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Capillary vessel wall in CADASIL angiopathy

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Abstract

The study was aimed at investigating the morphology of capillaries in four skin and muscle biopsy specimens obtained from CADASIL patients. In all cases diagnosis confirmed at the ultrastructural level, and additionally in three cases, the genetic test revealed the Notch3 gene mutations. Using histological and immunohistochemical (IHC) markers for components of capillary vessel wall we showed the reduction and loss of pericytes and and fibrous vessel wall including the thickened basement membrane.

The thorough ultrastructural study revealed the presence of widespread GOM deposits in capillary wall, but less numerous than in arterioles. They were located in the vicinity of pericytes and also in pericyte infolding like vascular smoth muscle cells (VSMC) in arterioles. Sometimes GOM deposits were observed near endothelial cells. The endothelial cells, damaged but not lost, were also observed while most of capillaries revealed only residual pericytes. The destruction and loss of pericytes in capillary wall, like those of VSMC in arteriole wall, was the main vascular pathology in our the examined cases consistent to that pericytes functionally correspondent to VSMC. The Notch3 receptor is expressed on VSMC and pericytes, the results of our study confirm that in capillaries devoid of VSMC, pericytes are the primary morphological target of the Notch3 gene mutation. It should be indicated that in diagnostic ultrastructural examinations of skin and/or skeletal muscle biopsies, not only arterioles but also capillaries, occurring in a larger amount, should be thoroughly analysed, because such an approach may facilitate the detection of GOM deposits – the pathognomonic feature of CADASIL.

Key words: CADASIL, GOM, pericytes, capillary vessel wall, immunochemistry, ultrastructure.

Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited systemic vascular disorder, whose typical morphological changes are manifested by basophilic granular degeneration of VSMC at light microscopic level mainly in arterial and arteriolar vessel wall and at ultrastructural level by deposits of granular osmiophilic material (GOM) in vessel wall, as well as by the pathology of vascular smooth muscle cells. However, GOM-deposits, the pathognomonic feature of the disease, were found not only in arteries and veins, but also in capillaries of the brain and other organs [6-8,14]. The capillaries deprived of VSMC are composed of only endothelial cells, peri-

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cytes and basement membrane. Moreover, in adult human blood vessels, pericytes like VSMC, showed the expression of the Notch3 receptor [5,6]. Only few papers have also revealed the degeneration of pericytes [6,15]. Thus, the key pathological changes in CADASIL vessels are not limited to VSMC, but they also include other compounds of the small vessel (arterioles and capillaries) wall [1,9]. Assuming that GOM deposits originate in capillaries deprived of VSMC, which has been observed in our study, as well as by other authors, it may be presumed that other cells and components of vessel wall can be the target for the Notch3 gene mutations.

The aim of our study was to examine thoroughly capillaries and especially pericytes from CADASIL-affected skin and muscle biopsies.

Material and methods

The study was performed on four skin and four muscle biopsy specimens derived from CADASIL patients, aged 44-54 years.

Light microscopic examination was carried out on the tissue samples fixed in 4% formaldehyde and embedded in paraffin. Morphology of small vessels was essayed with routine histological stains (HE, PAS) and immunohistochemical methods. Immune reactions with antibodies against smooth muscle actin (SMA, DAKO 1:35), collagen IV (DAKO 1:35) and ubiquitin (DAKO 1:200) were investigated.

For electron microscopy, skin and muscle samples were fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide. They were then dehydrated and embedded in Spurr. Ultra thin sections double-stained with uranyl acetate and lead citrate were examined with an Opton DPS 109 electron microscope.

Results

In all cases, electron microscopy examination allowed to definite diagnosis CADASIL. This examination showed in vessel walls granular osmiophilic material, typical of CADASIL. Moroover, genetic examinations revealed the Notch3 gene mutation in three patients.

Light microscopic studies of the skin and muscle specimens showed pathological changes in numerous capillaries. Swelling of endothelial cells and fibrous capillary walls was found as well as in PAS staining and ubiquitin immunoreaction. Pericytes were observed only in some capillaries. (Fig. 1A, B). The variable smooth muscular actin (SMA) – immunoreactivity of capillary wall indicated the degree of mural changes in lost or maintained pericytes. The strongest immunoreactivity was observed in capillaries with visible pericytes while weak indicated the capillaries without them (Fig. 2A, B).

The vessels of varied calibre with thickened and fibrous wall showed different immunoreactivity to collagen IV. Some of them, arterioles as well as capillaries revealed circumferential collagen IV granular deposits in the expected position of a thickened basement membrane (BM). Only the capillaries with thickened and fibrous wall showed narrow lumen (Fig. 3A, B).

At the ultrastructural level, GOM deposits in several capillaries were found in all the CADASIL-affected skin and muscle biopsy specimens. The majority of GOM-expressed capillaries revealed a thickened BM. Thickening of basement membrane in some capillaries was extensive and its appearance was usually homogenous, but sometimes it took a multi-layer form (Figs. 4-6). Collagen fibres were visible on the periphery of BM (Fig. 5).

GOM deposits, located in basement membrane and quite frequently in the vicinity of pericytes (Figs. 6, 7) or in pericyte membrane infolding (Fig. 8), differed both in their number and size. Sometimes, pericytes showed pinocytic vesicles in GOM-facing areas (Fig. 9). Degeneration and extensive loss of pericytes were noted in the majority of vessels. These capillaries exhibited only residues of pericytes of different size (Figs. 6, 10) or no pericytes at all (Figs. 11, 12). Pericytes with nucleus were sporadicaly seen (Fig. 5). GOM was only sporadically seen near endothelial cells (Fig. 13).

Endothelial cytoplasm was usually very thin with few organelles, and sometimes, nuclei were also seen (Fig. 14). Typical findings in these cells were intracytoplasmatic vacuoles with varied size and number. Some of them revealed fine grey material (Figs. 12, 15). On the other hand, some endothelial cells manifested the swelling of cytoplasm and mitochondria accompanied by the presence of only few organelles, while the loss of endothelial cells was not visible (Fig. 16). The capillaries without GOM deposits were also damaged.

Discussion

Until recently, there have been no systematic studies concenring the abnormalities of capillaries in

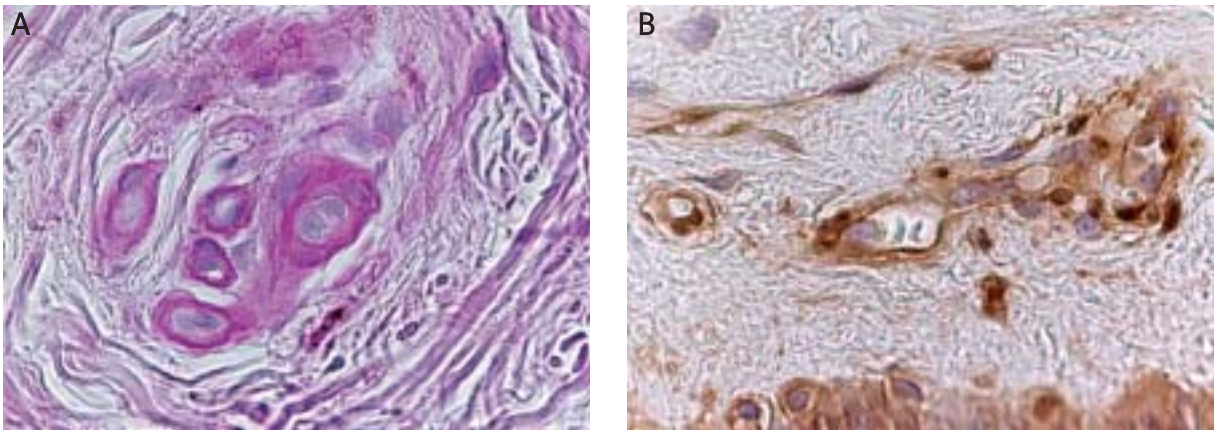


Fig. 1. A, B. Swollen endothelial cells and only residual pericytes in basal membrane of thickened and fibrous capillary walls. A. PAS × 630, B. Ubiquitin × 630.

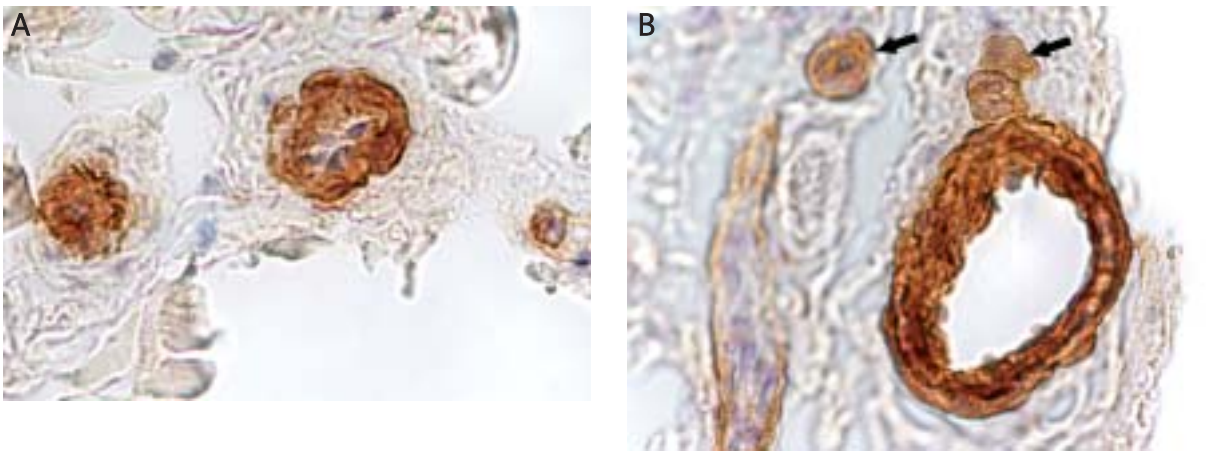


Fig. 2. A, B. Varied SMA-immunoreactivity of capillary wall. A. Strong positive with visible pericytes. B. Weak or negative without pericytes (arrows). SMA × 630.

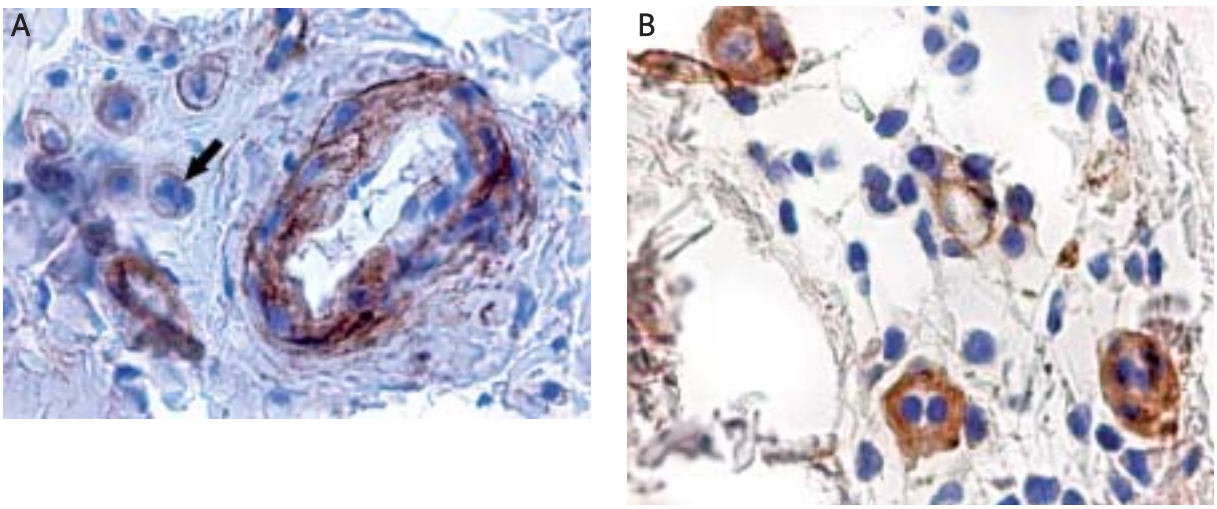


Fig. 3. A, B. Narrow of capillaries lumen (arrow) and circumferential collagen IV granular deposits in the expected position of a thickened basement membrane in capillaries as well arterioles. Collagen IV × 630.

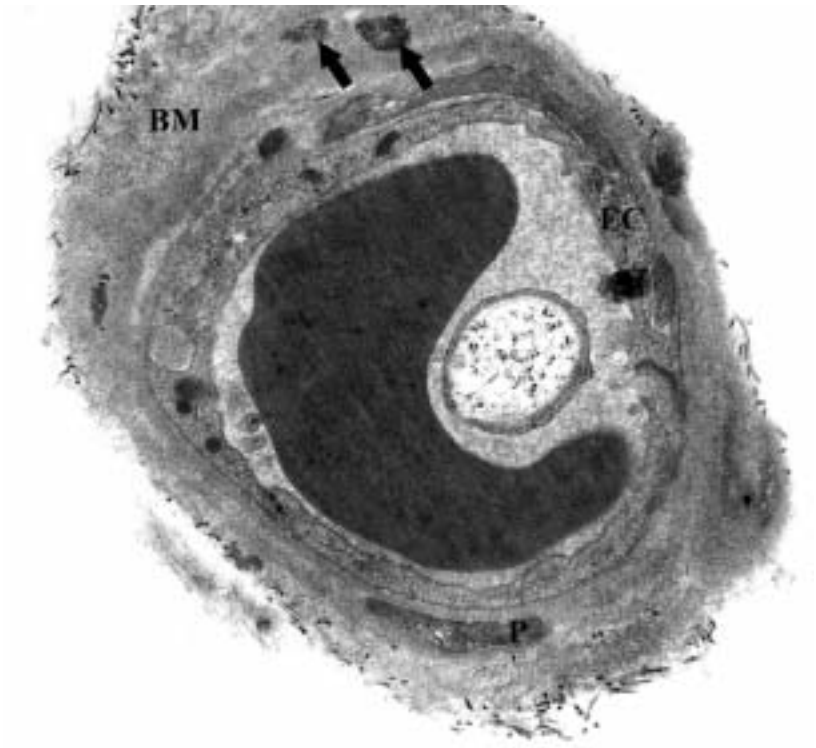


Fig. 4. Capillary with thickened basement membrane (BM) and residual pericyte (P). Endothelial cell (EC), GOM (arrows). Orig. magn. × 7000.

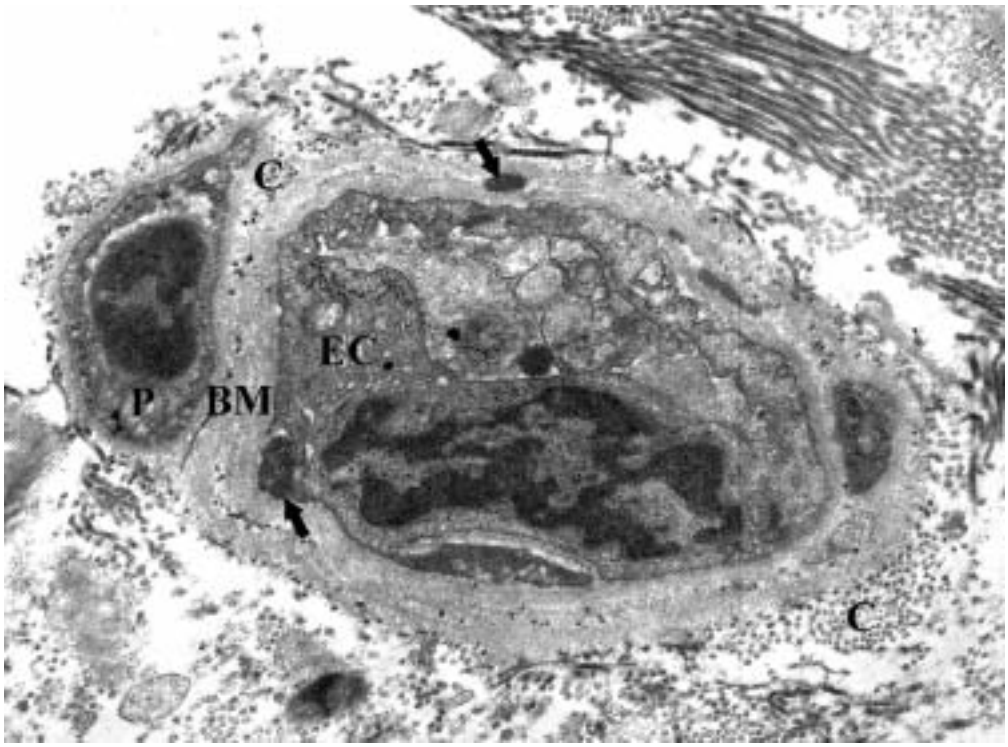
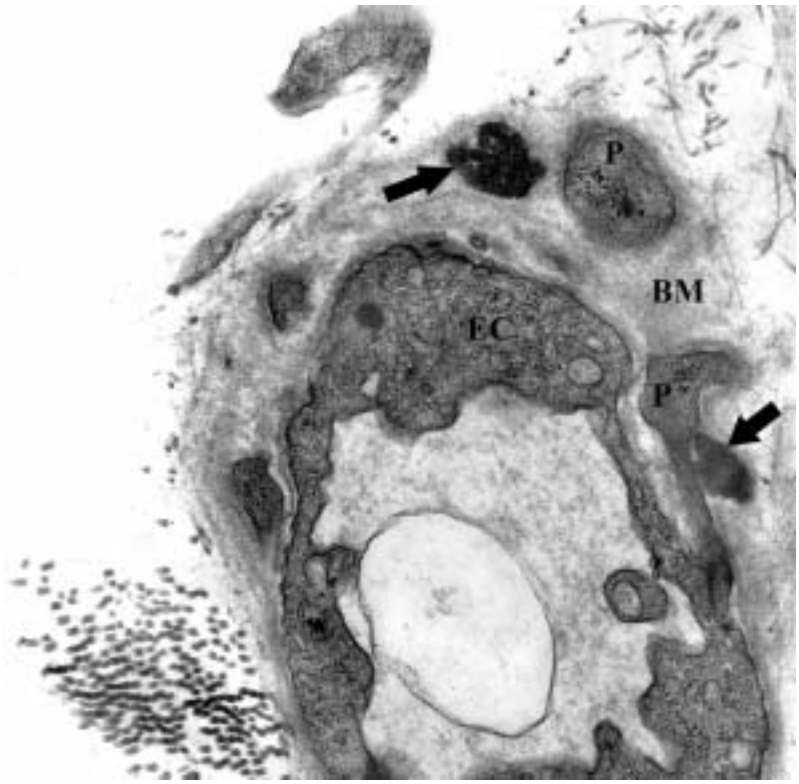
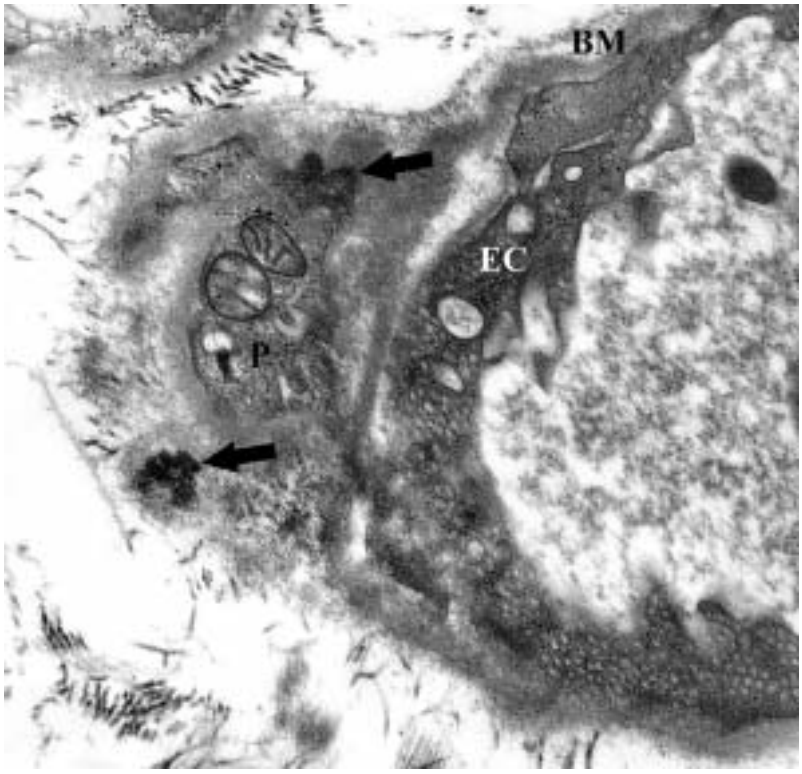


Fig. 5. Capillary with pericyte (P) and collagen fibres (C) in thickened basement membrane (BM). GOM (arrows), endothelial cells (EC). Orig. magn. × 7000.





Figs. 6, 7. Capillaries with GOM deposits (arrows) located within basement membrane (BM) and in vicinity of pericytes (P). Endothelial cells (EC). Orig. magn. 6 × 12 000, 7 × 7000.

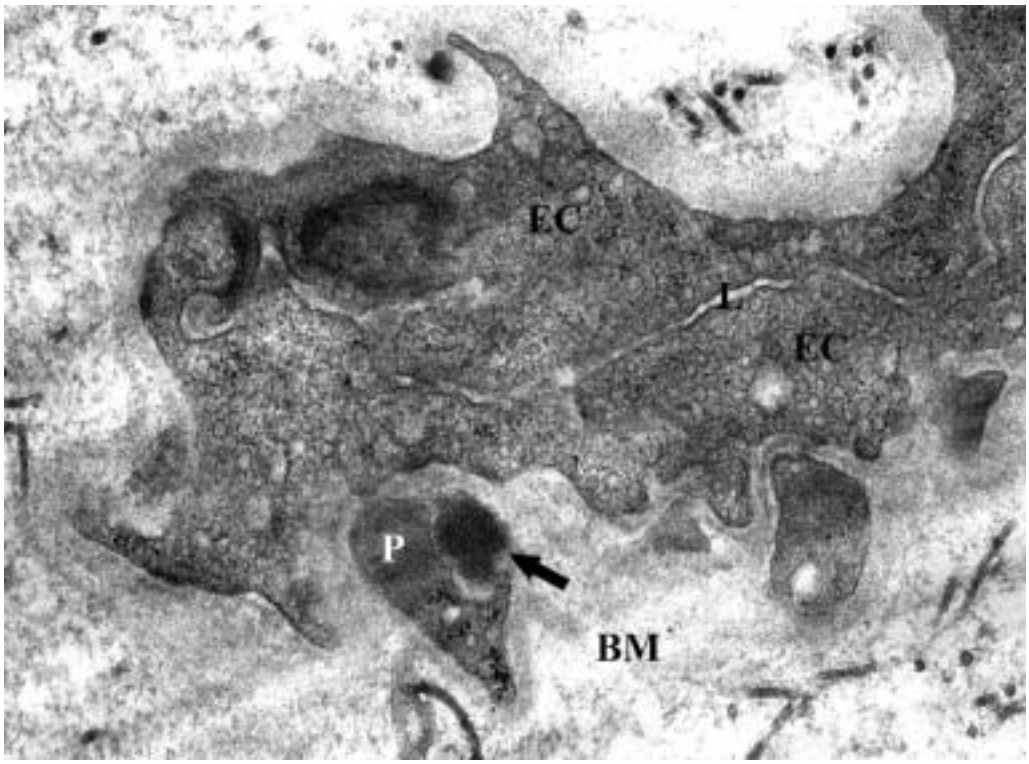


Fig. 8. Capillary with creviced lumen (L) and GOM deposit (arrow) in pericyte infolding. Basement membrane (BM), endothelial cells (EC). Orig. magn. × 12 000.

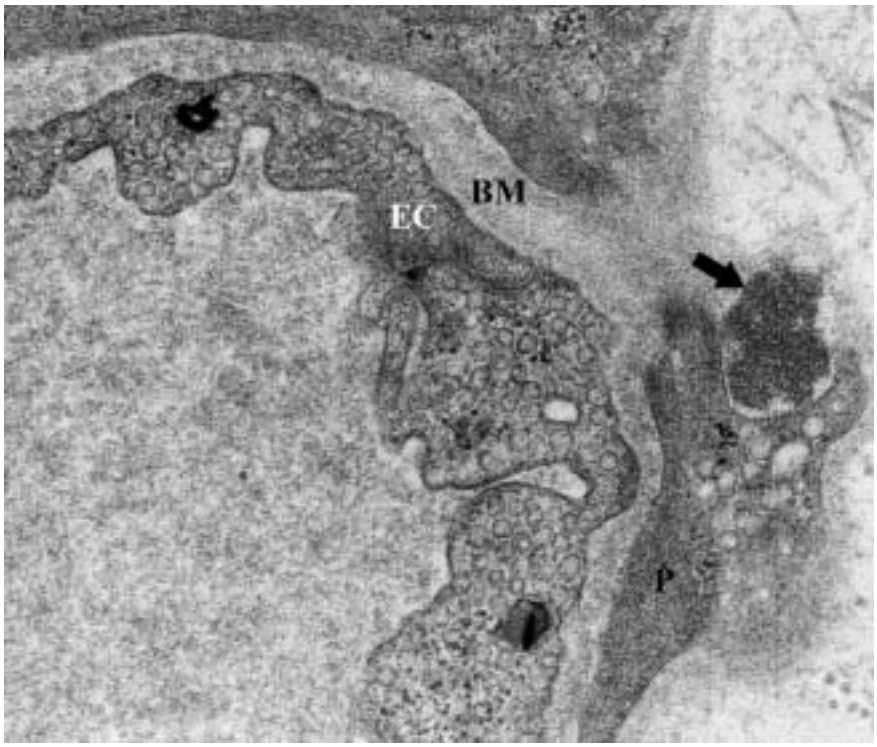


Fig. 9. GOM (arrow) in pericyte infolding (P). Visible pinocytic vesicles in cytoplasm of the pericyte. Endothelial cell (EC), basement membrane (BM). Orig. magn. × 20 000.

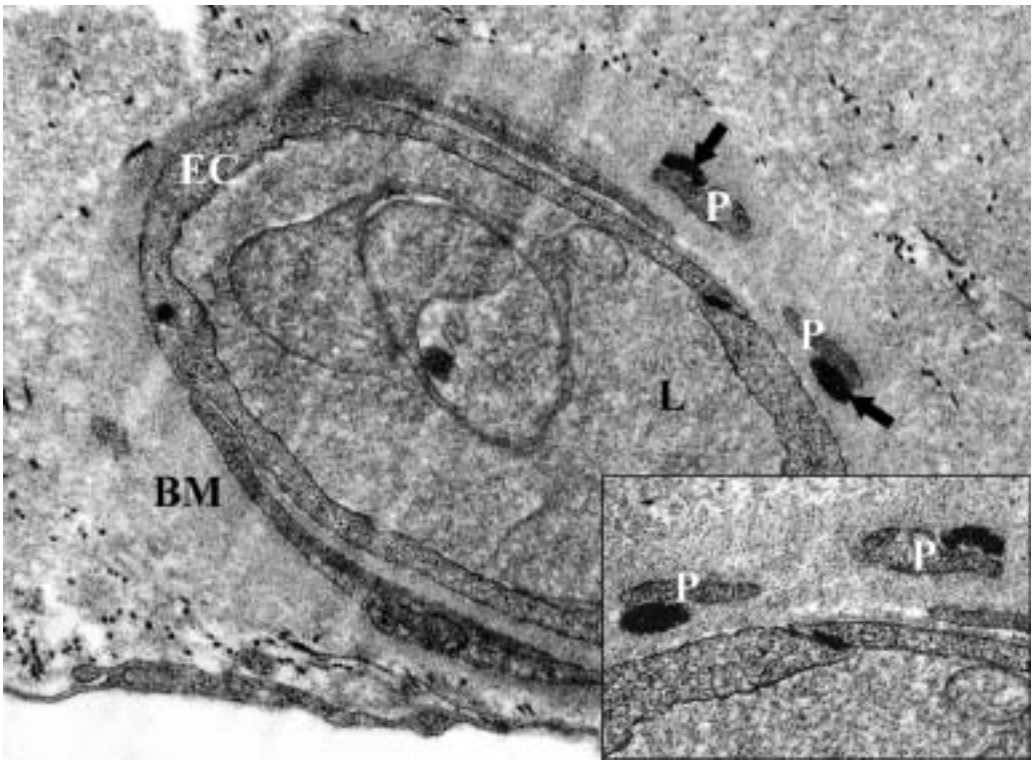


Fig. 10. Capillary with residual pericytes (P). Endothelial cels (EC), basement membrane (BM), GOM (arrows). Insert: Hight magnification of residual pericytes with GOM. Orig. magn. × 7000.

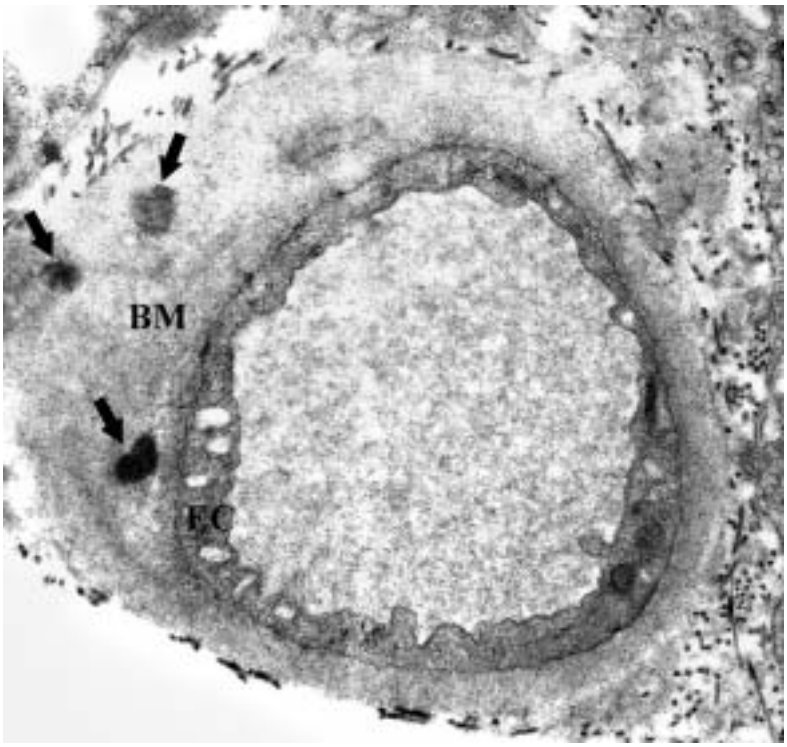


Fig. 11. Capillary without pericytes. Endothelial cell (EC), basement membrane (BM), GOM deposits (arrows). Orig. magn. × 7000.

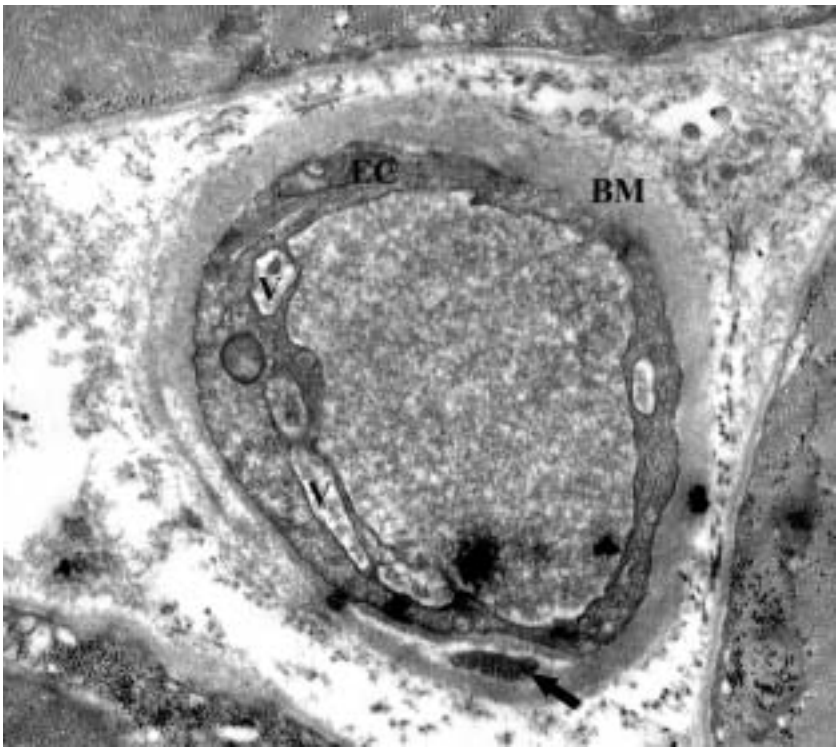


Fig. 12. Capillary with vacuoles (V) in endothelial cytoplasm (EC) and invisible pericyte. GOM (arrow), basement membrane (BM). Orig. magn. × 7000.

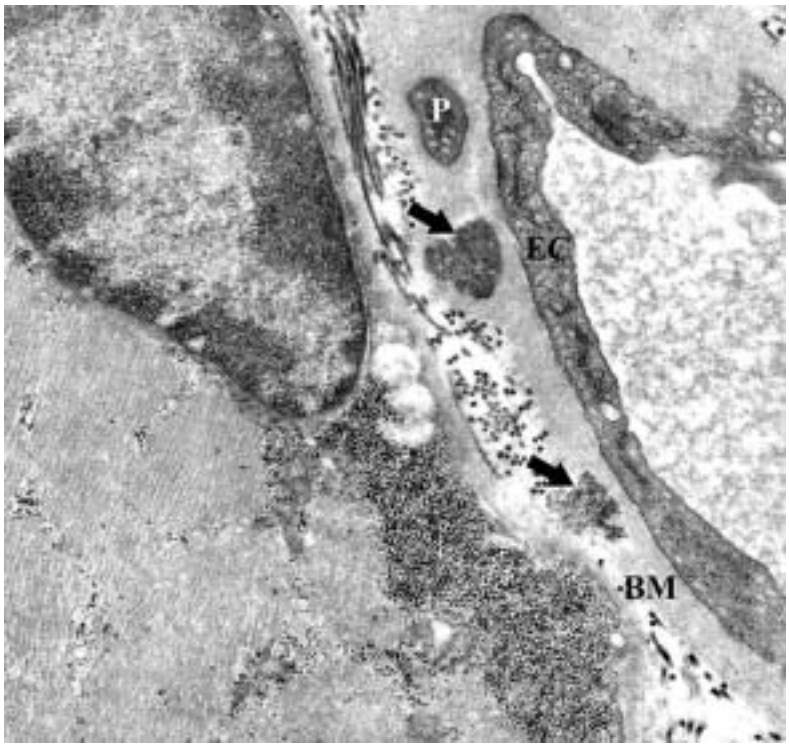


Fig. 13. Capillary with GOM deposits (arrows) near endothelial cells (EC). Basement membrane (BM), pericyte (P). Orig. magn. × 12 000.

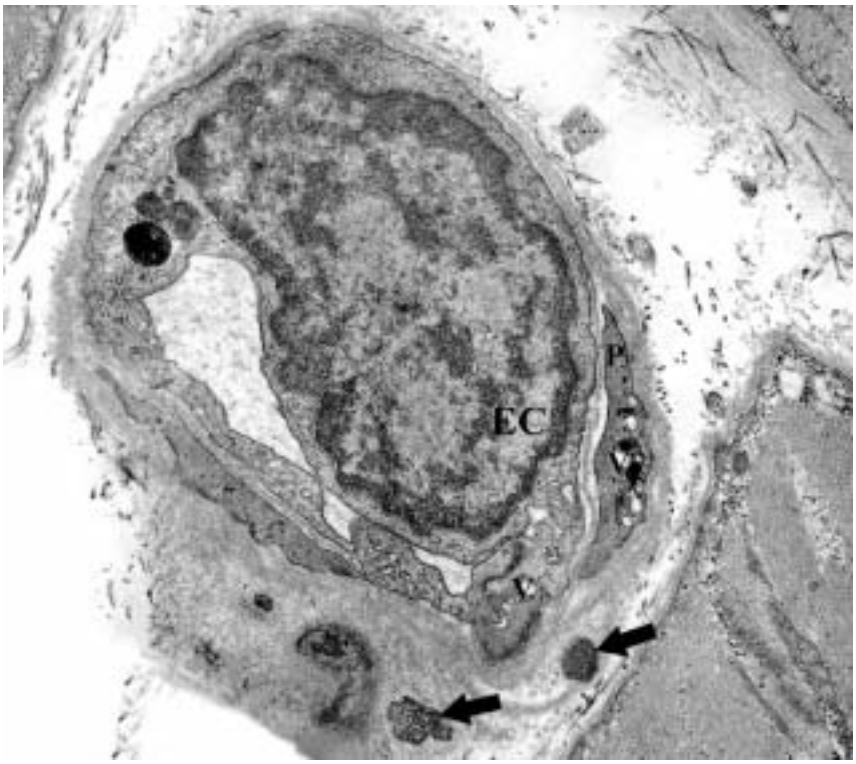


Fig. 14. Capillary showing nucleus in endothelial cell (EC) with vacuoles (V). GOM deposits (arrows), pericyte (P). Orig. magn. × 7000.

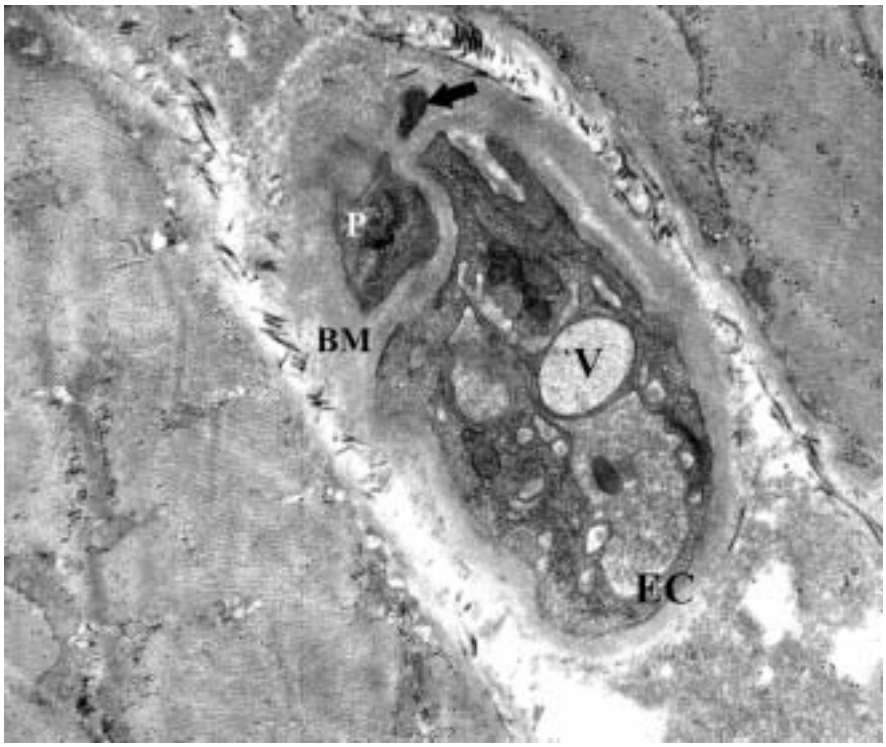


Fig. 15. Capillary with promient vacuoles (V) in endothelial cells (EC). Pericyte (P), basement membrane (BM), GOM (arrow). Orig. magn. × 7000.

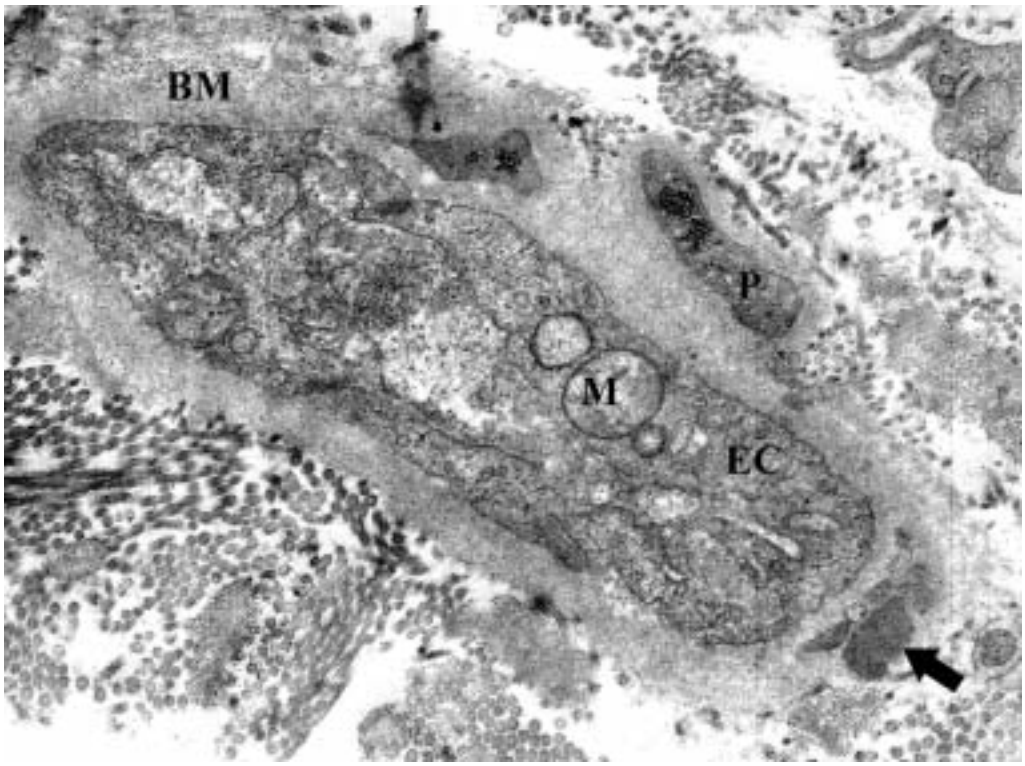


Fig. 16. Capillary showing endothelial cells (EC) with swollen cytoplasm and mitochondria (M). Pericyte (P), basement membrane (BM), GOM (arrow). Orig. magn. × 12 000.

CADASIL patients, although some authors indicated the presence of GOM deposits in capillaries [6-8]. While, more GOM deposits were visible in larger vessels with VSMC [2]. It has been postulated that VSMCs are not the only agent involved in CADASIL pathological process [1,10]. Our previus ultrastructural studies confirmed the presence of GOM deposits within the capillary wall in the skin and muscle biopsies from only one patient with CADASIL [6,15]. In the present study, we concentrated our attention on the capillaries in skin and muscle biopsies in four patients with CADASIL. Interestingly, the presence of GOM and other pathological changes in numerous skin and muscle capillaries was observed in all CADASIL patients.

In capillaries, GOM deposits were located within the thickened basement membrane, only sometimes in the vicinity of endothelial cells, the most often in close contact with pericytes, and even within an infolding of pericytes, resembling the GOM-VSMC relation in arterioles. Their number in capillaries was smaller than in arterioles suitable to small vessel wall morphology or stage of disease. One of reasons is probably a smaller number of pericytes in relation to numerous VSMC in arterioles, because, as already postulated, the Notch3 ectodomain occurs in close vicinity of GOM [4], or forms the major component of GOM deposits [3]. In other hand, the Notch3 receptor is expressed also on pericytes [4,15]. In our opinion this may explain different amounts of GOM in capillaries and arterioles.

PAS staining, as well as collagen IV immunoreactivity indicated thickened and fibrous vessels of the wall with circumferential collagen IV granular deposits in the expected position of thickened BM, narrowing the lumen in vessels. Basement membrane thickening in capillaries was also revealed in our ultrastructural studies. It was usually homogenous but sometimes it took a multiple layers form and decoratet by GOM deposits. The multilaminated capillar basement membrane can manifested possibility of regeneration stage in degenerative process of small vascular vessel wall.

Our extensive studies of all skin and muscle biopsies from CADASIL patients revealed that destruction of pericytes is the main feature of capillar pathology.

Our found prominent degeneration and loss of pericytes in the capillaries, in which pericytes formed the characteristic structural element [10,11]. The capillaries exhibited varied degrees of actin immunostaining due to the loss of pericytes equipped with contractile filaments and restricted to vessels devoid of muscular cells. Strong actin positive pericytes were observed only in a small number of well preserved vessels. At the ultrastructural level, the majority of capillaries exhibited only residual pericytes indicating their loss. However, the capilaries not exhibiting any pericytes, but with GOM within the basement membrane were also found. Like VSMCs, GOM deposits were observed around residual pericytes. Moreover, GOM was also located within infoldings of their membrane.

The degeneration and loss of VSMCs in arterioles, accompanied by accumulation of Notch3 and GOM in the vicinity of these cells, are regarded as the main features of CADASIL [4,14]. The resemblance of morphological changes in capillaries pericytes and arterial VSMCs may be explained by the fact that the Notch3 expression in adult humans is also found on pericytes [4,5] and that functionally pericytes correspond with VSMCs [15,16]. The degeneration and loss of pericytes contribute to the impairment of endothelial blood-barrier and auto-regulatory brain vessel response, which is essential for cerebral blood flow.

In capillaries with degenerated pericytes the damaged endothelial cells were seen. They revealed significantly thinned cytoplasm, the presence of vacuoles different in size and shape and dense mitochondria, sometimes difficult to distinguish. However, in some capillaries, EC showed swollen cytoplasm and mitochondria and only few other organelles. However, the loss of EC was not visible. The results of the previous EM analysis of endothelial cells both in CADASIL patients and in transgenic mice with the mutant Notch3 gene (only in arterioles) also demonstrated their injury [12,13]. It has been suggested that EC revealed impaired permeability that may play a role in the destruction of VSMCs [13]. However, it is not known whether these damages are primary or secondary to changes of other structures of the vessel wall [9]. Our results seems to confirm that in capillaries devoid of VSMC pericytes are the primary target of the Notch3 mutation, however, the relationship between the Notch3 gene mutation and degeneration of pericytes remain unclear, like the relationship between Notch3 mutations and VSMC in arterioles [4].

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