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Small cerebral vessel disease in familial amyloid and non-amyloid angiopathies: FAD-PS-1 (P117L) mutation and CADASIL. Immunohistochemical and ultrastructural studies

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Abstract

Three patients (of two unrelated Polish families) with early-adult onset dementia were subjects of the study. Two cases, previously diagnosed as familial Alzheimer's disease (FAD) with cerebral amyloid angiopathy (CAA), were confirmed by genetic and neuropathological studies, and one case of CADASIL was ultrastructurally confirmed by the presence of vascular granular osmiophilic material. Now the brain autopsy material has been reinvestigated using immunohistochemical (IHC) markers for vascular smooth muscle cells, paying special attention to collagen markers for extracellular matrix components and ultrastructural microvascular changes. In both diseases, IHC examination showed a reduction or loss of expression of smooth muscle actin (SMA) in tunica media of the cerebral arterioles. Fibrous thickening of the wall of the small meningeal arteries, intracerebral arterioles and numerous capillaries, with amyloid or granular deposits, drew our attention. In these vessels, marked expression of fibrillar collagen type III as well as strong immunoreactivity of the basement membrane (BM) component collagen type IV were found. The most damage was observed in the FAD/CAA double-barrel vessel wall and in some CADASIL arterioles changed by fibrinoid necrosis. The fibrous changes of the small vessels were more distinct in CADASIL than in FAD/CAA. In FAD, electron microscopic examination revealed both amyloid and collagen fibres within the thickened BM of capillaries and the small arterioles. Clusters of collagen fibres between lamellae of BM, frequently in a pericyte position, were observed, and some were seen in the degenerated pericytes as well. Typical changes of the pericytes were accumulation of lipofuscin-like material and their degeneration. The mitochondria of the pericytes and of the endothelium were rare and swollen, with damaged and reduced cristae. The VSMCs of the arteriolar walls exhibited degenerative changes with atrophy of the cellular organelles. The fibrous, collagen-rich CADASIL small cerebral vessels, despite the weakness of the vessel wall due to reduction of VSMCs, appeared to be stronger than in FAD/CAA. These findings may suggest an accelerated process of transformation of the small cerebral vessels in which early onset of VSMCs loss is a predominant feature of the vascular changes in both presented diseases.

Key words: *hereditary (familial) cerebral amyloid angiopathy, CADASIL, collagen, extracellular matrix, angiopathies, immunohistochemistry, ultrastructure*

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Introduction

The "muscularis" tunica media characterised for cerebral and leptomeningeal arteries and arterioles contains numerous smooth muscle cells in which elastic fibres predominate. This vessel wall layer is mainly involved in several sporadic and inherited vascular diseases contributing to various dementia syndromes.

A progressive loss of vascular smooth muscle cells (VSMCs) replaced with vascular amyloid or non-amyloid protein deposits and fibrosis in the tunica media is characteristic of two different hereditary forms of angiopathy: cerebral amyloid associated with rare familial form of AD (FAD/CAA), and systemic non-amyloid, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Recently, attention has turned to progressive changes, and also other elements of the vessel wall [24,29,43]. VSMCs are surrounded by extracellular matrix (ECM) which appears to increase to replace the VSMCs, progressive with aging and disease-related vessel wall destruction [3].

Although both presented small cerebral vessels diseases are heterogeneous in aetiology, their clinical manifestations are similar to recurrent, mainly ischaemic or haemorrhagic cerebral episodes leading to early-adult onset dementia and death [32,45].

Cerebral amyloid angiopathy (CAA) presented in our two FAD patients were caused by presenilin-1 gene mutation (P117L) found on chromosome 14, reported previously in the first Polish FAD family [41]. At present, there are known three of various pathogenic mutations mainly responsible for early onset familial AD usually with co-occurrence of CAA: of the amyloid precursor gene (APP) on chromosome 21, the PS-1 gene on chromosome 14, and the PS-2 gene on chromosome 1 [12,25]. Presenilin is a unique multifunctional protein, including a role as an intramembrane-cleaving protease that may facilitate membrane protein turnover [36]. In the central nervous system, PS-1 protein is principally located in neurones, mainly in the membrane of the endoplasmic reticulum, (ER) [31] and is involved in βAPP processing and Aβ generation [10]. Presenilins are a gene family of the key mediators of Notch signalling, processing both Notch and APP [36]. Mutation in the PS-1 gene especially facilitates amyloid angiopathy, early-adult onset disease and vessel destruction [36,37], then familial CAAs are generally more severe than sporadic forms [31,35,45].

CADASIL is a hereditary small vessel angiopathy characterised similarly to FAD/CAA by recurrent subcortical ischaemic strokes leading to vascular dementia and death [17,33]. The underlying lesion is a systemic angiopathy characterised by prominent alterations of VSMCs. In our CADASIL patient from the first Polish CADASIL family ultrastructural diagnosis was reported previously and GOM deposits were found also in capillary walls [32,34]. The responsible mutations in CADASIL involve the *Notch3* gene on chromosome 19q12 [14,15,16,34] which encodes a transmembrane receptor, protein Notch3. This protein is a member of the large Notch family of transmembrane receptors involved in signalling that control cell fate decisions during embryonic and postnatal development including neurogenesis, survival as well as apoptosis of cells. Notch signalling cascades are also linked to organ-specific angiogenesis and vascular remodelling [5].

The gold standard to confirm CADASIL diagnosis is to identify a mutation of the *Notch3* gene or alternatively, detection of an electron dense granular osmophilic material (GOM) deposit (diagnostic marker) around VSMCs in electron microscopic examination of a skin/muscle biopsy or in autopsy brain study [3,24,32,33]. Recently, an immunoelectron microscopic study demonstrated that the major component of GOM deposits on the outer surface of a smooth muscle cells comprises the Notch3 ectodomain peptide (gene product) [13,15].

Although different in their genetic aetiology, morphological criteria in both diseases comprise several similar changes leading to impairment of the integrity of small vessel walls. The integrity of the cerebral vasculature is crucial to the maintenance of brain perfusion needs for normal cerebral functions [20]. Aging and several neurodegenerative (Alzheimer's disease, AD; Parkinson disease, PD) and cerebrovascular (hypertension, CADASIL) disorders impair integrity of cerebral vessels [7]. It is now widely accepted that progressive damage of the vascular integrity, especially of the small cerebral vessels including arterioles responsible for autoregulation and capillaries creating the structural base of the blood-brain barrier (BBB), may significantly contribute to impairment of cognitive functions. The aim of this report was a comparative study of cerebral small vessel wall changes in two genetically determined angiopathies involving mainly the tunica media of small vessels in which pathologic protein deposits were assembled.

Morphology of small cerebral vessels was compared, using IHC and ME, in the material from two FAD/CAA sisters with presenilin (PS-1) mutation, one CADASIL patient, and one control brain derived from a non-hypertensive age-matched patient. The early-adult onset of dementia and death (third-fourth/fifth decade) could suggest the slight contribution of age-related changes.

The brain autopsy material was reinvestigated using IHC markers for smooth muscles and extracellular matrix elements, the two types of collagen: fibrillar-type III and BMs component-type IV.

The results of the study show that the fibrous transformation of the arterial wall occurs with the increasing degeneration of the VSMCs and pericytes, which are their functional equivalent in capillaries. Clusters of collagen fibres and changes in BM of the small vessels in both genetically determined early-onset diseases may suggest an accelerated process of transformation of the small vessels similar to age-related changes.

Material and Methods

The study was performed on the autopsy material derived from 4 persons, 36-46 years old, including the brains of two patients suffering from FAD with PS-1 mutation (P117L), one CADASIL patient and one non-demented age-matched control patient without hypertension in which no vascular pathological changes were found apart from a small aneurysm of the cerebral artery.

Light microscopic examination was carried out on the brain autopsy material fixed in 4% formaldehyde and embedded in paraffin. Samples were collected from both hemispheres of the cerebrum and cerebellum. Morphology of the small vessels (arterioles and capillaries) and of brain tissue was assayed with Congo red, PAS, van Gieson, Klüver-Barrera and IHC reactions, including those doubled reaction with antibodies: Aβ, smooth muscle actin (SMA), collagen types III and IV and GFAP.

Brain samples from FAD patients were taken 3-4 hours after death. After fixation in 2.5% glutaraldehyde and postfixation in 2% OsO_4, they were routinely processed for electron microscopic examination. In the CADASIL patient, samples were taken from the formalin-fixed brain, postfixed in 2.5% glutaraldehyde and in 2% OsO_4 and routinely processed to Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a TURBO DPS 109 electron microscope.

Results

In both analysed diseases, morphological examination revealed brain atrophy and diffuse myelin loss of the hemispheres predominating in the CADASIL patient similarly to changes of *etat crible* and lacunar infarcts in the deep white matter and basal ganglia, respectively (Fig. 1A, C). In both FAD sisters, severe amyloid angiopathy (CAA) of leptomeningeal and intracerebral small vessels was widespread, particularly in the cerebellar samples (Fig. 1B). CADASIL non-amyloid angiopathy mainly involved arterioles penetrating the white matter; however, characteristic granular deposits in some leptomeningeal vessels were also found (Fig. 1D).

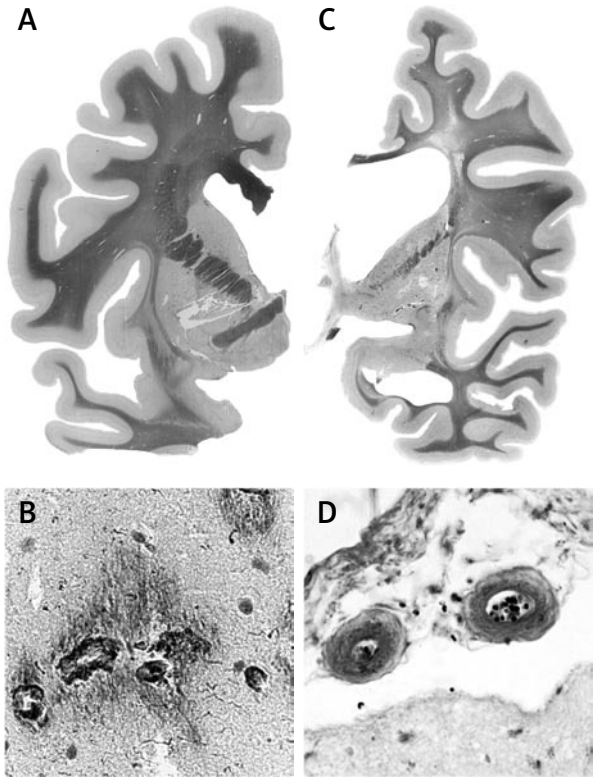

Fig. 1. (A-B) FAD/CAA; (C-D) CADASIL. (A, C) Cross section through the cerebral samples. Mild (A) and advanced (C) stage of the brain atrophy and lacunar infarcts. Klüver-Barrera. Magn. Glass. (B) β-amyloid diffuse deposits in the vicinity of the capillaries. Aβ × 40. (D) Granular deposits in tunica media of the small leptomeningeal arteries. PAS × 40

Arteries and arterioles of the control case characterised negative Congo red and Aβ reactions. Van Gieson and PAS staining were positive in adventitia fibres only, comparable to the initial stage of fibrous changes in FAD/CAA (Fig. 2E). Strong SMA expression was observed in the tunica media through all VSMCs layers. Antibodies to collagen III revealed a positive reaction in the arteriolar adventitia, whereas collagen IV was immunoreactive in the inner media of arterioles and in the walls of the capillaries (Fig. 2B-D).

FAD/CAA

In the brains of both FAD/CAA sisters, numerous β-amyloid-laden leptomeningeal and cortical small arteries, arterioles and capillaries, particularly in the cerebellar samples, were found. There were seen circumferential amyloid deposits in the media of some vessels, between endothelial and pericyte layers at the expected thickened BM position (Fig. 2A), and around Aβ-positive and fibrous vessels (Fig. 2I).

The thickened walls of the CAA arteries, arterioles and capillaries revealed positive van Gieson red staining; however, in the slight fibrous arterioles, positive staining was seen only in adventitia, comparable to the control case (Fig. 2E).

Smooth muscular actin (SMA)

There was variable immunoreactivity to actin, from strong in the control and initial stage CAA (Fig. 2B) to a weak or negative reaction due to the loss of VSMCs in the numerous affected arterioles (Fig 2B, F). The "vessel-within vessel" configuration of the small arteries and arterioles was often observed. Some of these double-barrel vessels revealed SMA positive subintimal media and SMA negative/Congo positive circumferential media and adventitia (Fig. 2J).

Collagen type III and IV

Strong collagen III immunoreactivity of the leptomeningeal and intracerebral arteriolar adventitia around the collagen III negative/Congo positive tunica media was usually observed; however, in some vessels collagen III immunoreaction throughout the arteriolar wall was seen (Fig. 2C, G, K). Immunoreactivity to collagen type IV in the inner media region, at the expected position of the BM, was more often observed than the reaction throughout the arteriolar wall (Fig. 2D, H, L). Strong collagens III and IV immunostaining of the numerous fibrous and thickened capillaries was associated with fibrous arteriolar changes, while the immunoreactivity of the other capillaries to collagen types III and IV was comparable to the control (Fig. 2H, K).

CADASIL

In the CADASIL patient amyloid angiopathy was not seen. The thickened walls of the numerous small arterioles in the cerebral white matter with the myelin loss contained PAS-positive granular deposits, particularly in the inner region of the media. Reactive GFAP (+) astrocytes and fibrous gliosis around the PAS positive arterioles in the white matter were observed (Fig. 3A, E).

Smooth muscular actin (SMA)

The thickened and slightly PAS positive tunica media of the arteries and the arterioles showed a reduced number of VSMCs and weak immunostaining to actin only of the inner part of the vessel wall (Fig. 3B).

Collagen type III and IV antibodies

Sometimes positive immunostaining to deposits of collagen III disclosed only the adventitia of the meningeal and of the larger intracerebral arterioles, but usually immunoreaction throughout the thickness of the wall was found (Fig. 3C, G). In some larger arterioles PAS (+) with collagen III positive adventitia, fibrinoid necrosis of the wall was found (Fig. 3F). The immunoreaction to collagen IV was seen in the inner part of the media of the fibrotic arterioles (Fig. 3D). In some terminal small white matter arterioles with stenosis, the strong collagen IV reaction throughout the thickness of the wall or thickened fibres at the expected position of BM and granular deposition were found (Fig. 3H). Thickened and fibrous, van Gieson-positive walls of the capillaries and frequently of the pericytes were also positive to collagens III and IV (Fig. 3C, G).

In FAD, at the ultrastructural level the VSMCs demonstrated different stages of morphological alterations. They revealed varied diameters and varying degrees of cellular structure preservation (Figs. 4-7). The cytoplasm of degenerated cells exhibited only dispersed filaments and reduced number of intracellular organelles including affected mitochondria (Figs. 4-5).

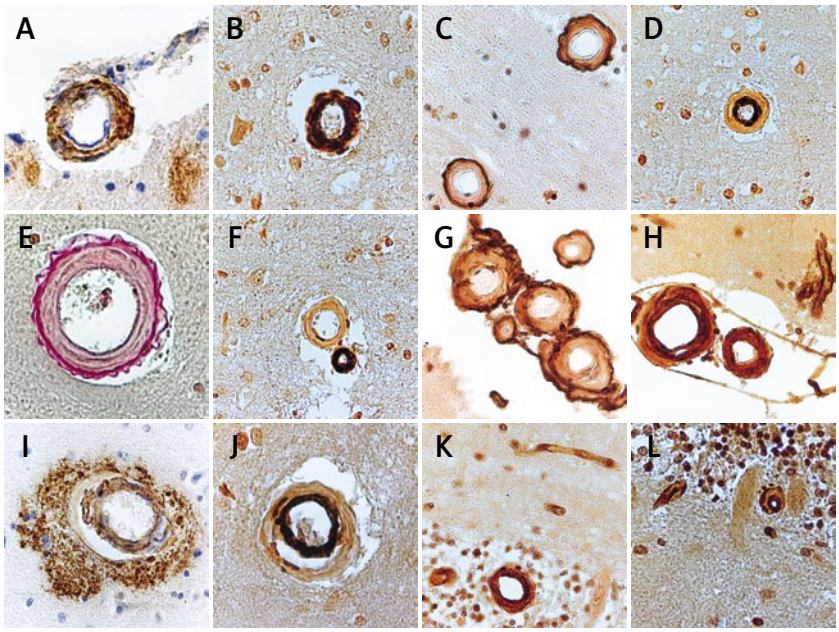

Fig. 2. (A-L) Familial AD with mutation PS-1. (A, E, I) Circumferential Aβ positive deposits in media at expected position of the basement membrane (A) and perivascular diffuse plaques around amyloid-laden arteriolar wall (I). Aβ × 40. Strong-red adventitia around the pale-red media in the initial stage of fibrous transformation vessel wall (E). van Gieson × 40. (B, F, J) Progressive loss of smooth muscle cells from strong SMA expression of preserved VSMCs in the initial CAA stage, comparable to control (B) to loss VSMCs (larger arteriole, F) and double-barrel arteriole with positive SMA only in subintimal muscle cells (J). SMA/Congo red × 40. (C, G, K) Progressive collagen III fibrosis of vessel wall from adventitia, comparable to control (C) to adventitia and outer media, amyloid-Congo positive arterioles (G, K). Collagen III/Congo red × 40. (D, H, L) Positive collagen IV expression in position of basement membrane from inner media, comparable to control (D) to expression of more damage to arterioles (H, L). Collagen IV/Congo red × 40

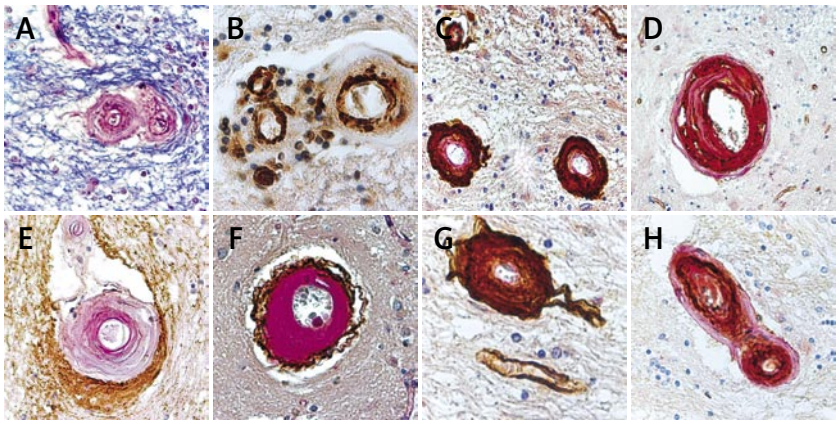

Fig. 3. (A-H) CADASIL. (A, E) Granular PAS-positive deposition inner media in small white-matter arterioles. Oedema and myelin loss (A). Kluver-Barrera/PAS × 40, perivascular fibrous changes of white matter. GFAP/PAS × 40. (B, F) Reduced VSMCs in arterioles of various size. Low SMA expression vessel walls with preservation only of subintimal VSMCs (B). SMA × 40. Collagen III positive adventitia surrounding media with fibrinoid necrosis and complete loss of VSMCs (F). Collagen III/PAS. (C, G) Progressive collagen III fibrosis of vessel wall from adventitia (C) to throughout the arteriole and capillaries walls with collagen III-positive granular deposits (G). Collagen III/PAS. (D, H) Thickened collagen IV positive fibres in arteriolar media in expected position of basement membrane and progressive luminal narrowing from larger arteriole (D) to small arterioles (H). Collagen IV/PAS

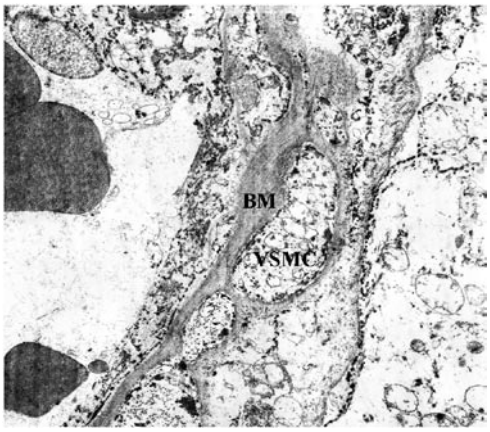

Fig. 4. FAD. Wall of arteriole with locally thickened basement membrane (BM) and degenerated VSMCs. Orig. magn. × 4400

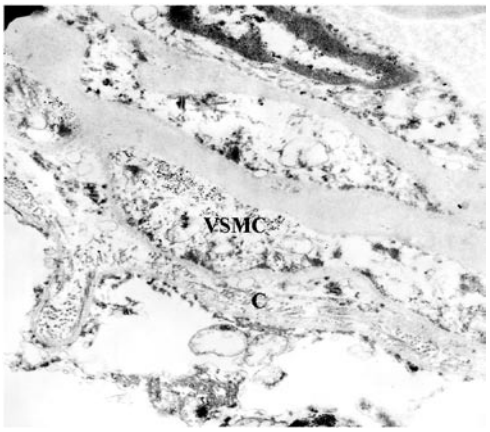

Fig. 5. FAD. Degenerating VSMCs and collagen (C) fibres in wall of arteriole. Orig. magn. × 4400

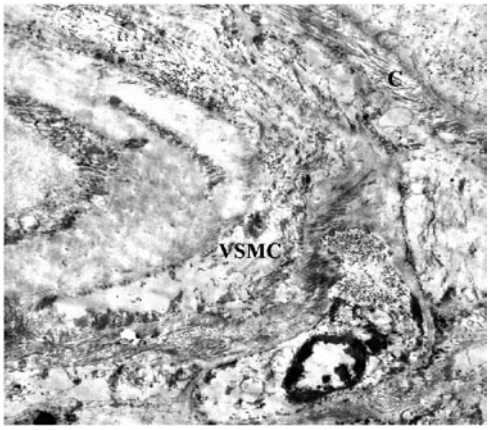

Fig. 6. FAD. Thickened wall of arteriole with numerous collagen fibres (C) and degenerated VSMCs. Orig. magn. × 4400

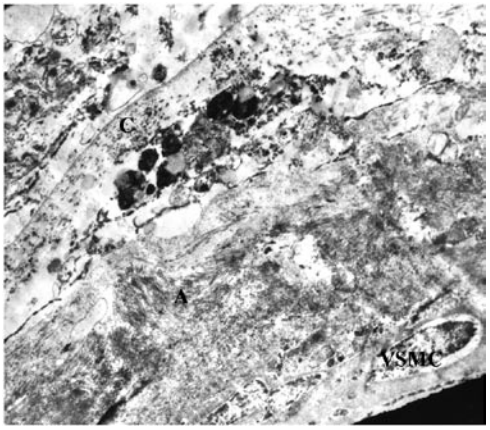

Fig. 7. FAD. Amyloid (A) and collagen (C) fibres and degenerated VSMCs in thickened wall of arteriole. Orig. magn. × 3000

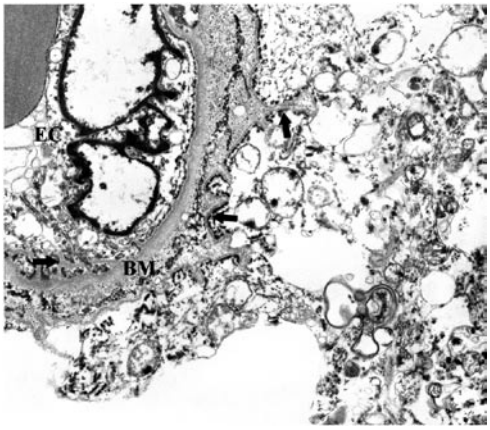

Fig. 8. FAD. Capillary with protrusions (arrows) of basement membrane (BM). Endothelial cell (EC). Orig. magn. × 4400

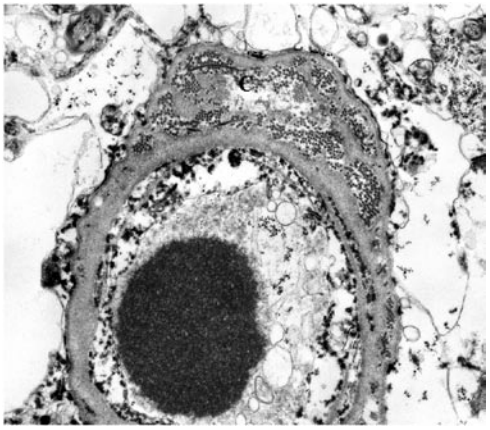

Fig. 9. FAD. Capillary with numerous collagen fibres (C) in a pericyte position. Orig. magn. × 4400

BM around smooth muscle cells and in capillaries was thickened to various degrees especially in the regions of numerous amyloid and/or collagen fibres (Figs. 6-7). Finger-like thickening of BM of different length, penetrating into the vessel lumen and/or surrounding tissue, were frequently observed (Fig. 8). Clusters of collagen fibres, often in a pericyte position or between vascular walls and parenchyma, as well as into BM, were also present (Figs. 9-10). Small and light areas of cytoplasm were seen into BM (Fig. 12). Some capillaries were surrounded by amyloid fibres (Fig. 11); however, another located near the amyloid plaque did not exhibit any amyloid fibres (Fig. 12).

Several pericytes demonstrated degenerative alterations and some of them exhibited almost normal morphology (Fig. 13). The cytoplasm of abnormal pericytes was filled with lipofuscin-like material, electron dense bodies, lipid drops and lysosomes (Figs. 14-16). Some pericytes with swelling cytoplasm and only a few organelles were visible (Fig. 15). These pericytes also exhibited deposits of collagen fibres in cytoplasm (Fig. 17). Endothelial cells showed a small number of mitochondria and changes in their structure. The mitochondria were most frequently swollen with light matrix and damaged and declining cristae (Figs. 8, 14). Endothelial cells with apoptotic morphology were also found (Fig. 18).

On electron microscopic examination CADASIL arterioles with numerous granular osmiophilic material (GOM) deposits of damaged VSMCs of small diameter, separated from each other by a very thick BM, contained numerous collagen fibres (Figs. 19-21). Since ultrastructural examination was carried out on the formalin-stored material, the morphological assay of vessel wall cells, and especially their organelles, is rather difficult. In the cytoplasm of pericytes, electron-dense and lipofuscin-like material was present.

Discussion

In the present study, with use of antibodies to the vascular smooth muscle actin, extracellular matrix components and electron microscopy, we found that in both genetically determined but aetiologically different diseases, affecting also (FAD) or mainly (CADASIL) blood vessels, several similar and distinct morphological vascular changes were observed.

Comparison of the vascular morphological changes in small cerebral vessels revealed that the characteristic "hallmark" disease-related amyloid or granular deposits and a low expression of smooth muscle actin due to the loss of VSMCs were associated with progressive changes, that lead to fibrous transformation and either weakness of the vessel walls or alternatively to marked thickening and stenosis. Additionally, in both diseases, the IHC and EM assays revealed the degeneration of pericytes, and adverse changes of endothelial cells in the arteriolar vessel wall, although of different grade due to the vessels' size and the distinct disease.

Structural remodelling of the cerebral arterioles comprised mainly: the degeneration and the loss of VSMCs, the fibrous thickening of the walls and significant collagen expression in adventitia and often in the arteriolar tunica media.

VSMCs

We revealed in this study that the VSMCs were destroyed in both presented diseases, with amyloid or GOM deposits respectively, and replaced by marked fibrosis of the arterial and arteriolar walls. In both diseases, we noticed low expression of the smooth muscle actin (SMA) due to the loss of VSMCs in numerous arterioles and the small- and medium-sized arteries. The marked loss of the smooth muscle cells and the most severe destruction of the vessel wall were seen in the CAA/FAD arterioles with amyloid deposits, reduced SMA reactivity, and the "double-barrel" appearance.

Ultrastructurally, VSMCs showed various degree of destruction, including degeneration of the cellular structures. The presence of amyloid fibres in the BM was linked to diminished size of the smooth muscle cells. VSMCs atrophy resulting from the accumulation of amyloid fibres in the vessel wall is regarded as a typical feature of CAA [2].

We found comparable, severe damage of CADASIL arteriole walls mainly in some larger arterioles devoid smooth muscular cells and damaged by fibrinoid necrosis. Our IHC and EM findings supported that in both vessel diseases CAA/FAD and CADASIL, VSMCs are a primary target of the pathogenic gene mutation [3,16,27]. The results of the recent EM analysis of the vessels in a CADASIL-animal model suggest that the cascade of changes leading to VSMCs degeneration may be initiated by the disruption of normal VSMCs anchorage to components of the extracellular matrix [33].

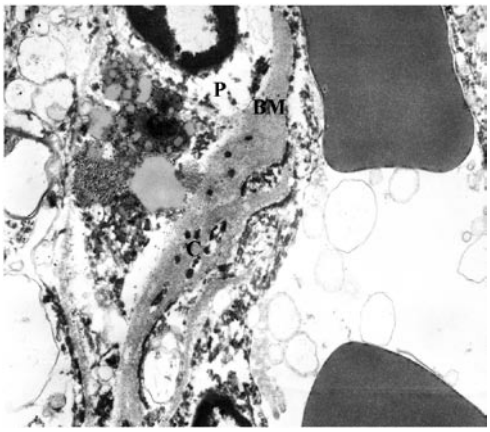

Fig. 10. FAD. Thickened basement membrane (BM) showing collagen fibres (C). Pericyte-(P). Orig. magn. × 7000

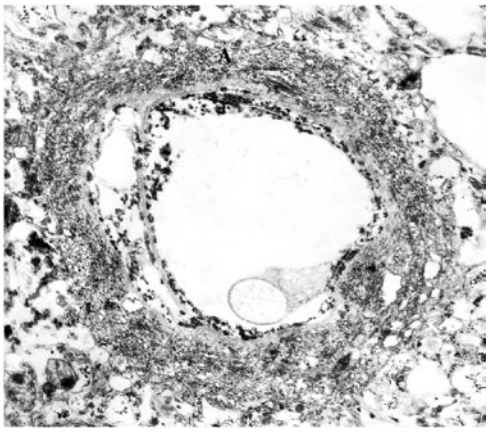

Fig. 11. FAD. Blood vessel surrounded by amyloid fibres (A). Orig. magn. × 7000

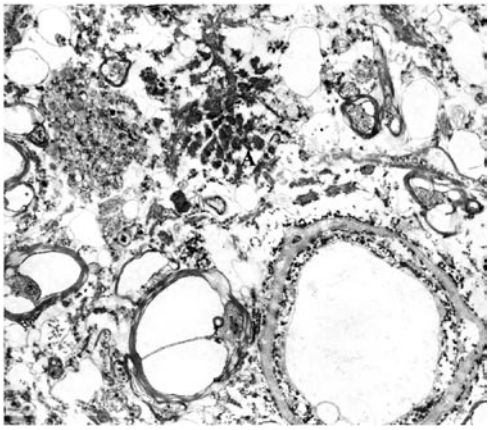

Fig. 12. FAD. Capillary located near amyloid plaque (A). Orig. magn. × 4400

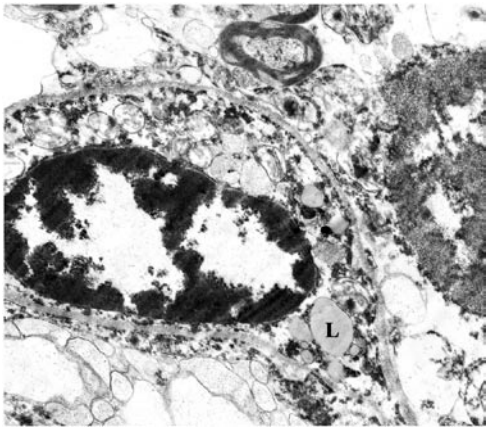

Fig. 13. FAD. Pericyte with mitochondria and lipid drops (L). Orig. magn. × 7000

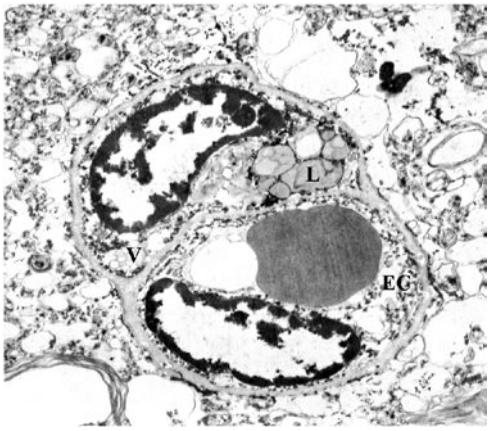

Fig. 14. FAD. Pericyte with lipid drops (L) and vesicles (V). Endothelial cell (EC). Orig. magn. × 4400

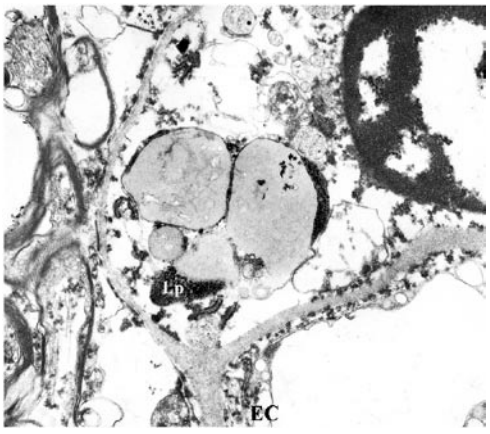

Fig. 15. FAD. Pericyte showing swollen cytoplasm, destroyed organelles and lipofuscin (Lp). Endothelial cell (EC). Orig. magn. × 7000

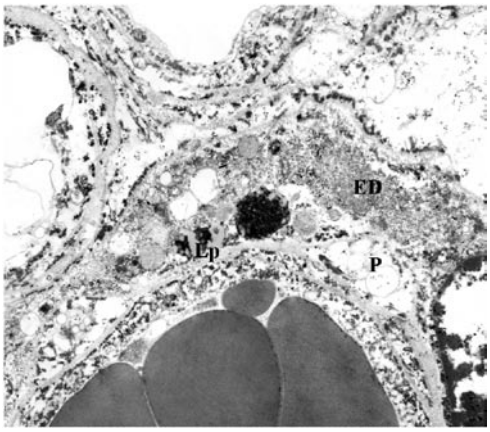

Fig. 16. FAD. Pericyte (P) filling lipofuscin (Lp) and electron-dense material (ED). Orig. magn. × 4400

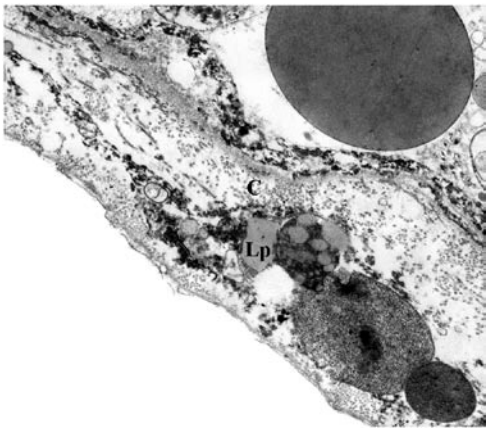

Fig. 17. FAD. Degenerated pericyte with lipofuscin (Lp), lisosomes and numerous collagen fibres (C). Orig. magn. × 4400

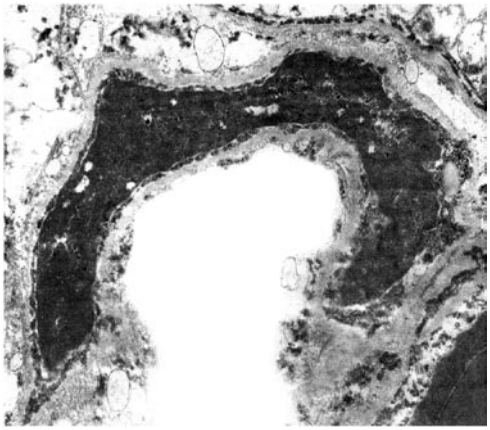

Fig. 18. FAD. Endothelial cell with apoptotic morphology. Orig. magn. × 7000

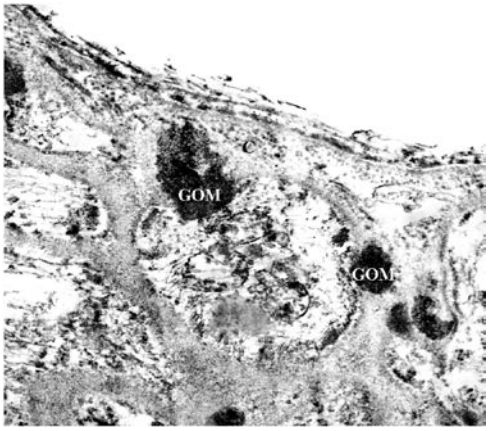

Fig. 19. CADASIL arteriole showing thickened wall, GOM and numerous collagen fibres (C) and degenerating VSMC. Orig. magn. × 7000

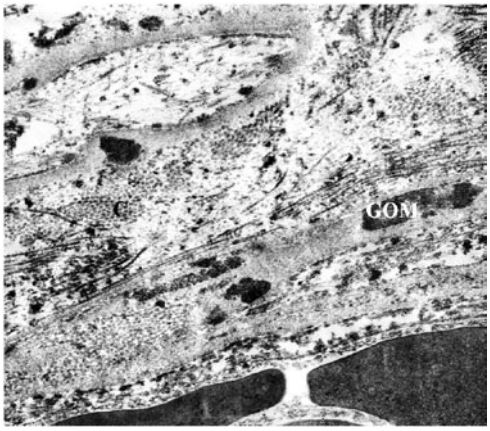

Fig. 20. CADASIL arteriole showing thickened wall, GOM and numerous collagen fibres (C) and degenerating VSMC. Orig. magn. × 7000

Fig. 21. CADASIL arteriole showing thickened wall, GOM and numerous collagen fibres (C) and degenerating VSMC. Orig. magn. × 7000

Pericytes, GOM and capillaries

Vascular SMCs disappearance was observed in both presented angiopathies; however, fibrous replacement of the arteriolar tunica media and the strong immunoreactivity of the arteriolar and capillary walls to the various types of collagen antibodies were more pronounced in CADASIL. Our present and previous ultrastructural studies confirmed that GOM deposits can be found not only in the white and grey matter arterioles, but also in the capillaries, in which pericytes form the characteristic structural element [28,32]. Pericytes equipped with contractile filaments are restricted to vessels devoid of muscular cells. They functionally correspond to VSMCs and have been shown to be multifunctional cells. The degeneration and loss of pericytes contribute to the impairment of endothelial blood-barrier and autoregulatory brain vessel response significant for cerebral blood flow. Our ultrastructural examinations indicated the significant destruction of pericytes in FAD vessels. The presence of dense bodies and lysosomes in pericytes may be a sign of their phagocytic activity. On the other hand, in FAD we also observed pericytes with a significantly reduced number of intracellular organelles as well as small regions of the cytoplasm in BM, which are interpreted as degenerated pericytes [7]. The degenerated pericytes often contained collagen fibres. Despite revealed collagen fibres in the basement membrane and endothelial apoptotic changes, ultrastructural evaluation of pericytes as well as other cells of the blood vessels in CADASIL was impossible because the brain was preserved in formalin.

Damage to the endothelial cells of the vessels in FAD was less frequent, but sometimes they exhibited apoptotic-like morphology. In some vessels, the endothelium cytoplasm was lighter on EM examination, with many vacuoles and a reduced number of organelles, including mitochondria. Typically observed swelling and reduction of the mitochondrial cristae seem to be common to all types of cells which form the vessel wall. The reduced amount and the destruction of the mitochondria lead to a diminished and damaged cellular energetic metabolism greatly contributing also to the development of familial Alzheimer's disease (FAD) in a similar way as in sporadic form (sAD) [1]. Moreover, the mitochondria play an important role in generating reactive oxygen species and consequently are responsible for the damage to the blood brain barrier (BBB) [4]. Changes in the endothelial morphology, pericytes and the smooth muscles of the small cerebral vessels were also observed in the presence and absence of amyloid fibres in the FAD vessel wall.

Extracellular matrix components (ECM) – collagen – basement membrane (BM)

The thickened walls of the CADASIL small arterioles penetrating alongside the subcortical and deep white matter usually showed pronounced fibrous changes. In this report we assessed the fibrous vascular changes with an emphasis on two forms of vascular collagen: type III, fibrillar; and type IV, one of the BM components.

These collagens are components of the extracellular matrix (ECM) and are found in the normal vascular wall, where they usually are weakly immunolabelled [20]. The marked expression of both collagen types is a characteristic feature in the fibrous vessel walls [15,16,31]. We found pronounced fibrous thickening of the arteriolar walls and their strong immunoreactivity to the collagen antibodies in both examined diseases; however in the CADASIL patient they were somewhat more prominent than in the FAD/CAA patients. Fibrous changes were observed in vessels of various size, but in CADASIL, mainly in white matter small arterioles, the same which contained the granular PAS (+) positive material corresponding with the GOM deposits.

Using double immunostaining reactions and the EM study we revealed a co-localisation of the different types of collagen/amyloid or collagen/granular-GOM deposits in some small vessel walls in both diseases [6]. Ultrastructurally in FAD, the conglomerations of the collagen fibres were located in the thickened BM, the degenerated pericytes and between BM lamellae in a pericyte position. In CADASIL large deposits of collagen fibres with typical morphology were visible near GOMs in the thickened vessel wall. Our finding may indicate the structural relationship between the basement membrane changes, deposits and collagen fibres, VSMCs and pericytes in the small arterioles and the capillaries.

Taken together, in this study we revealed that in both diseases the destroyed VSMCs are replaced by progressive fibrosis of the arteriolar walls, although with different intensify. In FAD patients, VSMCs loss and collagen assembly have been found both in ves-

sels with and without amyloid fibres. Corresponding data were found in a review of the literature [10,11,20]. Collagens of both types contribute to non-specific transformations of vessel walls. Collagen type IV accumulation in the BM have recently been reported in aging [20,38]. Fibrillar collagen type III contributes to vessel walls' structural integrity and small vessels' lumen narrowing in arteriosclerosis [30].

At present, pathogenesis of the distinct form of vascular collagenous changes is not known. The vessel wall response to heterogeneous disorders of various aetiology may activate one of three or all components of the vessel wall: endothelial cells (EC), vascular smooth muscle cells (VSMCs) and various collagen components of extracellular matrix (EMC). Morphologically, the vascular response may be mainly degenerative with cellular loss, or proliferative, associated with cell proliferation and ECM expansion, or mixed, similar to the immunologically mediated, inflammatory-productive response leading to "degenerative" age-related arteriosclerosis [30]. It is assumed that early-onset cascade of the significant loss of the VSMCs and collagen accumulation presented in both our familial angiopathies may be comparable to the fibrous age-related changes in the recently described "too much and not enough" collagen accumulation [30,31,35].

We suggest that FAD arterioles devoid of VSMCs throughout all the vessel wall are a weakness and cause more vulnerability and predispose to splitting and double-barrel forms during "not enough" collagen accumulation, whereas the CADASIL arterioles markedly thickened during "too much" collagen accumulation can be rigid and stronger.

So, the results of our present study and other recent studies indicate that in both assessed diseases, the fibrous transformation of small vessel walls lead to weakness and/or thickness of arteriolar walls and may be a non-specific process that may be associated with several disorders and with age-related vascular changes. However, this non-specific vascular transformation may contribute to impairment of the vessel walls' integrity, which may be significant for severe clinical forms of the presented diseases. The variable intensity of the fibrous changes of brain vessels, which are more intense in CADASIL than in FAD, as well as the damage of the endothelial cells and pericytes, may play a prognostic and therapeutic role. The fibrous, collagen-rich CADASIL small cerebral vessels, despite the weakness of the vessel wall due to reduction of VSMCs, appeared to be stronger than in FAD/CAA.

Less intensive fibrosis in the FAD/CAA vessels may predispose them to higher destruction and splitting of the tunica media into two inner and outer rings with double-barrel appearance and microbleets formation. It makes the brain biopsy in CAA a potentially invasive study. The rigid collagen rings in the thickened basement membrane suggest dysfunction of the arteriolar autoregulation response and the early-onset progressive alteration in the cerebral microcirculation in FAD/CAA and CADASIL [8,18,26,43]. Additionally, multilayer collagenous fibres separate from various components in the vessel walls including the basement membrane from the surrounding astrocytic foot may contribute to the impairment of blood-barrier transport mechanisms in both diseases [4,38].

Recently, IHC studies of an interrelation between collagen type IV and CAA-amyloid deposits revealed that some ECM vascular components, presumably collagen type IV, are inducers of a disassembly of Aβ fibrils, and collagen IV additionally inhibits assembly of Aβ in vessel walls. The results of this study suggest that fibrous transformation of the vessel walls may be an element of the cleaning mechanisms of amyloid proteins in the natural repair process of the affected vascular walls, which creates therapeutic implications [21,22].

In conclusion, the results of our present IHC and ultrastructural study show that both analysed familial angiopathies are aggressive, progressive and devastating diseases, leading to early-onset and widespread "presenile" fibrous transformation of the small cerebral vessels. It is likely that clinical implications in the presented genetically determined and aetiologically distinct angiopathies are related both to the aggressive range of results of gene mutation, short time of disease (4-6 years in FAD *versus* 6 years in CADASIL) and age-onset of pathological changes and dementia (29-30 years in FAD *versus* 39 years in CADASIL) and death-time (36 years in FAD versus 45 years in CADASIL). Very intense extracellular amyloid deposits both vascular and parenchymal on atypical topography (basal ganglia and cerebellum) in our FAD/CAA patients supported other reports that P117L PS-1 mutations accelerate amyloid formation and are associated with the most aggressive form of CAA [31,41]. Similarly, both presented forms of primary angiopathy in our CADASIL patient, the necroticans (fibrinoid, PAN-like) and pathognomic granular depo-

sits corresponding with GOM in the tunica media and fibrosis in this vessel layer, support the most aggressive form of this genetically determined disorder.

Although in both presented disorders small cerebral vessels diseases are heterogeneous in aetiology, their clinical manifestations and vascular fibrous transformation are similar despite different intensity. At present there are known numerous interrelations between both genes whose mutations are responsible for FAD and CADASIL and probably also responsible for several similarities in these diseases. Presenilins are not only involved in Aβ generation [10] but are a gene family of the key mediators of Notch signalling and processing of both Notch and APP [10,36].

Although the small cerebral vessels diseases presented in this report are distinct in genetically determined aetiology, intensive studies in recent years of three proteins, presenilin, Notch and APP, have led to the recognition of a direct interrelation between early development and late-life neurodegeneration [36].

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