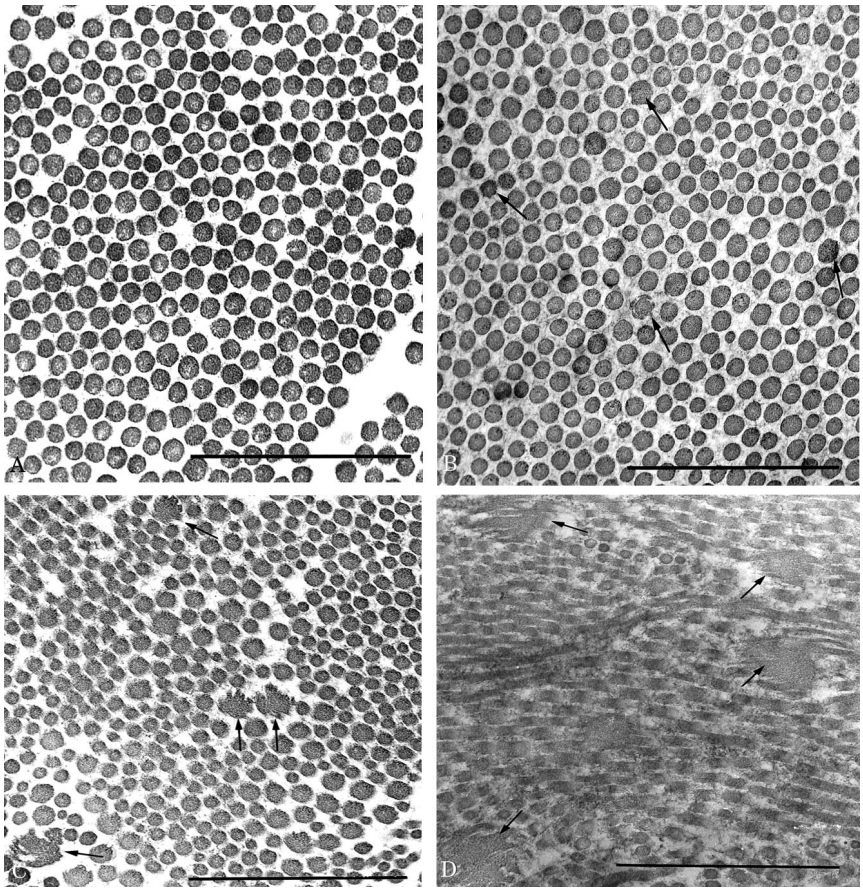


*Figure 1. (A) Normal collagen bundles within reticular dermis consist of large, densely packed collagen fibrils with uniform cross-sections. Control. Electron microscopy (EM); original magnification ×43,000. Bar = 1 μm. (B) Mild aberrations ("+" according to the table) consisting of collagen bundles within the reticular dermis containing single or several fibrils with irregular contours and composite (flower-like) fibrils (arrow). The calibers of the aberrant fibrils vary. EM; original magnification ×43,000. (C) Severe aberrations, including numerous composite fibrils (arrows) within the middermal collagen bundles and enlarged diameters of the composite fibrils. EM; original magnification ×43,000. Bar = 1 μm. (D) Collagen aberrations in a patient with spontaneous arterial dissection and minor stigmata of Ehlers–Danlos syndrome type II (small pseudo-molluscoid scars at elbows). Collagen bundles within the reticular dermis contain frequent composite (flower-like) fibrils (arrows). EM; original magnification ×31,000. Bar = 1 μm.*

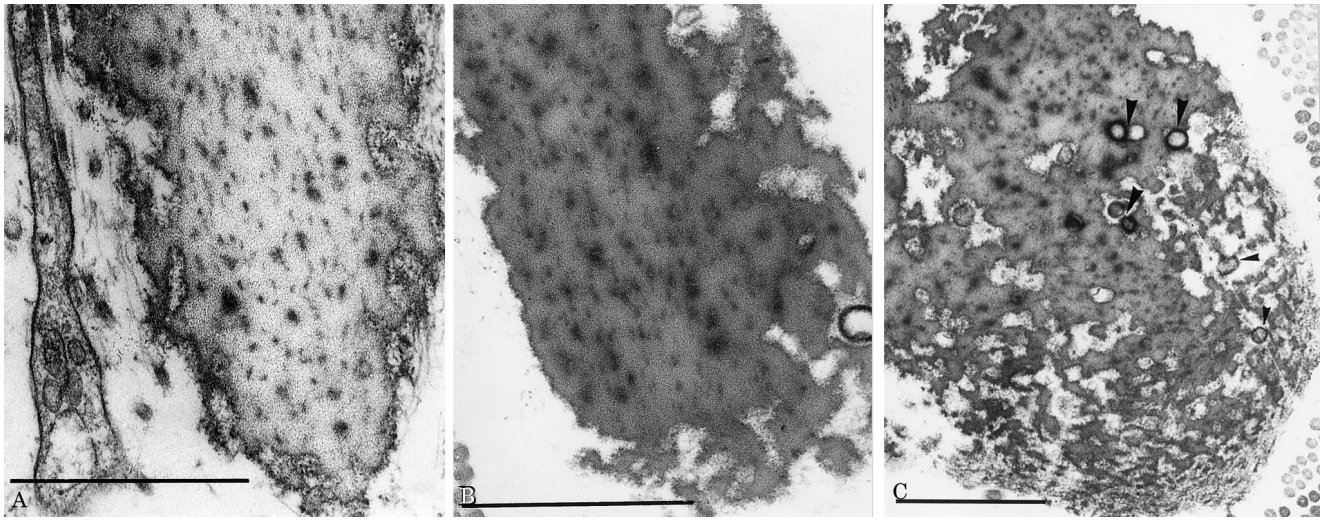


*Figure 2. (A) Normal mature elastic fiber in the reticular dermis presents with an amorphous elastin core surrounded by elastotubules. Control. Electron microscopy (EM); original magnification ×26,000. Bar = 1 μm. (B) Regular but mild irregularities of the contours of the elastic fibers; focal electron-dense deposit (arrowhead). EM; original magnification ×20,000. Bar = 1 μm. (C) Focal electron-dense deposits representing minicalcifications (arrows) and pronounced fragmentation with a moth-eaten, porous aspect. EM; original magnification ×9,000. Bar = 1 μm.*

had received local radiation therapy. Ten patients (15%), however, remembered some kind of recent sudden head movement before the event.

According to light microscopy findings, the overall architecture of the dermal connective tissue and microvasculature was normal in all but two patients with familial CAD in whom the dermis was very thin. All dermal arterioles and fibroblasts we examined on electron microscopy had no substantial changes. Ultrastructural aberrations of the connective tissue, however, were identified by electron microscopy in 36 of 65 patients (55%) with CAD. Because such abnormalities were not present in any patient with other etiologies of acute cerebral ischemia (figures 1A and 2A), we consider the association between CAD and the ultrastructural aberrations to be significant. A total of 20 patients had aberrations of both collagen fibrils and elastic fibers. In 10 patients isolated collagen changes and in six patients isolated elastic fiber abnormalities were seen. The collagen aberrations were pronounced (n = 9) or severe (n = 6) in 15 patients and mild in 15. Mild aberrations consisted of the regular occurrence of collagen bundles containing single composite fibrils with a variable diameter and a flower-like cross-section (see figure 1B). Marked aberrations included numerous composite fibrils within middermal collagen bundles and enlarged diameters of composite fibrils (see figure 1C). In four of these patients, the abnormalities were so severe that they resembled those found in patients with EDS type III or even II. In one of the patients with severe ultrastructural aberrations characteristic of the mild classic type of EDS (formerly EDS type II) (see figure 1D), small pseudo-molluscoid scars at the elbows were the only signs of a connective tissue disease detected only by a second dermal inspection after the electron microscopy findings. Two other patients had a very thin dermis on biopsy with frequent small-diameter collagen fibrils in the ultrastructure, as often seen in EDS type IV patients.

Elastic fiber abnormalities (see figure 2, B and C) were present in 26 patients. In 14 patients these aberrations were classified as mild and in 7 as pronounced; a severe fragmentation similar to the moth-eaten, porous aspect in the marfanoid hypermobility syndrome was found in five patients (see figure 2C).

All eight patients in whom a second biopsy was performed showed identical ultrastructural findings and were ranked at the same level of pathomorphologic severity in both analyses. In the six cases with familial CAD, similar findings were seen: four had mild aberrations (EDS IV-like in two), and two did not have any distinct pathologic findings. Connective tissue abnormalities were not found more frequently in patients with multivessel CAD (see the table). Connective tissue aberrations were more frequently found in male patients with CAD than in female patients and were associated with recurrent CAD ($p < 0.05$) but not with age and vascular risk factors.

**Discussion.** *Dissections and connective tissue abnormalities.* The pathogenesis of spontaneous CAD, an important cause of stroke in younger patients, is still unclear.[1,12] The hypothesis of an underlying connective tissue disorder leading to a structural instability of the arterial wall is supported by the high incidence of dissections and cerebral aneurysms in patients with known heritable connective tissue disorders such as EDS type IV.[16,19] As a definite molecular genetic diagnosis has not been established for many types of connective tissue disorders, skin biopsy for ultrastructural analysis serves as a routine diagnostic tool in patients at risk, for example, cutis laxa, pseudo-xanthoma elasticum (PXE), and EDS.[17,20,21]

The findings of patients with a history of CAD presented here confirm a strong association of spontaneous CAD and connective tissue abnormalities in an extended group of patients from different regions. Some 36 of 65 more patients (55%) showed ultrastructural aberrations of collagen fibrils and elastic

fibers within the reticular dermis. Moreover, follow-up biopsies taken from a different location did not disclose any intraindividual variability over time. This suggests that the abnormalities in the dermal connective tissue represent a constant finding in these patients. Familial CAD with connective tissue abnormalities showed a similar pathomorphology. Furthermore, in addition to the patients with familial CAD, we recently established identical connective tissue abnormalities in skin biopsies in three of four so far unaffected children of a patient with CAD who showed pronounced ultrastructural aberrations.[22] The obvious inheritance shown supports the hypothesis of a genetic origin of CAD, at least in a subgroup of patients. Our previous results with 17 of 25 (68%) positive for ultrastructural connective tissue abnormalities were recently confirmed by another group for spontaneous CAD, with 12 positive of 22 (54%) patients.[8,23] As reported by these authors, only one of the other 23 patients with traumatic CAD showed these aberrations on electron microscopy,[23] indicating there is likely a different pathogenesis for the two types of CAD abnormalities.

As the mechanical stability and elasticity of the vessel wall are provided by connective tissue elements,[20,24] structural deviations in the main components collagen and elastic fibers may lead to functional impairment, predisposing to dissection of the arterial wall at given points of minor resistance. The regular presence of composite collagen fibrils and fragmented elastic fibers in patients with CAD points to basic defects within the extracellular matrix.[17,20,24,25] Ultrastructural studies in the cervical arteries of patients with CAD could be the next step in correlating the dermal findings with the ultrastructure of the arterial walls. We speculate that patients with CAD with negative skin biopsies possibly show connective tissue abnormalities in arterial specimens. A combination of an underlying arteriopathy as genetic predisposition and temporarily active factors may be necessary for CAD to occur at a certain point in time. The significant association of CAD and recurrence rate with connective tissue abnormalities could offer some information on patients or relatives at risk. This and the question of a correlation to the severity of the connective tissue abnormalities are addressed in a long-term follow-up study.

*Ultrastructural dermal pathomorphology.* The ultrastructural dermal findings, however, are not specific for any distinct connective tissue disorder or genetic defect.[17,20,24,25] Further quantitative morphometric analysis of these findings might be helpful but, to our knowledge, is not used for diagnostic electron microscopy in connective tissue disorders at present. Qualitatively similar deviations may be seen in patients with various heritable connective tissue diseases (i.e., composite collagen fibrils in patients with EDS, osteogenesis imperfecta type I, and PXE[17,18,20,21,24-26]) and are also found in some acquired conditions but in restricted areas only (e.g., lymphedema and rheumatoid arthritis).[27,28] Fragmented elastic fibers with focal osmiophilic deposits are found in patients with PXE, Marfan syndrome, and marfanoid hypermobility syndrome as well as in patients with severe EDS types I and II.[17,18,20,24-26] It is the specific pattern of structural deviations that characterizes a connective tissue disorder.[17,18,20,25,26] The overall pattern and combination of the ultrastructural aberrations of collagen and elastic material combined with the clinical phenotype of our patients are unique and not yet known for a defined heritable connective tissue disorder. In some of our patients, the pronounced ultrastructural aberrations with frequent composite collagen fibrils resembled those found in EDS type III or even II; however, differing clinical features with vascular manifestation and lack of phenotypic signs of these types of EDS point to a distinct connective tissue disorder of a yet unknown nature. Whether this is a new disease entity or just a minor variant of a known connective disorder is speculative. Strikingly, similar morphologic changes were detected recently in healthy heterozygote carriers in families with recessive PXE.[21]

*Phenotypic expression.* Similar to a vascular phenotype of PXE,[29] the clinical manifestation of a connective tissue disorder might be limited to the vascular system as in our group of patients showing no external signs of a known connective tissue disease.[16,19,30] Also, very subtle signs might be recognized only by experienced examiners. Indeed, one of our patients with CAD who showed pronounced ultrastructural collagen fibril aberrations in the skin biopsy corresponding to EDS type II had only mild cardinal symptoms of EDS type II, with small hypertrophic pseudo-molluscoid scars at the elbows. No other patient except one with a marfanoid appearance and another with signs characteristic for EDS showed external stigmata for a known connective tissue disorder. In a prospective series, in three of 15 patients (20%) with CAD, phenotypic signs for a heritable connective tissue disease were found, such as a marfanoid appearance, hyperextensible skin with abnormal scars, joint hypermobility, or femoral hernia.[31] Further classification of these still unnamed disorders by biochemical studies for collagen I and III and fibrillin-1 abnormalities, however, was unsuccessful. In another patient with CAD and no phenotypic signs other than slightly bluish sclerae, a mutation characteristic of osteogenesis imperfecta type I was identified.[32] Unfortunately, electron microscopy was not performed in either study.

*Molecular genetics of connective tissue disorders and CAD.* The mechanisms and molecular genetics underlying composite collagen fibril formation are still under investigation.[33,34] A variety of candidate genes encoding fibrillar and interstitial collagens and enzymes involved in normal collagen fibril organization are known to bear mutations in some heritable connective tissue disorders.[33-43] Mutations in genes coding collagen V (*COL5A1* and *COL5A2*) known to modulate collagen fibril diameter could be identified in approximately 20% of patients with

classic EDS and ultrastructural dermal connective tissue abnormalities resembling qualitatively those found in some of our patients.[42,43] Screening for *COL5A1* mutations in our patients, however, was negative.[44] Several patients carry missense mutations in *COL5A2*, but as these mutations were also detected in healthy control persons, their role in the development of CAD is uncertain. Corresponding to the negative results in patients with PXE, we also excluded a mutation in the *elastin* gene, which could have been responsible for the elastic fiber abnormalities.[45] Interestingly, the ultrastructural abnormalities did not resemble the typical morphologic features of EDS type IV,[46] the "vascular" EDS, except in two patients. This is consistent with the negative results concerning biochemistry and molecular genetics of collagen III.[8,47,48] The combined results of these and other studies make it unlikely that mutations affecting collagen III are a major cause of CAD.[49]

The significance of the high proportion of the connective tissue abnormalities in male patients and also the higher prevalence of male patients in ours and some other series of patients with CAD is not clear.[50,51] An X-chromosomal linked mutation might be involved in the pathogenesis of CAD. No mutation responsible for the majority of patients with CAD has been identified yet in mutation screening, however. The recently detected molecular defect in patients with PXE demonstrates the possible involvement of unexpected candidate genes.[52] Pedigree studies and linkage analyses in patients with CAD and ultrastructural connective tissue aberrations who have large families represent a possibly more successful basis of mutation search.

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