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Ultrastructure of granular osmiophilic material deposits (GOM) in arterioles of CADASIL patients

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Abstract

Granular osmiophilic material (GOM) is a pathognomonic feature of CADASIL that may be a consequence of pathological processes triggered by Notch3 mutations. Since knowledge of the effects of CADASIL-associated GOM deposits is important to understand the molecular pathogenesis of this disorder, we performed a thorough ultrastructural analysis of GOM morphology in the skin and muscle arterioles in CADASIL patients. Electron microscopy revealed numerous GOM deposits with different morphology including size, shape and osmiophilic density. Osmiophilic granular material of high density was frequently observed in part of GOM deposits located near vascular smooth muscle cells (VSMC) while a part localized distally from the cell body was less dense and loose. On the basis of our observations we postulate that GOM can be formed on the surface of VSMC in the arteriolar wall and penetrate from these cells into the basement membrane and/or extracellular matrix. The dispersion of granules, which form GOM deposits, may be one of the factors triggering the thickening and changes in the basement membrane and/or extracellular matrix.

Key words: *CADASIL, arterioles, GOM deposits, skin and muscle biopsies.*

Introduction

CADASIL is a systemic vascular disease caused by mutations in the NOTCH 3 gene. The gene encodes a large transmembrane receptor protein [9] whose extracellular domain ($N3^{ECD}$) gradually accumulates on the surface of degenerating vascular smooth muscle cells (VSMC) [8]. Apart from $N3^{ECD}$ accumulation, deposits of granular osmiophilic material (GOM) in the vessel wall are another characteristic morphological feature. They have been described only in CADASIL and constitute a pathognomonic feature for the disease.

In CADASIL, ultrastructural investigations revealed GOM deposits not only in cerebral arteries and veins, but also in cerebral capillaries and vessels of other internal organs [13,15]. They were located within the basement membrane near VSMC and pericytes, often in cell membrane infoldings [12,13]. Their origin, chemical nature and function are mysterious. It has only been identified that GOM deposits do not

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contain amyloid, elastin, chromatin, calcium or iron [11]. Also, the relationship between GOM deposits and Notch3 remains unknown. In the opinion of some authors, $N3^{ECD}$ is accumulated in close proximity to GOM deposits [2,8] while others claim it consists a component of GOM lodgements [7]. Likewise, it is still uncertain how GOM deposits cause changes in the vessel wall and what is the relationship between them and damage to VSMC. It is only known that there is no apparent correlation between the presence and number of GOM deposits and severity of VSMC damage [12,16].

An analysis of vessels from transgenic mice expressing mutant Notch3 showed that VSMC damage preceded Notch3 and GOM accumulation [16]. Also in CADASIL patients dysfunction of VSMC is followed by morphological changes in the vessel wall. These observations suggest that GOM deposits may be a consequence of pathological processes triggered by NOTCH3 mutations. Since knowledge of the effects of CADASIL-associated GOM deposits is important to understand the molecular pathogenesis of this disorder, we performed a thorough ultrastructural analysis of GOM morphology in the skin and muscle vessels in CADASIL patients.

Material and methods

Using electron microscopy, we assessed changes in small blood vessels in tissues from the skin and muscle biopsies of eight CADASIL patients, aged 39-57 years. According to the CADASIL diagnostic criteria [4], the disease was diagnosed on the basis of the results of the ultrastructural vessel examination. Genetic examination was performed in only 2 patients and confirmed by mutations in the NOTCH3 gene.

Tissue samples were fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide, dehydrated in ascending grades of ethanol and embedded in Spurr. Semithin sections were stained with toluidine blue for selecting blood vessels. Ultrathin sections double-stained with uranyl acetate and lead citrate were examined in a transmission electron microscope (Opton DPS 109).

Results

In all the examined cases numerous deposits of GOM located near VSMC or in their membrane infoldings were found (Fig. 1). GOM deposits were also observed within the thickened basement membrane at some distance from the degenerated cells (Fig. 2). GOM deposits with electron density resembling the density of the basement membrane were seen (Fig. 3). Sometimes, they were seen near the external edge of the arterial media (Fig. 4). A detailed ultrastructural examination of the vessel wall revealed different morphology of GOM deposits. They exhibited different size, shape and osmiophilic density. Some of them were round, small and very dense, but others revealed irregular shape and diverse density of osmiophilic granules (Figs. 2, 4). In the latter, light areas surrounded by numerous dark granules were observed (Fig. 4). In some GOM deposits exhibiting bizarre shapes, various electron density of the accumulated granular osmiophilic material was seen (Figs. 5A,B). The osmiophilic material of high density was usually observed in GOM deposits located near the VSMC body or within cell membrane infoldings (Figs. 1, 5B). Some GOM deposits situated within the basement membrane and more distant from the cells showed low electron density, and osmiophilic granules more dispersed and sometimes merging with collagen fibres (Figs. 4, 5A,B).

The presence of GOM deposits of mixed morphology was the most interesting finding. The deposits were composed of two parts. One, located in close contact with the VSMC body or inside cell membrane infoldings, revealed very electron-dense and condensed aggregates of osmiophilic granules. The second, localized far from the cell body, was less dense and loose (Figs. 4, 5B). In GOM deposits of mixed morphology the density of granules gradually decreased with increasing distance from the cell body.

Discussion

In all the examined cases we found many blood vessels with numerous extracellular deposits of GOM exhibiting various size, shape and morphology, as well as different localization in vessel walls. This heterogeneous ultrastructural picture of GOM deposits was the most prominent and interesting finding of our investigation.

The key question in CADASIL is what GOM deposits are and how they arise. It is also interesting to know what morphological changes they undergo in the course of the disease. To date, no systemic investigations on morphological diversity and changes in GOM deposits have been reported in the available literature,

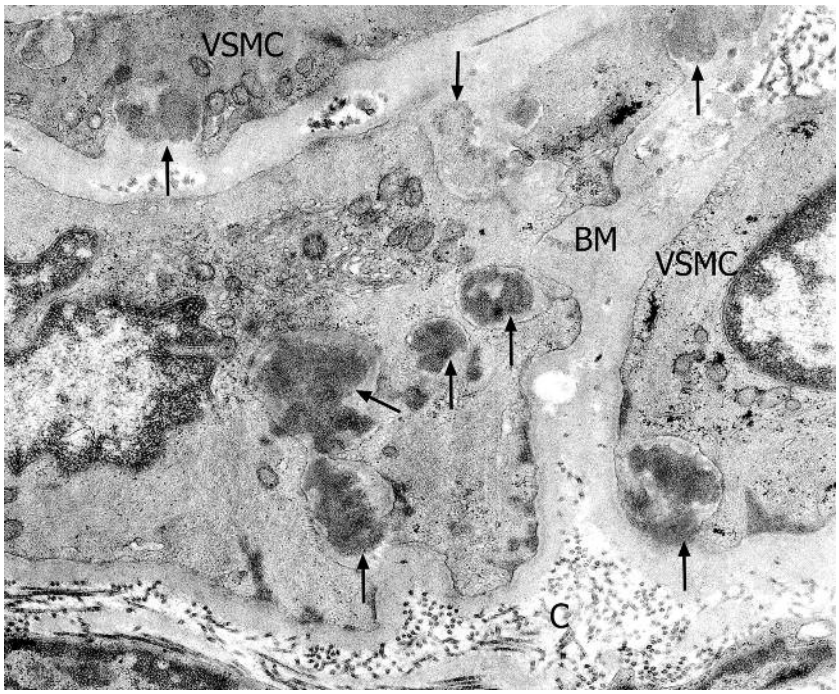

Fig. 1. VSMC of arteriole with GOM deposits (arrows) located near VSMC particularly in their membrane infolding. BM – basement membrane, C – collagen. Orig. magn. × 7000.

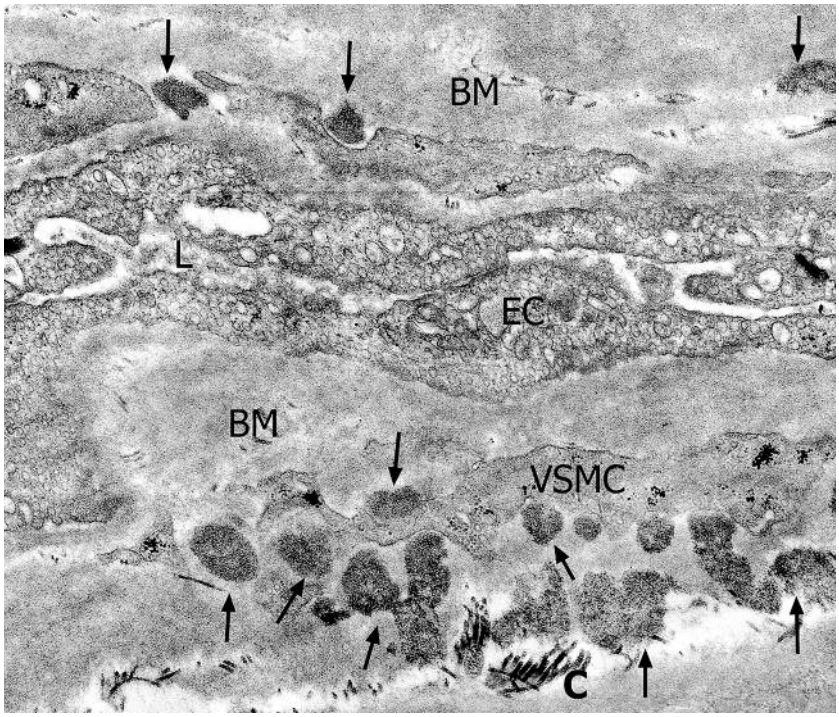

Fig. 2. An arteriole with degenerated VSMC and numerous GOM deposits (arrows) of various shape, size, electron density and localization. EC – endothelial cell, BM – basement membrane, C – collagen, L – lumen. Orig. magn. × 7000.

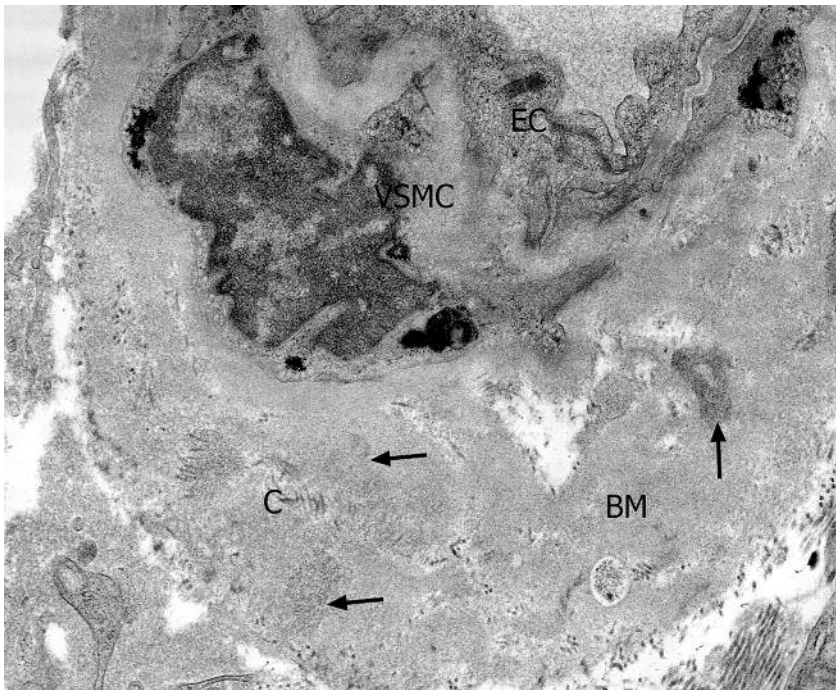

Fig. 3. An arteriole with degenerating and loose VSMC and very thickened basement membrane (BM). GOM deposits (arrows) located in BM revealed various electron density, sometimes resembling density of BM. Orig. magn. × 7000.

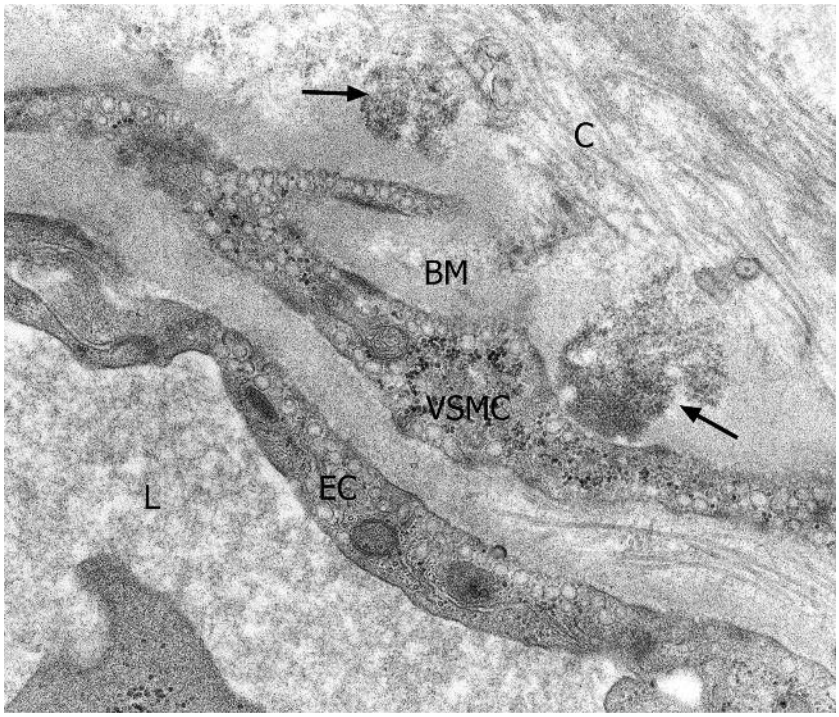

Fig. 4. An arteriole with GOM deposits (arrows) of various electron density and shape located near VSMC and on the external edge of arterial media. L – lumen, EC – endothelial cell, C – collagen. Orig. magn. × 12 000.

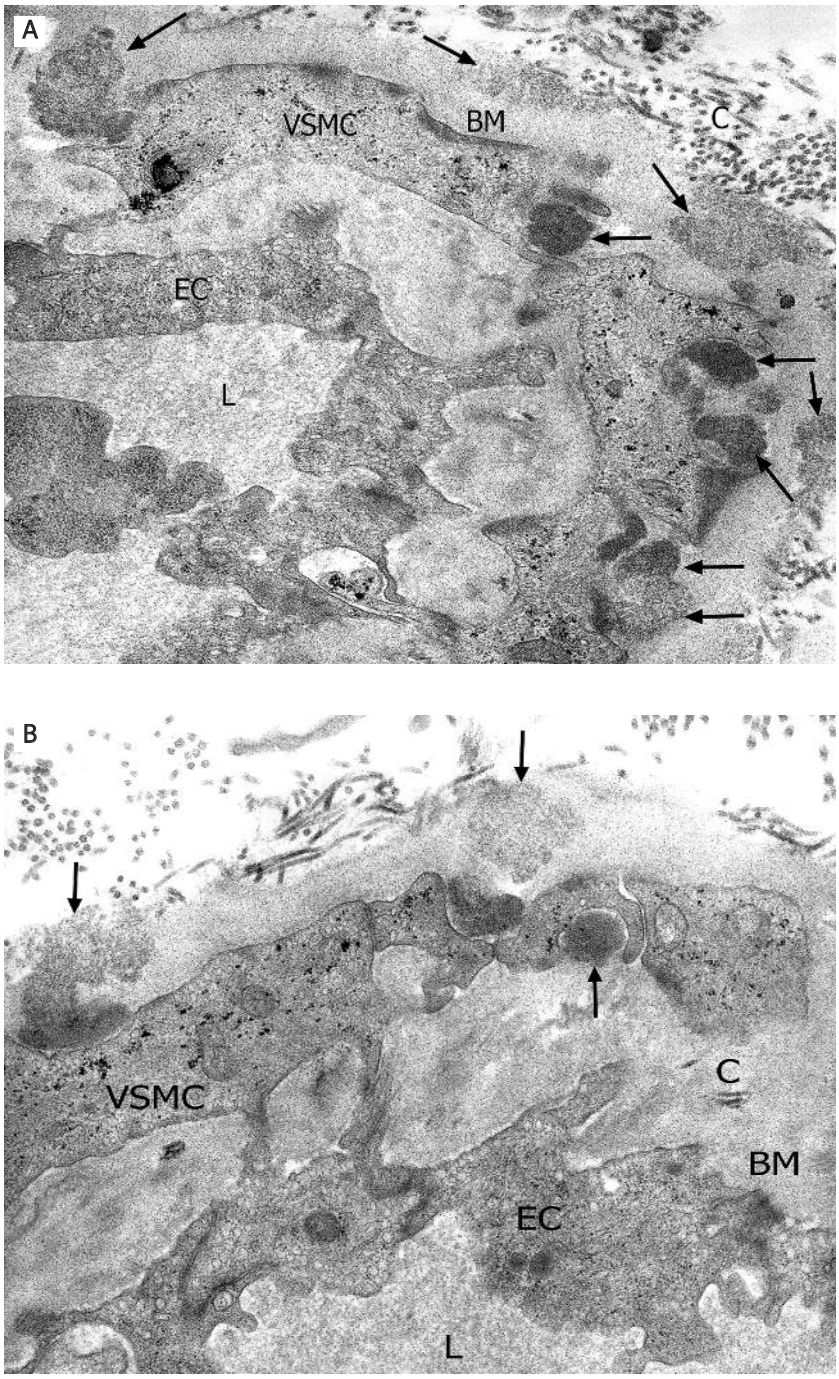

Fig. 5 A,B. Arterioles showing GOM deposits (arrows) with different electron density and size, some of them showing bizarre shapes. L – lumen, EC – endothelial cell, BM – basement membrane, C – collagen. Orig. magn. × 12 000.

although various shapes, sizes and electron density of these pathognomonic structures in CADASIL have been described by some authors [3,10,17].

Various electron density of GOM deposits and their bizarre shapes is a very striking finding. With increasing distance from the cell body the deposits became gradually less dense and loose. The rarefaction of granular material in GOM deposits, progressing with growing distance from the VSMC and accompanied by characteristic changes in their shapes, suggests a gradual breakdown of GOM lodgements. Since in vessels with a striking loss of VSMC, GOM deposits located at the external edge of arterial media had electron density resembling the density of the basement membrane, it may be concluded that this finding may support not only our hypothesis on the gradual disintegration of GOM deposits, but also suggests their origin. As GOM deposits were detected around the degenerated VSMC or in the indentations of these cells, but not within the cells, it is possible that their formation occurs on the cell surface. This process may be connected with (1) the dysfunction of TACE enzyme which proteolytically cuts $N3^{ECD}$ from the Notch3 receptor after ligand binding or (2) the disturbed endocytosis which in normal conditions removes $N3^{ECD}$ from the cell membrane [6]. It is also supposed that GOM deposits may be formed in the endoplasmic reticulum and released in the extracellular space by the disruption of degenerated cells expressing Notch3 receptor. However, the presence of GOM deposits near the relatively well preserved VSMC is against this hypothesis.

Aggregation and accumulation of abnormally folded proteins has been recognized as a key pathological event in various neurodegenerative diseases. Recently, it has been reported that CADASIL-associated mutations significantly enhance Notch3 multimerization [14]. If GOM deposits contain $N3^{ECD}$, lodgements of GOM may be the spontaneously formed oligomers of mutated Notch3 protein. Mutant Notch3 aggregates are resistant to degradation [19] and, like in many disorders associated with the accumulation of abnormally folded proteins, their formation may lead to cellular dysfunction and eventually to death. But not only VSMC degeneration and loss may lead to impairment of small vessel wall integrity in CADASIL. The vessel hyalinization, fibrosis with strong positive immunostaining to collagens III and IV, and abnormal enlargement of the space between cells in the vessel wall observed on light or electron microscopy [1,3,5,18] may also participate in this process.

In conclusion, on the basis of our thorough ultrastructural study of GOM deposits morphology, we suggest that granular osmiophilic material can be formed on the surface of VSMC in the vessel wall and penetrate from the cells into the basement membrane and/or extracellular matrix. The dispersion of granules which form GOM deposits may be one of the factors triggering the thickening of and changes in the basement membrane and/or extracellular matrix.

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