

Ultrastructural picture of blood vessels in muscle and skin biopsy in CADASIL

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Abstract

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy) is an inherited systemic vascular disorder affecting mainly the central nervous system. We performed detailed ultrastructural examination of the small vessels in the skin and skeletal muscle of a 51-year-old patient with bilateral cerebral white matter lesions, who had a history of two ischaemic strokes. The arterioles were characterized by degeneration and loss of vascular smooth muscle cells (VSMCs). GOM deposits, varied in size and shape, were located in the neighbourhood of the smooth muscle cells, often within an infolding of the cell membrane. No apparent correlations between presence, size or number of GOM deposits and damage severity of vascular smooth muscle cells were seen. Moreover, in some capillaries there were GOM deposits which were seen in the basement membrane near pericytes and endothelial cells. On the other hand, lesions of VSMCs and/or endothelial cells were also visible on the sections of blood vessels devoid of GOM deposits. Genetic tests detected a mutation in exon 4 of the *Notch3* gene. It confirmed the initial diagnosis which had been suggested on the basis of the clinical and MRI findings.

Key words: CADASIL, GOM, arterioles, capillaries, vascular smooth muscle cells, ultrastructure

Introduction

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy) is a rare inherited systemic vascular disease affecting mainly the central nervous system. The disease is characterized by recurrent subcortical ischaemic events, in the absence of amyloid or atherosclerotic vasculopathy, as well as risk factors such as hyper-

tension, diabetes and dislipidaemia [12,17]. Often migraine attack with/without aura is an initial symptom, followed by ischaemic attacks [11,14]. Afterwards mood disorders, memory disturbances and progressive stepwise dementia appear in the course of illness.

In CADASIL, pathomorphological changes of the wall of small and medium vessels were observed not

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only in the brain but also in the skin, muscles and internal organs: heart, kidney, lung, liver [4,15,18,25].

CADASIL is caused by mutation in the Notch3 gene localized on chromosome 19 [13,32]. The affected gene consists of 33 exons and encodes a transmembrane receptor whose expression is highly restricted to vascular smooth muscle cells in cerebral and extracerebral vessels [13–15]. Until now, most mutations, in approximately 70% of cases of CADASIL, have been found in exons 3 and 4 of the Notch3 gene [13].

Morphologically CADASIL is characterized by degeneration of VSMCs and accumulation of granular osmiophilic material (GOM) [2,24]. Diagnostic criteria for CADASIL involve presence of mutation in the Notch3 gene and/or deposits of GOM in walls of both the cerebral and peripheral arteries. So the final diagnosis must be confirmed either by ultrastructural examination of blood vessels, e.g. of skin or muscle, and/or by genetic analysis.

In this paper we present ultrastructural evaluation of the blood vessels from the skin and skeletal muscle biopsies taken from a patient suspected of CADASIL disease.

Case report

We present a 51-year-old woman who was admitted to our Department of Neurology to establish diagnosis of diffuse white matter changes

observed for ten years with corresponding progressive deterioration in cognitive functions. She had two ischaemic strokes (in 1994 and 1996) with right hemiparesis and aphasia. She also suffered from angina pectoris and rheumatoid arthritis. On admission the patient was alert, but disorientated to time, place and situation. She complained of headaches and constant pain in the lumbar region. Mild paresis in the right lower face and the right arm were present. Papilledema was detected in both eyes. No skin changes were noted. No urinary incontinence was reported. Psychological tests confirmed diffuse cognitive function impairment.

The brain MRI (Fig. 1 A, B) showed diffuse white matter changes in both hemispheres compared to previous brain imaging, and enlargement of all 4 ventricles with patent aqueductus (last CT was done in 2003). Suspecting CSF flow disturbances, lumbar MRI was performed and revealed two tumours in the spinal canal at L1 and L2-L3 level. The patient underwent neurosurgery and the tumours were removed. Histopathological examination revealed Schwannoma.

In order to explain the etiology of white matter changes, metabolic diseases of white matter were firstly excluded: metachromatic leukoencephalopathy, Krabbe disease, GM-1 and GM-2 gangliosidosis and alfa-mannosidosis. Finally systemic angiopathy of the small vessels was suspected. Skin and skeletal muscle biopsies were undertaken for ultrastructural exami-

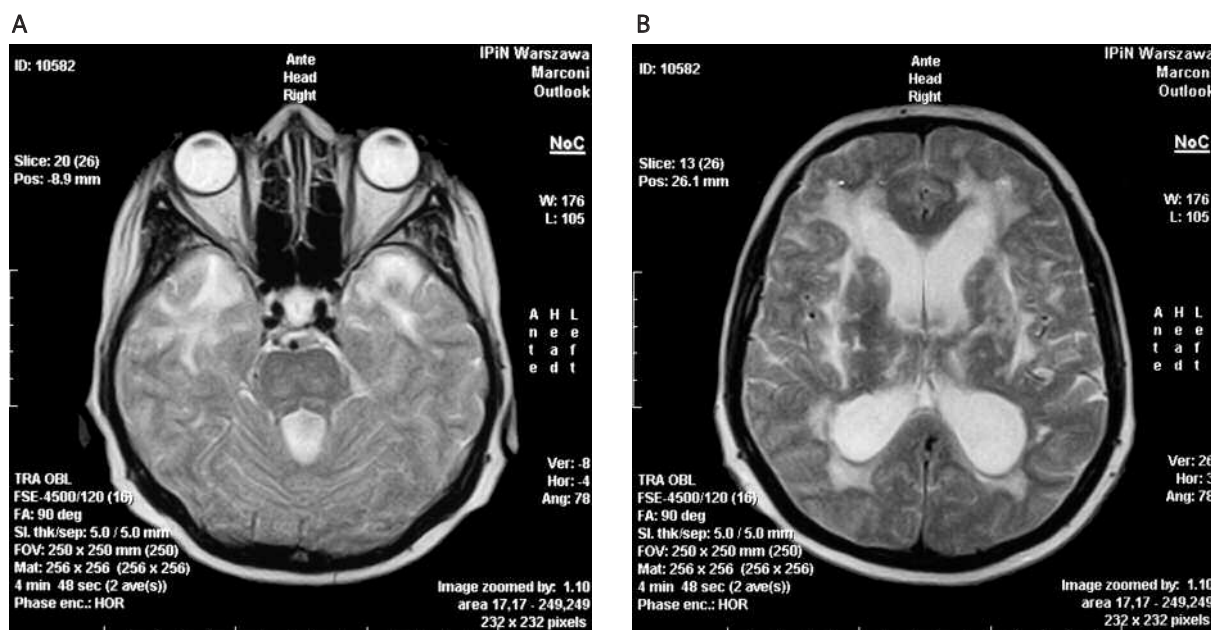


Fig. 1 A, B. T2- weighted MR image: diffuse white matter hyperintensities

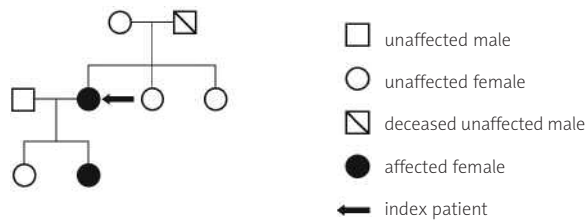


Fig. 2. Family history. Members of the family with CADASIL and without confirmed mutation in exons 3 and 4 of the Notch3 gene

nation and the diagnosis of CADASIL was established. The diagnosis was confirmed by a genetic test sequencing of exon 4 of the Notch3 gene, which showed a mutation of CADASIL type leading to the replacement of an arginine by a cysteine at position 475 (Arg 133Cys) in EGF 3 (Fig. 2). Genetic tests were performed in Laboratoire de Genetique Moleculaire Hospital Lariboisiere, Paris, France by Prof E. Tournier-Lasserre.

Material and methods

Skin and skeletal muscle biopsies from the 51-year-old woman from the upper arm were fixed in 2.5% glutaraldehyde and postfixed in 2% osmium tetroxide. After dehydration the tissues were embedded in Epon 812. Semithin sections stained with toluidine blue were analyzed under a light microscope to select the area with blood vessels. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (Opton DPS109).

Results

In the skin and muscle biopsy numerous blood vessels were damaged. The basement membrane of these vessels was often thickened with collagen fibrils and granular osmiophilic material (GOM) accumulation (Fig. 3). GOMs variable in size, shape and number were located both in the arterioles and capillaries; around pericytes, endothelial cells and VSMCs (Figs. 3–7, 12). Endothelial cells were thin with more osmiophilic cytoplasm and irregularly shaped nuclei showing a lot of heterochromatin (Fig. 8). In their cytoplasm numerous pinocytotic vesicles, vacuoles and dense granular material were present, and processes protruding into the lumen were seen

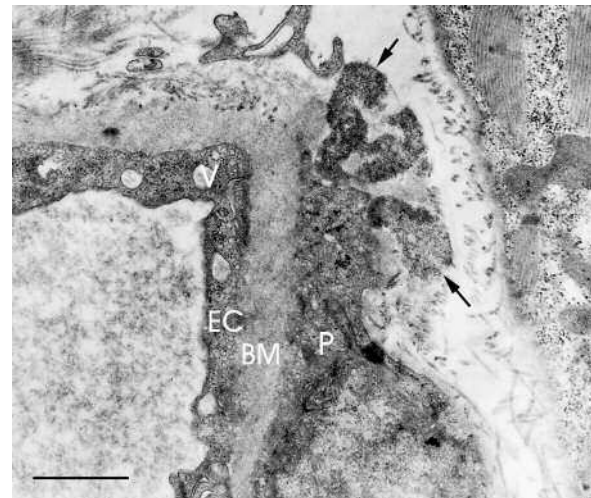


Fig. 3. Capillary with thickened basement membrane (BM) showing collagen fibrils. GOM deposits (arrows) in close contact with the pericyte (P). EC – endothelial cells; V – vacuoles. Muscle biopsy. Bar represents 1 μm

(Figs. 8,9). Sporadically, the endothelial cells were degenerated and appeared “empty” (Fig. 10).

In the arteries many vascular smooth muscle cells were degenerated and appeared to have disappeared from the vessel walls (Figs. 11, 12). Extremely VSMCs



Fig. 4. Capillary with GOM deposit (arrow) located in pericyte (P) infolding. Endothelial cells (EC) are thin. Muscle biopsy. Bar represents 1 μm

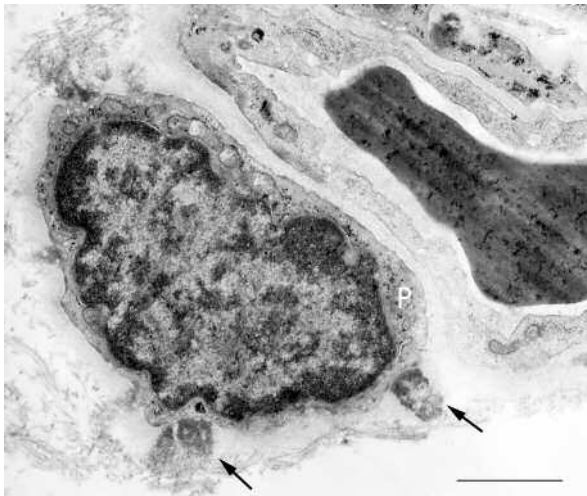


Fig. 5. Capillary with GOM deposits (arrows) close to pericyte (P). Muscle biopsy. Bar represents 1.6 μ m

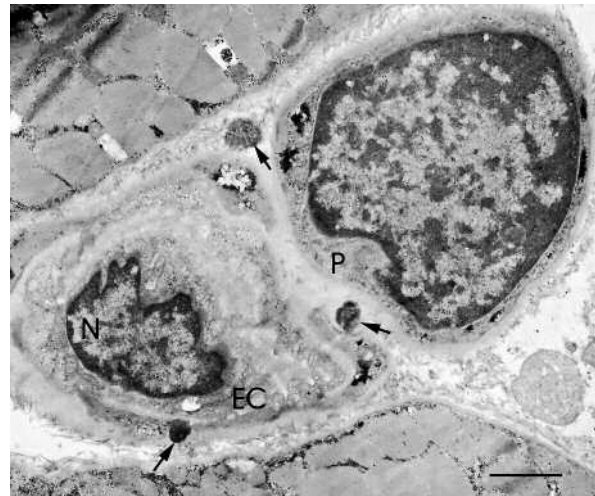


Fig. 6. GOM (arrows) are deposited close to endothelial cell (EC) and pericyte (P). Muscle biopsy. Bar represents 1.6 μ m

assumed the shape of very narrow processes with shrunken and denser cytoplasm (Fig. 13).

The damaged VSMCs contained the conglomeration of mitochondria as well as glycogen grains and different size vacuoles (Fig. 14). In VSMCs, sometimes nuclei with a great amount of dense chromatin were seen (Figs. 11, 12). The smooth muscle cells were so

loosely packed and separated from each other that only GOM deposits marked their original position (Fig. 15). GOM deposits were located close to the VSMCs, often within infoldings of their cell membrane (Fig. 16). Some of them were found some distance off smooth muscle cells. Around the smooth muscle cells, within the thickened basement membrane, from one up to fifteen GOM deposits per blood vessel section were observed (Fig. 16). Some of the vessels without GOM were also damaged (Fig. 17).

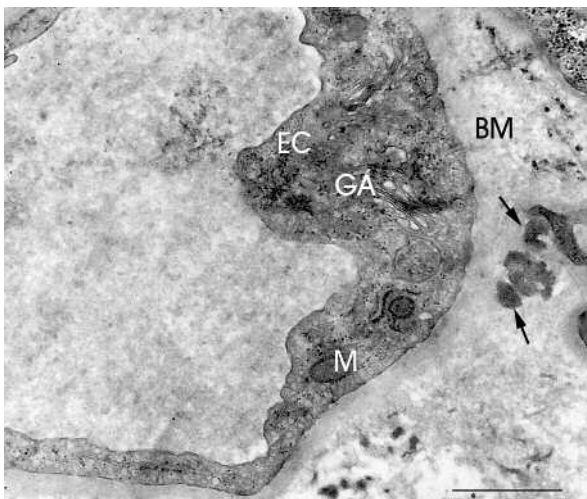


Fig. 7. Fragment of capillary. GOM deposits (arrows) within basement membrane (BM) surrounding the endothelial cells. Cytoplasm of endothelial cell (EC) with mitochondria (M) showing dense matrix and prominent Golgi apparatus (GA). Muscle biopsy. Bar represents 1 μ m

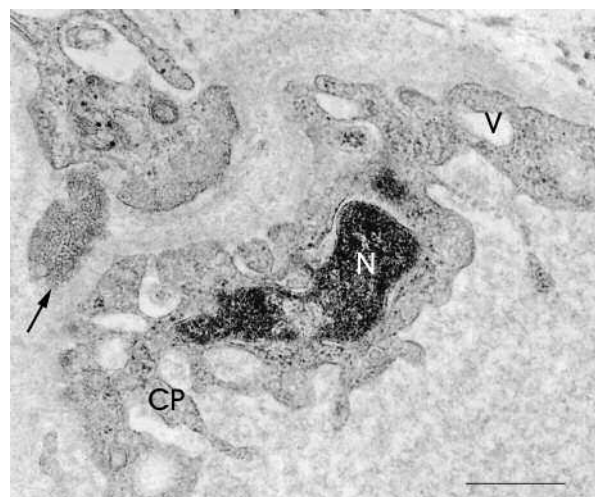


Fig. 8. Endothelial cell with lobular nucleus showing dense chromatin (N). Cytoplasm is filled with vacuoles (V). Cellular protrusions (CP), GOM deposit (arrow). Skin biopsy. Bar represents 0.5 μ m

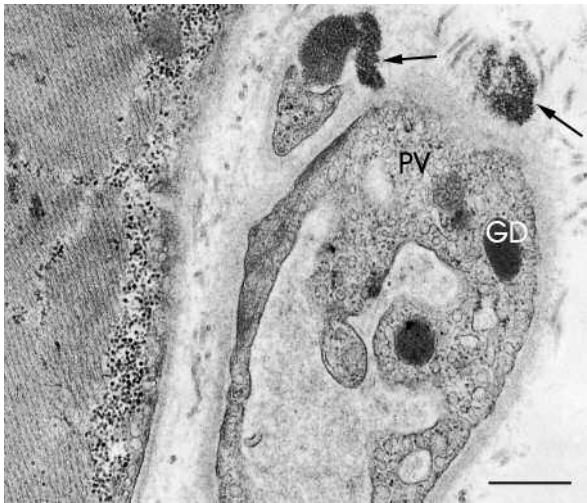


Fig. 9. Endothelial cell with numerous pinocytotic vesicles (PV) and conglomerations of granular dense material (GD). GOM deposits (arrows). Muscle biopsy. Bar represents 0.5 μ m

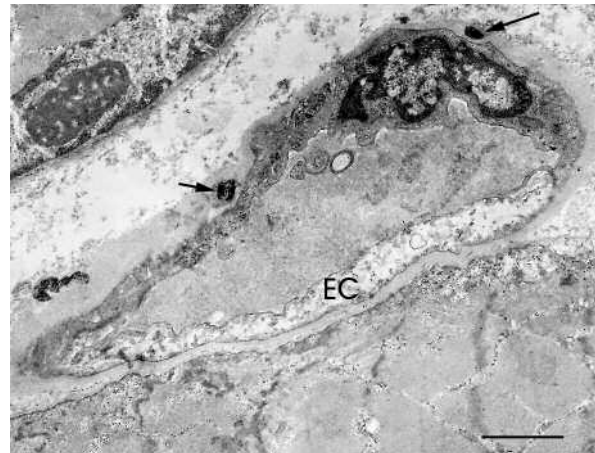


Fig. 10. Capillary with degenerating endothelial cell (EC). GOM deposits (arrows). Muscle biopsy. Bar represents 1.6 μ m

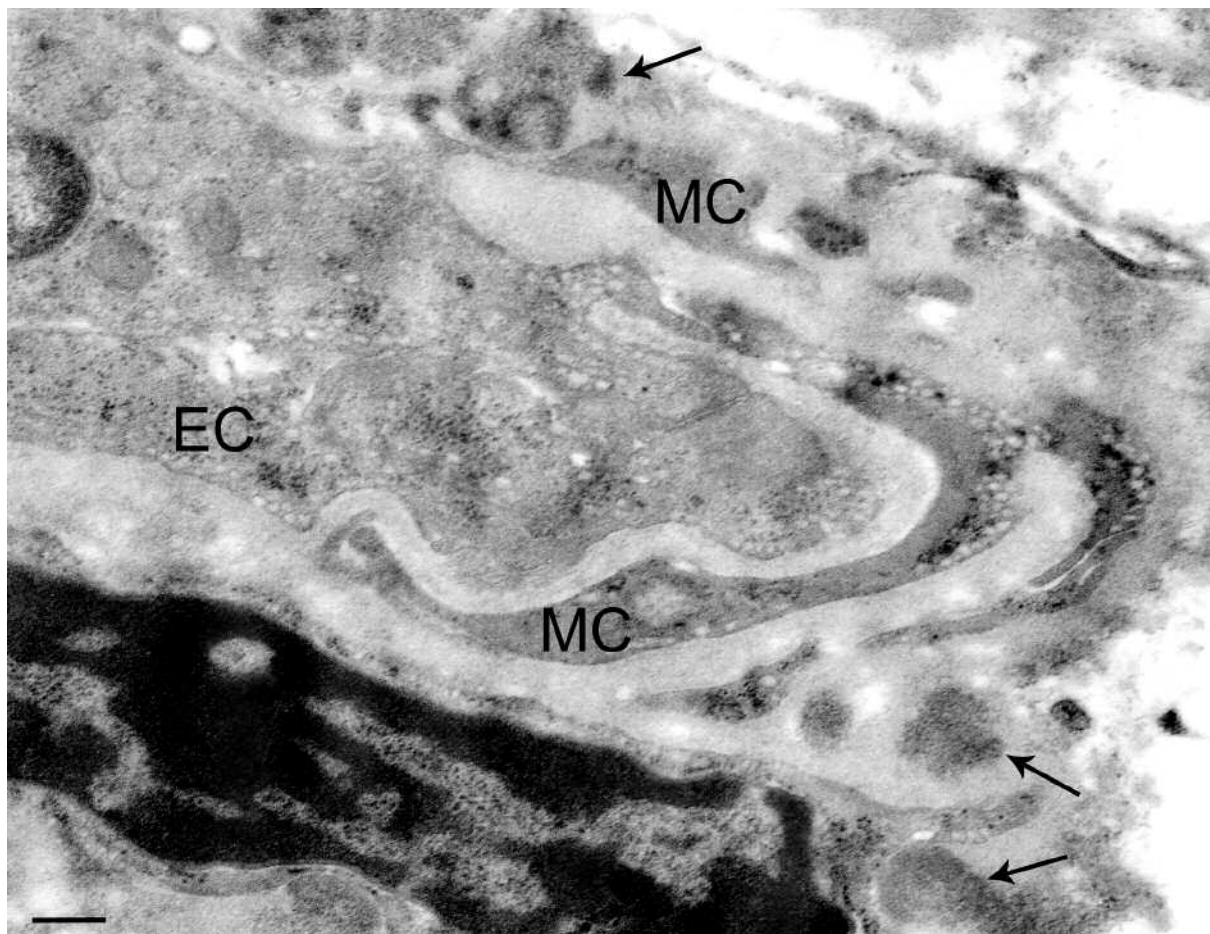


Fig. 11. An artery with GOM deposits (arrows) located in basement membrane around the VSMCs (MC) and in VSMC infolding. Endothelial cell (EC). Skin biopsy. Bar represents 0.6 μ m

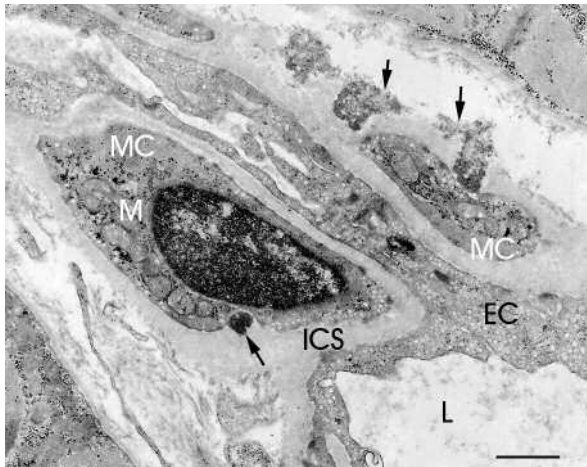


Fig. 12. A small arteriole with numerous GOM deposits (arrows) located around smooth muscle cells (MC). In cytoplasm of VSMCs the conglomeration of mitochondria (M) is seen. Intersmooth muscle space (ICS) is enlarged. The endothelial cells (EC) are thin with numerous pinocytotic vesicles. L-lumen. Muscle biopsy. Bar represents 1 μ m

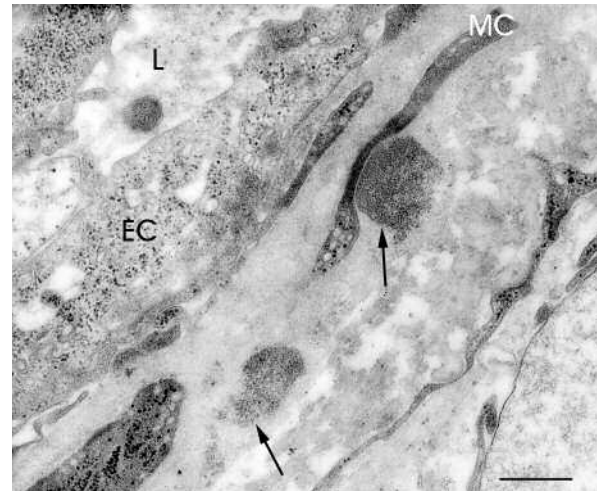


Fig. 13. GOM deposits (arrows) located close to thin and irregular shape smooth muscle cells (MC). L-lumen, EC-endothelial cell. Skin biopsy. Bar represents 0.6 μ m

Discussion

Our patient presented the clinical symptoms and diffuse cerebral white matter lesions in MRI, as well as GOM deposits in blood vessels, that are features

essentially indicating CADASIL. CADASIL appears to be underdiagnosed or misdiagnosed and it should be taken into consideration in patients diagnosed for stroke at young age or suspected vascular dementia. In such cases an MRI examination is indicated [1,5,6]. It detects the changes which are highly suggestive of CADASIL diagnosis even at a very early stage of the disease [17,31].

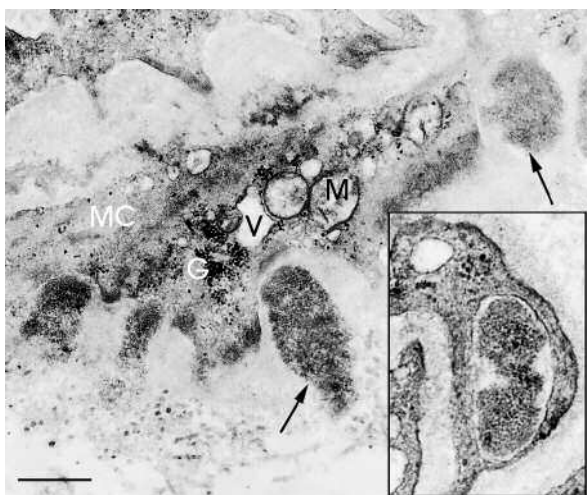


Fig. 14. Vessel wall with GOM deposits (arrows) near and in smooth muscle cell infolding. Cytoplasm of smooth muscle cells (MC) showing swollen mitochondria (M), vacuoles (V) and glycogen (G). Skin biopsy. Insert: GOM deposit in VSMC infolding. Bar represents 0.5 μ m

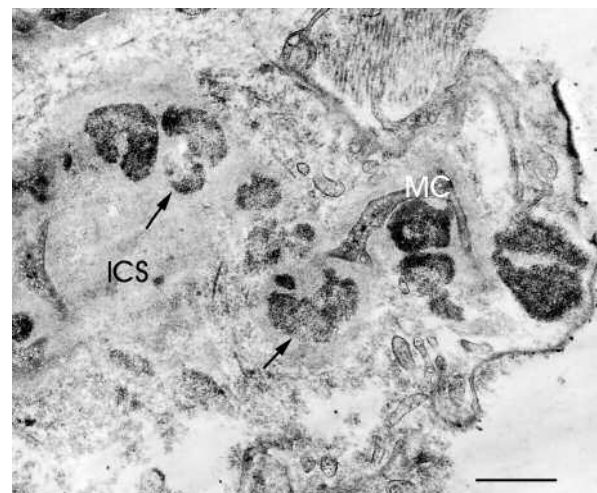


Fig. 15. Numerous GOM deposits (arrows) in enlargement of the intersmooth muscle cell space (ICS). The smooth muscle cells (MC) are irregular in shape and very narrow. Muscle biopsy. Bar represents 1 μ m

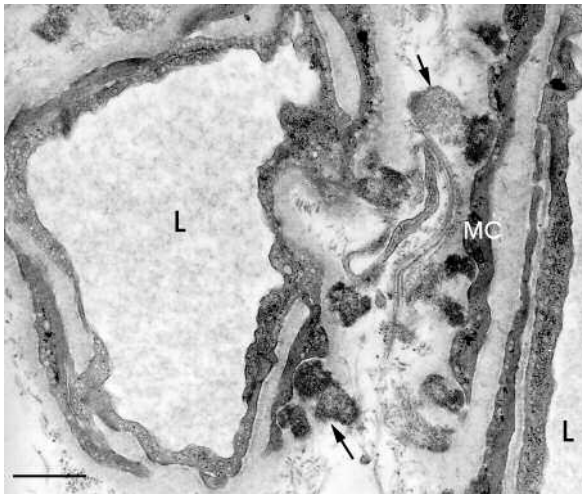


Fig. 16. Narrow smooth muscle cells (MC) with dense cytoplasm. Numerous GOM deposits (arrows) are located near VSMCs. L-lumen. Muscle biopsy. Bar represents 1 μ m

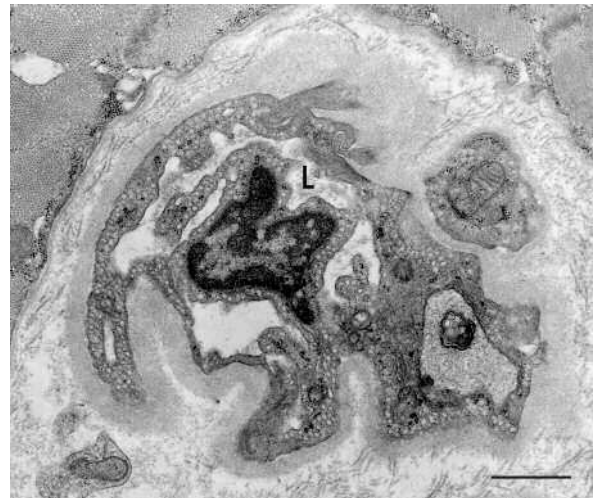


Fig. 17. Degenerating blood vessel not showing GOM in basement membrane. L-lumen. Muscle biopsy. Bar represents 1 μ m

The white matter and deep grey matter are most prone to tissue destruction since these areas are supplied by relatively long penetrating arteries of the end-artery type. Infarcts result from a thickening and fibrosis of the vessel wall of small and medium arteries with consequent obliteration and/or thrombosis [17,24]. The changes seem to correspond with the clinical state and progression of the illness; hence MRI may not show any abnormalities in the prodromal phase [6,17,29].

In the present study the ultrastructural examination revealed both in skin and muscle blood vessels lesions typical for CADASIL. In addition to GOM deposits which varied in size and shape, destruction of the VSMCs as well as the endothelial cells were found. The presence of GOM deposits is accepted as the morphological signal confirming the diagnosis of CADASIL disease [2,3,8,20]. However, the occurrence of GOM may be focal, requiring a thorough evaluation of the entire biopsy specimen [29]. Vessels having GOM deposits revealed irregular contour of vascular lumen, thickened basement membrane, degenerated VSMCs and endothelial cells. On the other hand, lesions of VSMCs and/or endothelial cells were also visible on the section vessels devoid of GOM. Degeneration and loss of VSMCs make them wide packed, finally leading to an abnormal enlargement of the intersmooth muscle space and of the subendothelial space. Molecular pathways linking degeneration of VSMCs to Notch3

gene mutations are as yet poorly understood [16]. However, according to some authors the destruction of vascular muscle cells is associated with presence of GOM. It is commonly accepted that GOM deposits are located within the basement membrane nearby VSMCs, often within an infolding of the cell membrane [9,10,20,21,24,26]. The chemical nature, origin and function of GOM deposits are still unknown. Some results from immunohistochemical studies suggest the GOM may come from VSMCs or may be debris of degenerating muscle cells or basal lamina [18,19,29]. It has also been suggested that GOM deposits and/or excess of Notch3 protein may be toxic for VSMCs [30]. However, electron microscopy analysis of vessels in transgenic mice, as animal model of CADASIL, demonstrated that morphological alternations of VSMCs and endothelial cells precede Notch3 gene product accumulation and GOM deposits [27]. These findings indicate that in CADASIL VSMCs are a primary target of pathogenic processes and neither Notch3 accumulation nor GOM deposits are necessary for VSMC damage, but the cascade leading to VSMC degeneration may be initiated by disruption of normal smooth muscle cells anchorage [27]. Our data indicate that there is no apparent correlation between the presence and/or the number of GOM deposits and the severity of VSMC damage. Moreover, we also observed lesions of blood vessels without GOM deposits. It was observed in the arteries of the transgenic mice [27].

On the other hand, it is truly interesting that in our material GOM deposits were also found within skin and muscle capillary wall, near endothelial cells and pericytes, although capillaries do not have muscle cells in the wall. GOM deposits were also observed in brain capillaries [22,23] as well as skin and muscle capillaries [20]. The ultrastructural picture of some endothelial cells in arteries and capillaries suggests their degenerative changes. It is worth noticing that lesions of endothelial cells were also found in vessels devoid of GOM. Often, endothelial cells were significantly thinned and irregularly shaped with numerous protrusions bulging into the lumen of the vessels. Their nuclei were filled with dense chromatin. In the published literature, endothelial cells have been described only rarely in CADASIL patients [25,28]. It was suggested that a striking increase in bundles of filaments might possibly block and then impede the normal permeability of endothelial cells and transendothelial exchange could play an essential role in the pathogenesis of CADASIL. Impaired permeability in endothelial cells may play a role in the destruction of VSMCs [25]. Although in CADASIL all systemic vessels show deposition of GOM, the disorder affects predominantly arteries in the brain, suggesting that topographic differences in the vasculature as well as the blood-brain barrier may play a role in the pathogenesis of the disorder [12].

In our patient, along with electron microscopy, genetic tests detected the mutation in exon 4 of the Notch3 gene, confirming the diagnosis of CADASIL. It has been well known for several years that CADASIL arteriopathy is due to mutations of the Notch3 gene, located on chromosome 19; still the important question about the relation between formation of GOM deposits and/or Notch3 accumulation and VSMCs destruction remains obscure. In normal vessels Notch3 gene expression is restricted to VSMCs, and probably in patients with mutation of the Notch3 gene abnormal accumulation of protein at the VSMCs membrane is located in the vicinity of the GOM [15,30].

It should be pointed out that no correlations between the presence or number and size of GOM and severity of damage of VSMCs were found, and GOM deposits were also located in the basement membrane of capillaries. The increasing number of reports where GOM are present within the capillaries deprived of smooth muscle cells implies expanding

the approach to other elements of the vascular wall. Because the mechanisms and sequences of pathological changes in blood vessels wall and particularly the mechanisms of VSMCs degeneration remain unclear, future investigation of the biopsies taken from patients clinically suspected of having CADASIL are necessary. The pathological studies in human patients were mostly focused on the end-stage of the disease, when the CADASIL phenotype became apparent; therefore we believe that analysis of the biopsy taken from the patient at different age and disease stages may lead to recognition of mechanisms and the sequence of changes in the blood vessel wall, which may indicate both faster means of diagnosis and approach to more effective therapy of CADASIL disease.

References

1. Auer DP, Putz B, Gossel C, Elbel G, Gasser T, Dichgans M. Differential lesion patterns in CADASIL and sporadic subcortical arteriosclerotic encephalopathy: MR imaging study with statistical parametric group comparison. *Radiology* 2001; 218: 443–451.
2. Baudrimont M, Dubas F, Joutel A, Tournier-Lasserre E, Bousser MG. Autosomal dominant leukoencephalopathy and subcortical ischemic stroke. A clinicopathological study. *Stroke* 1993; 24: 122–125.
3. Brulin P, Godfraind C, Leteurtre E, Rouchoux MM. Morphometric analysis of ultrastructural vascular changes in CADASIL: analysis of 50 skin biopsy specimens and pathogenic implications. *Acta Neuropathol* 2002; 104: 241–248.
4. Brulin-Fardoux P, Godfraind C, Maurage CA, De Reuck J, Hauw JJ, Kaltner H, Bovin NV, Gabius HJ, Rouchoux MM, Kiss R, Camby I. Glycohistochemical characterization of vascular muscle cell destruction in CADASIL subjects by lectins, neoglycoconjugates and galectin-specific antibodies. *Neuropathol Appl Neurobiol* 2003; 29: 400–410.
5. Chabriat H, Tournier-Lasserre E, Vahedi K, Leys D, Joutel A, Nibbio A, Escaillas JP, Iba-Zizen MT, Bracad S, Tehindranarivelo A, et al. Autosomal dominant migraine with MRI white-matter abnormalities mapping to the CADASIL locus. *Neurology* 1995; 45: 1086–1091.
6. Chabriat H, Levy C, Taillia H, Iba-Zizen MT, Vahedi K, Joutel A, Tournier-Lasserre E, Bousser MG. Patterns of MRI lesions in CADASIL. *Neurology* 1998; 51: 452–457.
7. Dichgans M, Mayer M, Uttner I, Bruning R, Muller-Hocker J, Rungger G, Ebke M, Klockgether T, Gasser T. The phenotypic spectrum of CADASIL: clinical findings in 102 cases. *Ann Neurol* 1998; 44: 731–739.
8. Ebke M, Dichgans M, Bergmann M, Voelter HU, Rieger P, Gasser T, Schwendemann G. CADASIL: skin biopsy allows diagnosis in early stages. *Acta Neurol Scand* 1997; 95: 351–357.
9. Furby A, Vahedi K, Force M, Larrouy S, Rouchoux MM, Joutel A, Tournier-Lasserre E. Differential diagnosis of vascular leukoencephalopathy within a CADASIL family: use of skin

- biopsy electron microscopy study and direct genotypic screening. *J Neurol* 1998; 245: 734–740.
10. Goebel HH, Meyermann R, Rosin R, Schlote W. Characteristic morphologic manifestation of CADASIL, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy, in skeletal muscle and skin. *Muscle Nerve* 1997; 20: 625–627.
 11. Guidetti D, Casali B, Mazzei RL, Cenacchi G, De Berti G, Zuccoli G, Nicoli D, Conforti FL, Sprovieri T, Pasquinelli G, Brini M. An Italian case of CADASIL with mutation CGC-TCG in codon 1006, exon 19 Notch3 gene. *Neurol Sci* 2004; 24: 401–406.
 12. Hagel C, Groden C, Niemeier R, Stavrou D, Colmant HJ. Subcortical angiopathic encephalopathy in a German kindred suggests an autosomal dominant disorder distinct from CADASIL. *Acta Neuropathol* 2004; 108: 231–240.
 13. Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cecilion M, Marechal E, Maciazek J, Vayssiere C, Cruaud C, Cabanis EA, Ruchoux MM, Weissenbach J, Bach JF, Bousser MG, Tournier-Lasserre E. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature* 1996; 383: 707–710.
 14. Joutel A, Vahedi K, Corpechot C, Troesch A, Chabriat H, Vayssiere C, Cruaud C, Maciazek J, Weissenbach J, Bousser MG, Bach JF, Tournier-Lasserre E. Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. *Lancet*; 1997; 350: 1511–1515.
 15. Joutel A, Andreux F, Gaulis S, Domenga V, Cecillon M, Battail N, Piga N, Chapon F, Godfrain C, Tournier-Lasserre E. The ectodomain of Notch3 receptor accumulates within the cerebrovasculature of CADASIL patients. *J Clin Invest* 2000; 105: 597–605.
 16. Joutel A, Monet M, Domenga V, Riant F, Tournier-Lasserre E. Pathogenic mutations associated with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy differently affect Jagged1 binding and Notch3 activity via the RBP/JK signaling pathway. *Am J Hum Genet* 2004; 74: 338–347.
 17. Kalimo H, Viitanen M, Amberla K, Juvonen V, Marttila R, Poyhonen M, Rinne JO, Savontaus M, Tuisku S, Winblad B. CADASIL: hereditary disease of arteries causing brain infarcts and dementia. *Neuropathol Appl Neurobiol* 1999; 25: 257–265.
 18. Kaniakakis J, Thobois S, Claudy A, Broussolle E. CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy): a neurovascular disease diagnosed by ultrastructural examination of the skin. *J Cutan Pathol* 2002; 29: 498–501.
 19. LaPoint SF, Patel U, Rubio A. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *Adv Anat Pathol* 2000; 7: 307–321
 20. Mayer M, Straube A, Bruening R, Uttner I, Pongratz D, Gasser T, Dichgans M, Multer-Hocker J. Muscle and skin biopsies are a sensitive diagnostic tool in the diagnosis of CADASIL. *J Neurol* 1999; 246: 526–532.
 21. Mellies JK, Baumer T, Muller JA, Tournier-Lasserre E, Chabriat H, Knobloch O, Hackeloer HJ, Goebel HH, Wetzig L, Haller P. SPECT study of a German CADASIL family: a phenotype with migraine and progressive dementia only. *Neurology* 1998; 50: 1715–1721.
 22. Rafalowska J, Dziewulska D, Fidzianska A. CADASIL: what component of the vessel wall is really a target for Notch 3 gene mutations? *Neurol Res* 2004; 26: 558–562.
 23. Rafalowska J, Fidzianska A, Dziewulska D, Podlecka A, Szpak GM, Kwiecinski H. CADASIL or CADVaSIL? *Neuropathology* 2004; 24: 16–20.
 24. Ruchoux MM, Guerouaou D, Vandenhoute B, Pruvo JP, Vermersch P, Leys D. Systemic vascular smooth muscle cell impairment in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Acta Neuropathol* 1995; 89: 500–512.
 25. Ruchoux MM, Muraige CA. Endothelial changes in muscle and skin biopsies in patients with CADASIL. *Neuropathol Appl Neurobiol* 1998; 24: 60–65.
 26. Ruchoux MM, Brulin P, Leteurtre E, Muraige CA. Skin biopsy value and leukoencephalopathy. *Ann NY Acad Sci* 2000; 903: 285–292.
 27. Ruchoux MM, Domenga V, Brulin P, Maciazek J, Limol S, Tournier-Lasserre E, Joutel A. Transgenic mice expressing mutant Notch3 develop vascular alterations characteristic of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Am J Pathol* 2003; 162: 329–342.
 28. Schroder JM, Selhaus B, Jorg J. Identification of the characteristic vascular changes in a sural nerve biopsy of a case with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *Acta Neuropathol* 1995; 89: 116–121.
 29. Schultz A, Santoanni R, Hewan-Lowe K. Vasculopathic changes of CADASIL can be focal in skin biopsies. *Ultrastruct Pathol* 1999; 23: 241–247.
 30. Spinner NB. Notch signaling defect or protein accumulation problem? *J Clin Invest* 2000; 105: 597–605.
 31. O'Sullivan M, Jarosz JM, Martin RJ, Deasy N, Powell JF, Markus HS. MRI hyperintensities of the temporal lobe and external capsule in patients with CADASIL. *Neurology* 2001; 56: 628–634.
 32. Tournier-Lasserre E, Joutel A, Melki J, Weissenbach J, Lathrop GM, Chabriat H, Mas JL, Cabanis EA, Baudrimont M, Maciazek J. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy maps to chromosome 19q12. *Nat Genet* 1993; 3: 256–259.