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CADASIL patient with extracellular calcium deposits

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Abstract

We report the case of a 57-year-old male patient with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) diagnosed on the basis of ultrastructural and genetic examinations. Ultrastructurally, granular osmiophilic material (GOM) deposits, degeneration and loss of vascular smooth muscle cells (VSMC) and pericytes in small arterial and capillary vessels from skin-muscle biopsy typical of CADASIL were visible. Degeneration of pericytes and endothelial cells were often pronounced, which resulted in a complete disappearance of mural cells and extremely severe thickening of the basement membrane. Degenerative changes in blood vessels, especially evident in skeletal muscle arterioles, also included significant vacuolization of VSMC, misshapen nuclei both in vessel wall cells and skeletal muscle fibres, and deposits of a hyaline material and calcium in the vessel wall. Abundant calcium deposits were located in the vascular basement membrane and exhibited laminar morphology with abnormally arranged light and dark bands. In the basement membrane of the most severely affected microvessels, only clusters of calcium deposits and remnants of the mural cells were observed. Laminar calcifications were also observed within the basement membrane surrounding skeletal muscle fibres. Such abundant calcium deposits in CADASIL have not as yet been described. Morphological findings, described in this report, expand the spectrum of histopathological changes in this genetically determined angiopathy.

Key words: CADASIL, small blood vessels, calcification, skeletal muscle fibres.

Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited systemic non-amyloid, non-atherosclerotic arteriopathy that affects – contrary to its name – not only the brain but also other organs and structures. Its generalized nature enables the diagnosis through the ultrastructural examination of the skin or skeletal muscle vessels. At the ultrastructural level,

characteristic of CADASIL, the observed changes comprise degeneration and loss of vascular smooth muscle cells (VSMC) and pericytes as well as the accumulation of granular osmiophilic material (GOM), typical of this disease with different morphology in vessel walls [8,18,20,26].

CADASIL is caused by mutations in the *NOTCH3* gene encoding transmembrane receptor Notch3 [15]. Notch signalling plays an important role in the vas-

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cular development and human vascular disease [23]. Notch3 is one of four members of the Notch family receptors (Notch1-4) [16] found in human VSMC and pericytes [14,15], whereas Notch1 and Notch4 are also present in endothelium [28].

Notch3 is involved in an evolutionarily conserved signaling pathway essential for the cardiovascular development. Alternations in Notch signaling lead not only to abnormal vascular development but also to disturbed homeostasis of the cardiovascular system [5,13]. Experimental studies with Notch mutants display either vascular abnormalities or lethal cardiovascular defects [5,12,21]. In humans, NOTCH1 mutations lead to Alagille syndrome, in which cardiovascular anomalies are among the most common features [23]. NOTCH1 mutations were also described in aortic valve disease characterized by structural defects and severe calcification of the valve [10,24]. Moreover, mutations in JAGGED1 receptor involved in Notch signaling pathway have been found in patients with isolated congenital heart defects, including pulmonic stenosis or tetralogy of Fallot [1,9,17].

The aim of this paper is to describe atypical pathological changes in vessels from skin and muscle biopsy in the patient with genetically confirmed CADASIL.

Case report

A 57-year-old man was admitted to the Department of Neurology, few weeks after the first episode of ischemic stroke, to diagnose diffused white matter changes detected by magnetic resonance imaging (MRI) scan (Figs. 1 and 2). The patient also suffered from migraine headaches, postinfarction heart failure, severe coronary artery disease, atrial septal aneurysm and abdominal aortic aneurysm. For a few years he has demonstrated mood disturbances (apathy) and behavioural changes (initiative and social withdrawal, difficulties in performing daily activities). The patient's family history revealed epilepsy in his father and daughter, and migraine in his mother.

On neurological examination, pseudobulbar syndrome and right-sided hemiparesis were revealed. Neuropsychological evaluation showed significant deficits in attention, executive functions, and memory.

Diagnostic procedures performed during the hospitalization excluded metachromatic leukodystrophy, GM-1 and GM-2 gangliosidosis, adrenoleukodystrophy, and Krabbe, Fabry and Schindler diseases as the causes of the white matter abnormalities.

Genetic examination performed in the Neuroimmunology Laboratory, 2nd Department of Neurology, Institute of Psychiatry and Neurology, revealed a mutation in the 12 exon of the NOTCH3 gene.

Material and methods

Examination of the tissue samples was performed at the level of light and electron microscopy. Formalin-fixed and paraffin-embedded slides from the skin and skeletal muscle biopsy were stained with HE and Kossa method.
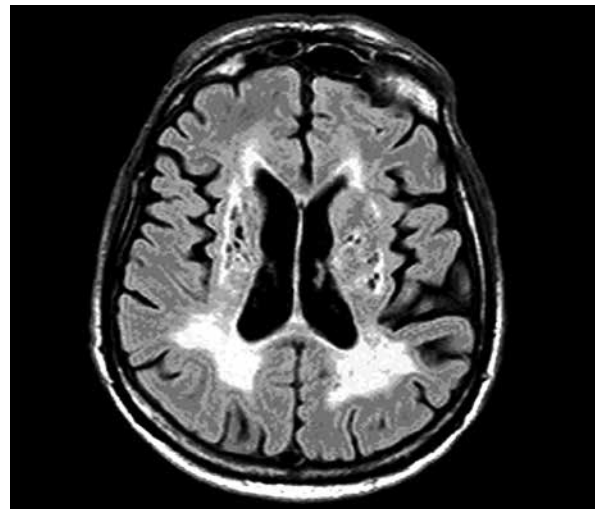

Fig. 1. Brain MRI. FLAIR sequence showing a confluent lesion in periventricular and subcortical white matter and external capsules.

Fig. 2. Brain MRI. FLAIR sequence showing relatively spread anterior temporal lobes.

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At electron microscopy the muscle and skin tissue samples were fixed in 2.5% glutaraldehyde with post-fixation in 2% osmium tetroxide, and routinely processed into epoxy resin. Semi-thin sections stained with toluidine blue were examined in the light microscope to select the areas required. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined in the transmission electron microscope (Opton DPS 109).

Results

Ultrastructural examination of muscle and skin vessels showed GOM deposits, numerous in arterioles but very rare in capillaries. Blood vessels in skeletal muscle were dramatically changed.

Skeletal muscle arterioles

The arterioles revealed degeneration, and loss of VSMC, thickening of the basement membrane. VSMCs were damaged to a varied degree and their ultrastructural abnormalities were highly diverse. In our CADASIL patient, the VSMC were enlarged and ballooned or often thin and irregular in shape (Figs. 3A-B). In VSMC cytoplasm complexes of swollen mitochondria with a reduced amount of cristae (Fig. 3A) were seen, but numerous vacuoles of different
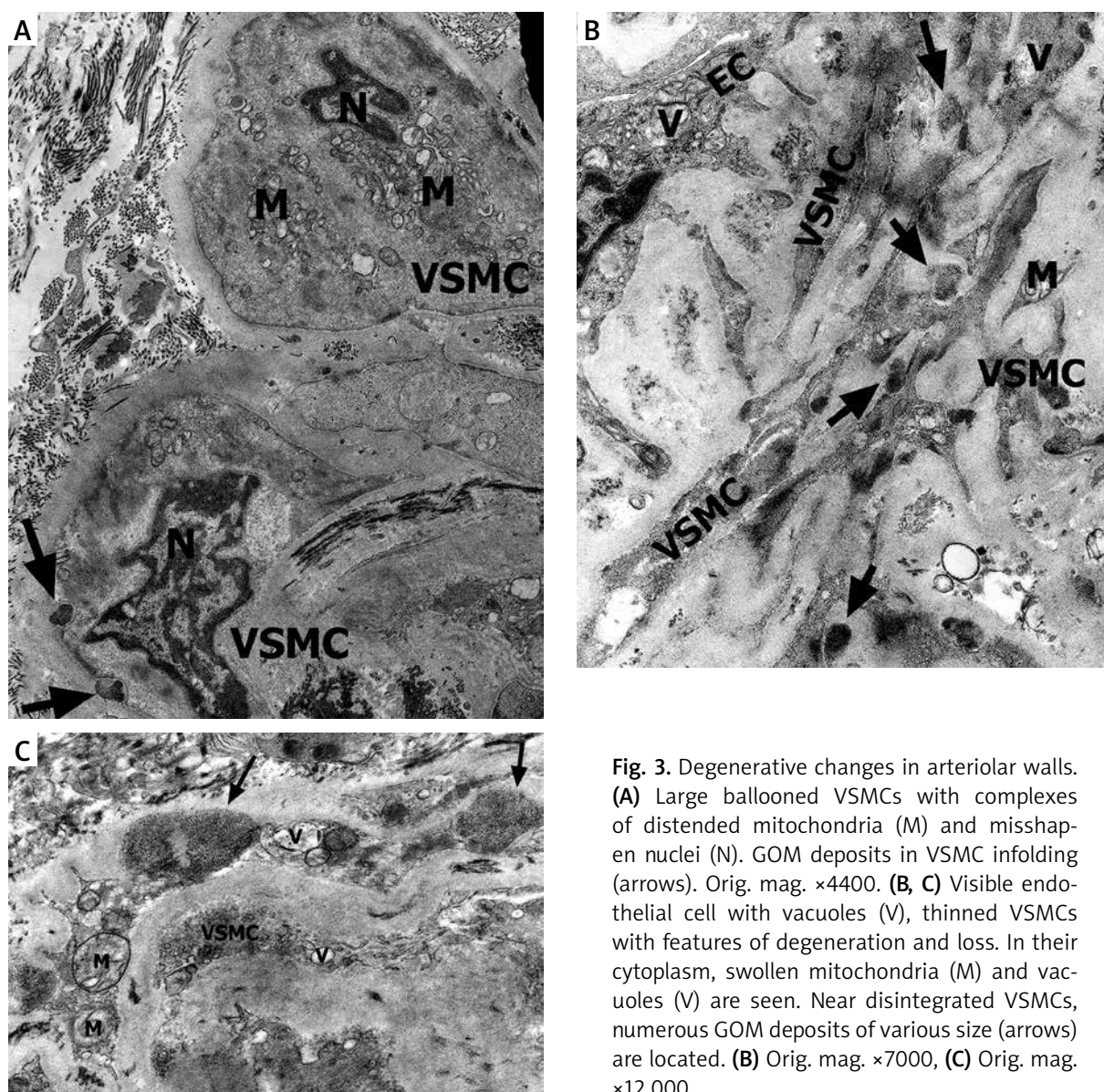

Fig. 3. Degenerative changes in arteriolar walls. **(A)** Large ballooned VSMCs with complexes of distended mitochondria (M) and misshapen nuclei (N). GOM deposits in VSMC infolding (arrows). Orig. mag. ×4400. **(B, C)** Visible endothelial cell with vacuoles (V), thinned VSMCs with features of degeneration and loss. In their cytoplasm, swollen mitochondria (M) and vacuoles (V) are seen. Near disintegrated VSMCs, numerous GOM deposits of various size (arrows) are located. **(B)** Orig. mag. ×7000, **(C)** Orig. mag. ×12 000.

size, including vacuoles containing mitochondria, were the most characteristic finding (Figs. 4A-C). Some giant vacuoles were surrounded by a double membrane similar to the mitochondria (Fig. 4B). Cytoplasm of the thinned and irregularly shaped VSMC showed few organelles, such as mitochondria (Fig. 3B). Nearby VSMC membranes or in their infoldings numerous deposits of GOM were visible (Figs. 4B-C). The endothelial cells (EC) also revealed pathological changes. In their cytoplasm, narrow lumen and numerous vacuoles were seen (Fig. 5).

Sometimes in arteriolar wall a focal deposition of hyaline material and non specific-granular debris were observed (Fig. 5). Interestingly, shrunken and irregularly shaped nuclei were present in all mural cells (Figs. 4A and 5).

Light microscopic examination revealed granular deposits in the wall of vessels (Fig. 6A). At the ultrastructural level, laminarly arranged calcium deposits were seen in prominently thickened basement membrane (Figs. 6B-D). They were found to be single (Fig. 6B) or in clusters near mural cells (Figs. 6C-D).
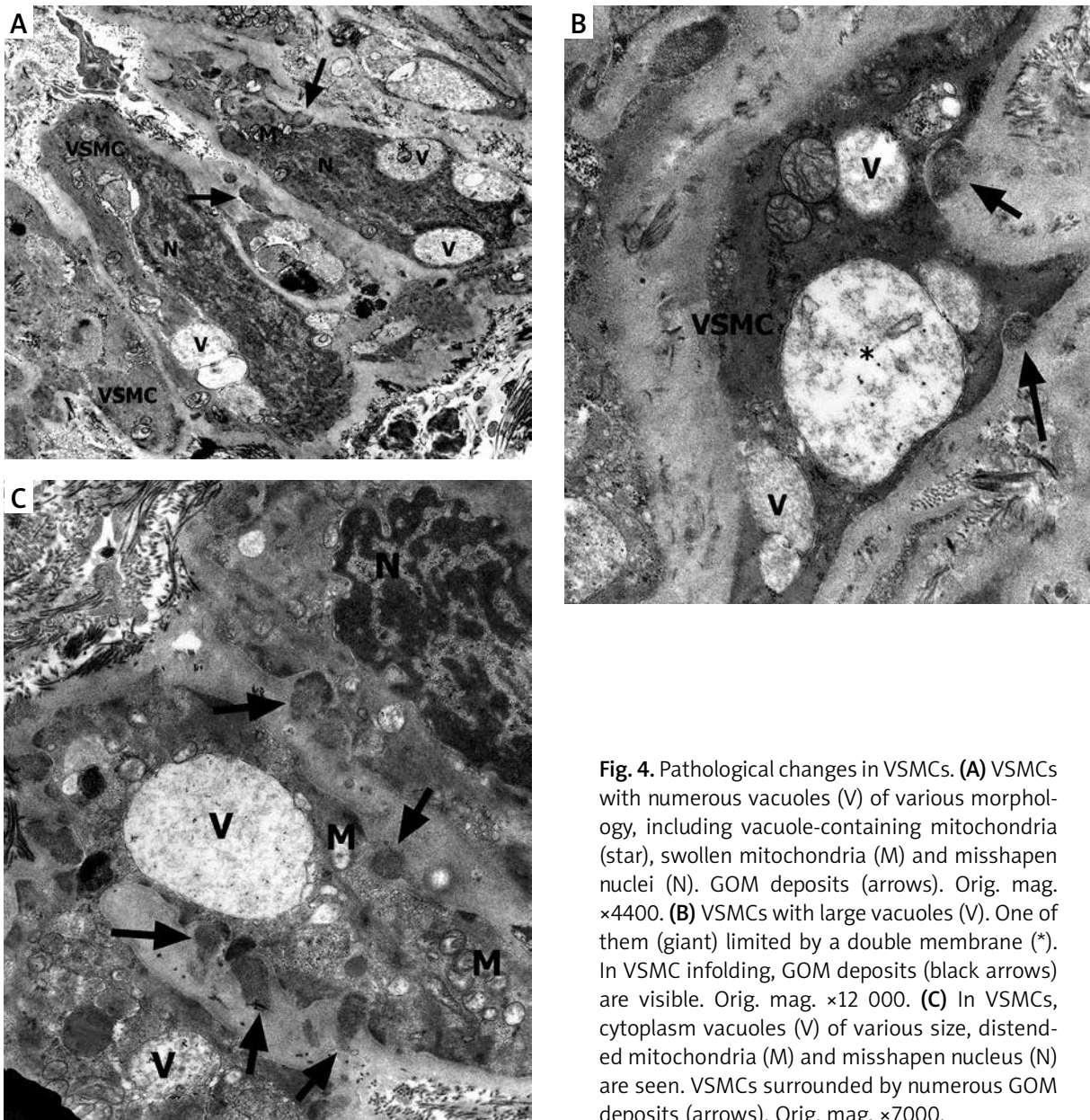

Fig. 4. Pathological changes in VSMCs. **(A)** VSMCs with numerous vacuoles (V) of various morphology, including vacuole-containing mitochondria (star), swollen mitochondria (M) and misshapen nuclei (N). GOM deposits (arrows). Orig. mag. ×4400. **(B)** VSMCs with large vacuoles (V). One of them (giant) limited by a double membrane (*). In VSMC infolding, GOM deposits (black arrows) are visible. Orig. mag. ×12 000. **(C)** In VSMCs, cytoplasm vacuoles (V) of various size, distended mitochondria (M) and misshapen nucleus (N) are seen. VSMCs surrounded by numerous GOM deposits (arrows). Orig. mag. ×7000.
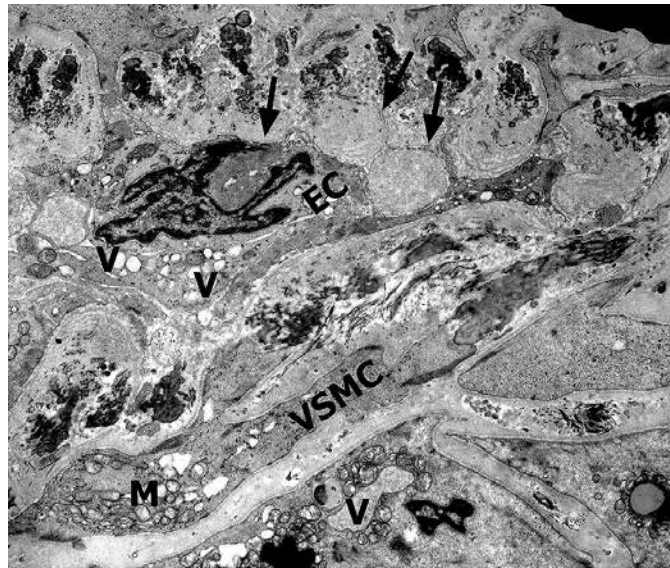
Fig. 5. Arteriolar wall with hyaline material (arrows) located near endothelial cells (EC). In endothelial cells' cytoplasm, numerous vacuoles (V) are visible. VSMCs with swollen mitochondria (M) and vacuoles (V). Orig. mag. ×4400.

Skeletal muscle capillaries

Capillary vessels showed very rare deposits of GOM. Usually they had a narrow lumen, very thick basement membrane and thin or swollen endothelial cells. Some endothelial cells were completely disintegrated. In the cytoplasm of the affected EC, only a few organelles, including vacuoles, pinocytic vesicles and mitochondria were seen (Fig. 7A). The pericytes were usually lost and in the majority of capillaries only residual pericytes were observed (Fig. 7B). In the thick basement membrane, laminar calcifications were visible (Fig. 7B). In extremely damaged vessels, only big conglomerations of basement membrane with laminar deposits of calcium, often located nearby the remains of mural cells, were seen (Fig. 7C).

Skeletal muscles

Besides muscle fibres of normal diameter and architecture, necrotic fibres were present. Staining with Kossa method showed granular material on the periphery of skeletal muscle fibres (Fig. 8A). Ultrastructurally, calcium deposits in the basement membrane, surrounding numerous pathological skeletal muscle fibres, were found (Fig. 8B). Several muscle fibres nuclei showed irregular shape, dense chromatin and were shrunken (nuclei with undulating surface) (Fig. 8C). In muscle fibres with normal structure, large clusters of mitochondria of altered morphology were seen (Fig. 8D).

Discussion

Our ultrastructural investigation of the skin and skeletal muscle biopsy in the patient with CADASIL, confirmed by the electron microscopy and genetic testing, revealed morphological changes of blood vessels typical of this disease. Morphologically, CADASIL is characterized by the accumulation of granular osmiophilic material, VSMC and pericyte degeneration and loss as well as thickening of the basement membrane [8,19,20,26]. Besides typical abnormalities we found calcium deposits in the thickened basement membrane in both vessels and muscle fibers. Compared with our earlier biopsies, this patient showed particularly severe damage to arterioles and capillaries. Degenerative changes of VSMCs were dramatic. Numerous VSMCs were narrow and irregular in shape and their nuclei were misshapen like in other cells including muscle fibres.

The presence of numerous vacuoles of different sizes, some of them very large, and clusters of swollen mitochondria is particularly worth emphasising. Some of vacuoles were limited by double membrane, which may suggest their origin from mitochondria. Sometimes vacuoles contained mitochondria presumably indicating their autophagic functions.

We also observed significant narrowing of the vessel lumen, damage to the vascular wall and prominent thickening of the basement membrane, probably due to deposition of hyaline material.
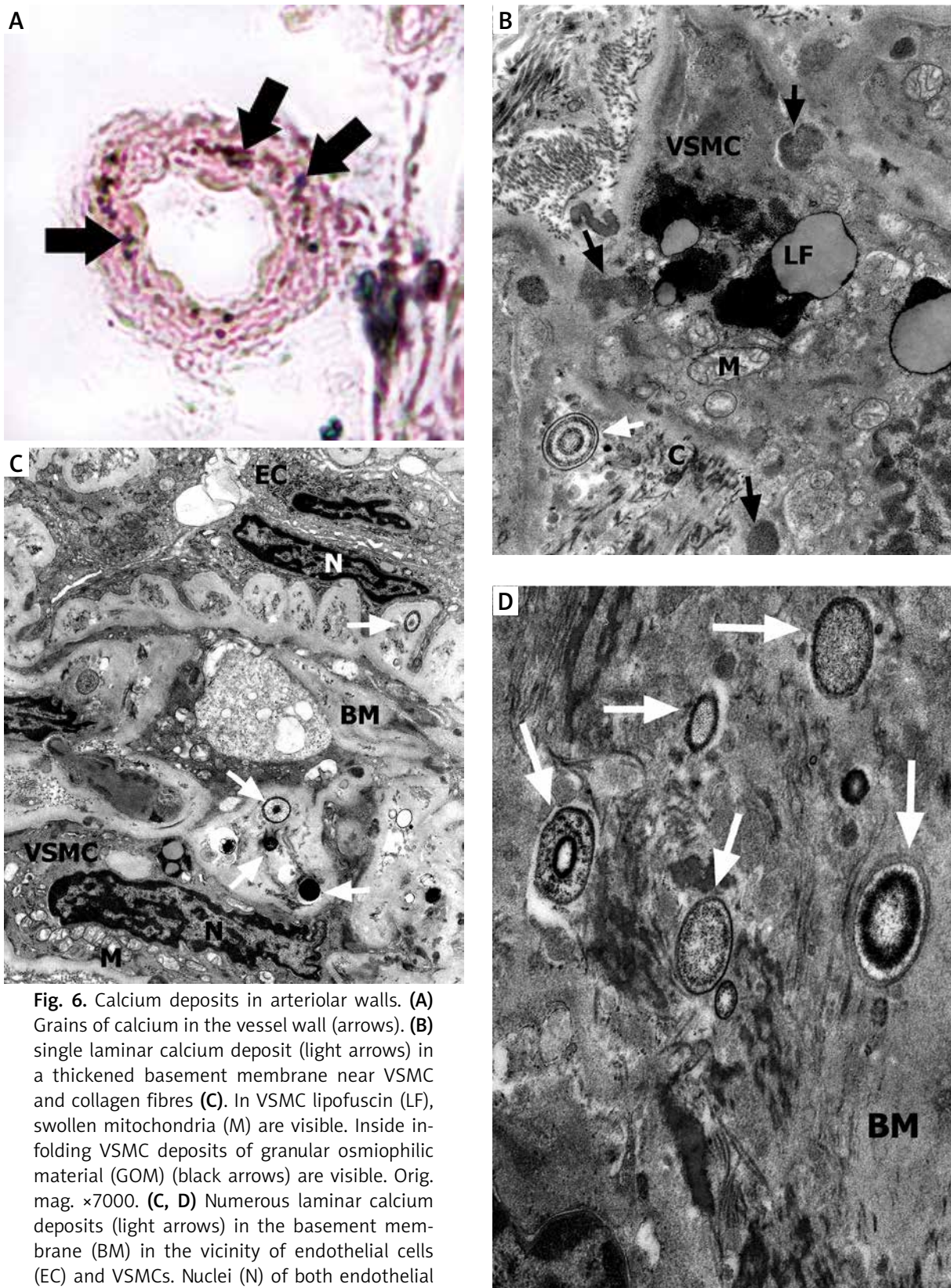
Fig. 6. Calcium deposits in arteriolar walls. **(A)** Grains of calcium in the vessel wall (arrows). **(B)** single laminar calcium deposit (light arrows) in a thickened basement membrane near VSMC and collagen fibres **(C)**. In VSMC lipofuscin (LF), swollen mitochondria (M) are visible. Inside infolding VSMC deposits of granular osmiophilic material (GOM) (black arrows) are visible. Orig. mag. ×7000. **(C, D)** Numerous laminar calcium deposits (light arrows) in the basement membrane (BM) in the vicinity of endothelial cells (EC) and VSMCs. Nuclei (N) of both endothelial cells and VSMCs are misshapen. **(C)** Orig. mag. ×4400, **(D)** Orig. mag. ×12 000.

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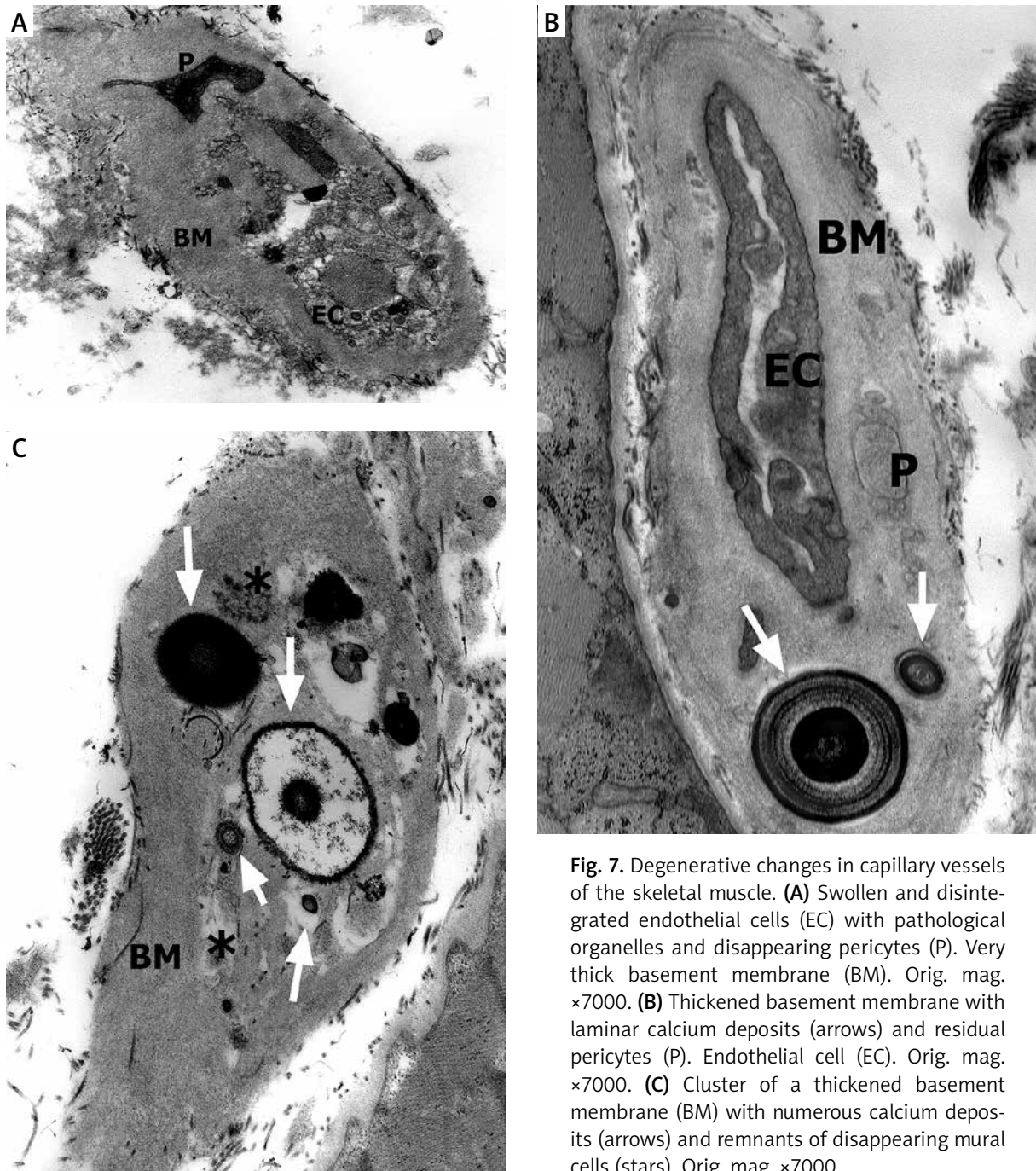

Fig. 7. Degenerative changes in capillary vessels of the skeletal muscle. **(A)** Swollen and disintegrated endothelial cells (EC) with pathological organelles and disappearing pericytes (P). Very thick basement membrane (BM). Orig. mag. ×7000. **(B)** Thickened basement membrane with laminar calcium deposits (arrows) and residual pericytes (P). Endothelial cell (EC). Orig. mag. ×7000. **(C)** Cluster of a thickened basement membrane (BM) with numerous calcium deposits (arrows) and remnants of disappearing mural cells (stars). Orig. mag. ×7000.

In many arterioles there was a myriad of GOM deposits in both indentations of VSMC cytoplasmic membranes or free within the thickened basement membrane as described previously [2,7,19,25]. Interestingly, GOM deposits were much less numerous in capillaries compared to our other patients, although the basement membrane was very thickened and degeneration and loss of pericytes was still visible.

Apart from morphological changes in the skin and skeletal muscle vessels typical of this disease, our CADASIL patient also showed unusual pathological changes. They included accumulation of hyaline material, large amounts of collagen fibres and calcium deposits in the thickened basement membrane. The presence of extracellular calcium deposits of different size and morphology in both vascular
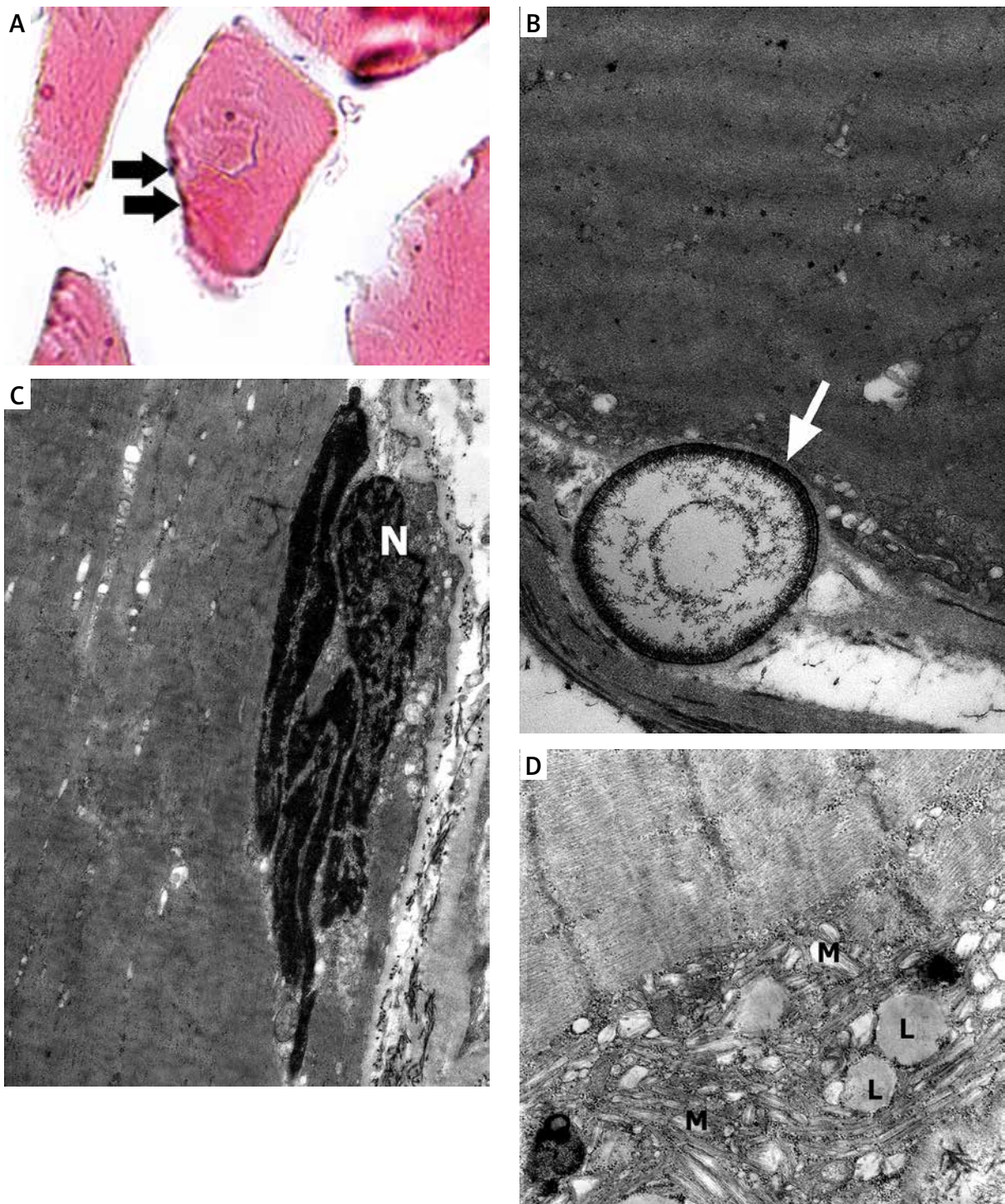
Fig. 8. Pathological changes in skeletal muscle fibres. **(A)** Grain of calcium under sarcolemma (arrows). **(B)** Calcium deposit (arrow) in the basement membrane surrounding skeletal muscle fibres. Orig. mag. ×7000. **(C)** Misshapen nucleus (N) in the skeletal muscle fibre. Orig. mag. ×4400. **(D)** Muscle fibres with a large cluster of pathological mitochondria (M) with changed morphology. Orig. mag. ×12 000.

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basement membrane and muscle fibers was very surprising. To our best knowledge, this is the first description of such deposits in CADASIL. The presence of small vessels, probably capillaries, almost completely devoid of mural cells was also a very interesting finding. The affected capillaries revealed only the conglomeration of basement membrane with calcium deposits and sometimes remnants of mural cells, which caused the dysfunction of these blood vessels.

Calcification of blood vessels is observed in many diseases, including artheriosclerosis, diabetes and chronic renal failure and may involve large as well as small vessels [3]. In our patient, the two latter diseases were excluded but the presence of mutation in 12 exon of the NOTCH3 gene could be an additional factor that increased vessel mineralization. Although there is no evidence that Notch3 mutations can be a causative factor contributing to vessel calcification, it is well known that Notch signaling pathway is involved in this process.

Many studies have revealed that vessel cells, including endothelial cells, vascular smooth muscle cells, myofibroblasts, and pericytes can undergo osteogenic differentiation. It is also known that molecular events associated with calcification of the vasculature resemble endochondral bone ossification [references in: 11]. Although the signals, which initiate vessel calcification are not well understood, Notch signaling pathway is involved in this process and enhances osteogenic conversion and mineralization of vessel wall cells, including VSMCs. VSMCs are not terminally differentiated and can undergo phenotypic changes in response to various physiological and pathological stimuli. Notch signaling makes the media muscle cells responsive to BMP2 (bone morphogenic protein 2), the protein essential for osteogenic differentiation [29] and induces osteogenic differentiation of VSMC in a manner dependent on osteogenic transcription factors, such as Msx2 [30] and Runx2 [31]. Therefore, it is possible that the dysfunction of the Notch3 system in CADASIL may increase VSMC calcification in the same manner as it is observed in aortic valve defects linked with NOTCH1 mutations [10].

Interestingly, the calcium deposition was not limited only to vessel walls but it also included the basement membrane surrounding muscle fibres. Usually the architecture of the fibres were altered, including myofibrillar and nuclear abnormalities and

accumulation of mitochondria with changed shape and morphology.

Earlier data from skeletal muscle biopsies of CADASIL patient revealed ultrastructural and biochemical abnormalities of mitochondria [4,6,22,27]. Moreover, it was assumed that mutations in the Notch3 gene may be related to mitochondrial DNA mutations [4] but this hypothesis needs further investigation. In our patient, mitochondrial pathology was found in both skeletal muscle and VSMCs although their nature differed, depending on the cell type. The increase in the number of mitochondria and their abnormal morphology in CADASIL VSMC might have an effect on vital cellular functions important for CADASIL pathology [33] and the NOTCH3 gene may influence mitochondrial metabolism [22].

Similarly to nuclei of vessel wall cells, the nuclei of skeletal muscle fibres were condensed, shrunken and irregular in their shape. However, ultrastructurally the picture of chromatin did not correspond with apoptotic changes. It is surprising that in various cell types nuclei changes are of the same character.

Conclusions

Skin and skeletal muscle biopsy specimens derived from the CADASIL patient showed severe damage to blood vessels, including a complete destruction of mural cells, accumulation of calcium deposits in very thickened basement membrane and abnormalities in skeletal muscle fibres.

The presence of small blood vessels almost completely devoid of mural cells was an interesting finding. The affected vessels revealed only a large conglomeration of basement membrane with calcium deposits and sometimes remnants of mural cells responsible for dysfunction of blood vessels.

Acknowledgements

References

1. Bauer RC, Laney AO, Smith R, Gerfen J, Morrissette JJ, Woyciechowski S, Garbarini J, Loomes KM, Krantz ID, Urban Z, Gelb BD, Goldmuntz E, Spinner NB. Jagged1 (JAG1) mutations in patients with tetralogy of Fallot or pulmonic stenosis. Hum Mutat 2010; 3: 594-601.
2. Baudrimont M, Dubas F, Joutel A, Tournier-Laserve E, Bousser MG. Autosomal dominant leukoencephalopathy and sub-

cortical ischemic stroke. A clinicopathological study. Stroke 1993; 24: 122-125.

3. Collett GD, Canfield AE. Angiogenesis and pericytes in the initiation of ectopic calcification. Circ Res 2005; 96: 930-938.

4. de la Peña P, Bornstein B, del Hoyo P, Fernández-Moreno MA, Martín MA, Campos Y, Gómez-Escalonilla C, Molina JA, Cabello A, Arenas J, Garesse R. Mitochondrial dysfunction associated with a mutation in the Notch3 gene in a CADASIL family. Neurology 2001; 57: 1235-1238.

5. de la Pompa JL, Epstein JA. Coordinating tissue interactions: Notch signaling in cardiac development and disease. Dev Cell 2012; 22: 244-254.

6. Dotti MT, De Stefano N, Bianchi S, Malandrini A, Battisti C, Cardaioli E, Federico A. A novel NOTCH3 frameshift deletion and mitochondrial abnormalities in a patient with CADASIL. Arch Neurol 2004; 61: 942-945.

7. Dziewulska D. Mysteries of CADASIL-the contribution of neuropathology to understanding of the didease. Folia Neuropathol 2009; 47: 1-10.

8. Dziewulska D, Lewandowska E. Pericytes as a new target for pathological processes in CADASIL. Neuropatholology 2012; 32: 515-521.

9. Eldadah ZA, Hamosh A, Biery NJ, Montgomery RA, Duke M, Elkins R, Dietz HC. Familial tetralogy of Fallot caused by mutation in the jagged1 gene. Hum Mol Genet 2001; 10: 163-169.

10. Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, King IN, Grossfeld PD, Srivastava D. Mutations in NOTCH1 cause aortic valve disease. Nature 2005; 437: 270-274.

11. Golub EE. Biomineralization and matrix vesicles in biology and pathology. Semin Immunopathol 2011; 33: 409-417.

12. High FA, Epstein JA. The multifaceted role of Notch in cardiac development and disease. Nat Rev Genet 2008; 9: 49-61.

13. Hofmann JJ, Iruela-Arispe ML. Notch signaling in blood vessels who is talking to whom about what? Circ Res 2007; 100: 1556-1568.

14. Joutel A, Andreux F, Gaulis S, Domenga V, Cecillon M, Battail N, Piga N, Chapon F, Godfrain C, Tournier-Lasserve E. The ectodomain of the Notch3 receptor accumulates within the cerebrovasculature of CADASIL patients. J Clin Invest 2000; 105: 597-605.

15. Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cécillion M, Marechal E, Maciazek J, Vayssiere C, Cruaud C, Cabanis EA, Ruchoux MM, Weissenbach J, Bach JF, Bousser MG, Tournier-Lasserve E. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature 1996; 383: 707-710.

16. Kalimo H, Miao Q, Tikka S, Mykkanen K, Junna M, Roine S, Viitanen M, Pöyhönen M, Baumann M. CADASIL: the most common hereditary subcortical vascular dementia. Future Neurol 2008; 3: 683-704.

17. Krantz ID, Smith R, Colliton RP, Tinkel H, Zackai EH, Piccoli DA, Goldmuntz E, Spinner NB. Jagged1mutations in patients ascertained with isolated congenital heart defects. Am J Med Genet 1999; 84: 56-60.

18. Lewandowska E, Dziewulska D, Parys M, Pasennik E. Ultrastructure of granular osmiophilic material deposita (GOM) in arterioles of CADASIL patients. Folia Neuropathol 2011; 49: 174-180.

19. Lewandowska E, Leszczyńska A, Wierzba-Bobrowicz T, Skowrońska M, Mierzewska A, Pasennik E, Członkowska A. Ultrastructural

picture of blood vessels in muscle and skin biopsy in CADASIL. Folia Neuropathol 2006; 44: 265-273.

20. Lewandowska E, Szpak GM, Wierzba-Bobrowicz T, Modzelewska J, Stępień T, Pasennik E, Schmidt-Sidor B, Rafałowska J. Capillary vessel wall in CADASIL angiopathy. Folia Neuropathol 2010; 48: 104-115.

21. MacGrogan D, Nus M, de la Pompa JL. Notch signaling in cardiac development and disease. Curr Top Dev Biol 2010; 92: 333-365.

22. Malandrini A, Albani F, Palmeri S, Fattapposta F, Gambelli S, Berti G, Bracco A, Tammaro A, Calzavara S, Villanova M, Ferrari M, Rossi A, Carrera P. Asymptomatic cores and paracrystalline mitochondrial inclusions in CADASIL. Neurology 2002; 59: 617-620.

23. McElhinney DB, Krantz ID, Bason L, Piccoli DA, Emerick KM, Spinner NB, Goldmuntz E. Analysis of cardiovascular phenotype and genotype-phenotype correlation in individuals with a JAG1 mutation and/or Alagille syndrome. Circulation 2002; 106: 2567-2574.

24. Mohamed SA, Aherrahrou Z, Liptau H, Erasmi AW, Hagemann C, Wrobel S, Borzym K, Schunkert H, Sievers HH, Erdmann J. Novel missense mutations (p.T596M and p.P1797H) in NOTCH1 in patients with bicuspid aortic valve. Biochem Biophys Res Commun 2006; 345: 1460-1465.

25. Rafałowska J, Fidziańska A, Dziewulska D, Podlecka A, Szpak GM, Kwieciński H. CADASIL or CADVaSIL? Neuropathology 2004; 24: 16-20.

26. Ruchoux MM, Guerouaou D, Vandenhaute B, Pruvo JP, Vermersch P, Leys D. Systemic vascular smooth muscle cell impairment in cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy. Acta Neuropathol 1995; 89: 500-512.

27. Schröder JM, Züchner S, Dichgans M, Nagy Z, Molnar MJ. Peripheral nerve and skeletal muscle in CADASIL. Acta Neuropathol 2005; 110: 587-599.

28. Shawber CJ, Kitajewski J. Notch function in the vasculature: insights from zebrafish, mouse and man. Bioessays 2004; 26: 225-234.

29. Shimizu T, Tanaka T, Iso T, Matsui H, Ooyama Y, Kawai-Kowase K, Arai M, Kurabayashi M. Notch signaling pathway enhances bone morphogenetic protein 2 (BMP2) responsiveness of Msx2 gene to induce. osteogenic differentiation and mineralization of vascular smooth muscle cells. J Biol Chem 2011; 286: 19138-19148.

30. Shimizu T, Tanaka T, Iso T, Doi H, Sato H, Kawai-Kowase K, Arai M, Kurabayashi M. Notch signaling induces osteogenic differentiation and mineralization of vascular smooth muscle cells role of Msx2 gene induction via Notch-RBP-Jk signaling. Arterioscler Thromb Vasc Biol 2009; 29: 1104-1111.

31. Tanaka T, Sato H, Doi H, Yoshida CA, Shimizu T, Matsui H, Yamazaki M, Akiyama H, Kawai-Kowase K, Iso T, Komori T, Arai M, Kurabayashi M. Runx2 represses myocardin-mediated differentiation and facilitates osteogenic conversion of vascular smooth muscle cells. Mol Cell Biol 2008; 25: 1147-1160.

32. Tang SCH, Jeng JS, Lee MJ, Yip PK. Notch signaliting and CADASIL. Acta Neurol Taiwan 2009; 18: 81-90.

33. Viitanen M, Sundstrom E, Baumann M, Poyhonen M, Tikka S, Behbahani H. Experimental studies of mitochondrial function in CADASIL vascular smooth muscle cells. Exp Cell Res 2013; 3: 134-143.