

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL)

A Hereditary Cerebrovascular Disease, Which Can Be Diagnosed by Skin Biopsy Electron Microscopy

Akira Ishiko, MD, PhD,* Atsushi Shimizu, DVM,* Eiichiro Nagata, MD, PhD,†
Kouichi Ohta, MD, PhD,‡ and Masaru Tanaka, MD, PhD*

Abstract: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited cerebrovascular disease characterized by recurrent subcortical ischemic strokes starting in the third or fourth decade as a result of mutations in the *Notch3* gene. Granular osmiophilic material (GOM) deposition around the vascular smooth muscle cells is a specific feature and electron microscopic observations of skin biopsies are useful for this diagnosis. A 39-year-old female with dizziness, abnormal visual fields, and hemiplegia, and a 42-year-old male with tinnitus and dizziness, were suspected of suffering from CADASIL based on MRI findings. Both cases were shown to have characteristic deposits of GOM, 200 to 800 nm in diameter, around the vascular smooth muscle cells of small arteries in the deep dermis, and thus the diagnoses of CADASIL were made, although there was no family history of cerebrovascular disorders or dementia. Dermatologists should be aware of these ultra-structural findings because this disease may occur sporadically and might be more common than initially thought.

Key Words: CADASIL, dementia, electron microscopy, MRI, skin biopsy

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL, OMIM #125310) is an inherited cerebrovascular disease characterized by recurrent subcortical ischemic strokes starting in middle age.^{1,2} It is caused by specific mutations in the *Notch3* receptor,³ which belongs to the highly conserved *Notch* receptor protein family involved in cell fate specification.⁴ The clinical symptoms of CADASIL usually begin with migraine headaches, and lead to transient ischemic attacks, subcortical

dementia, gait disturbances, pseudobulbar palsy, and subsequent progression of the disease leads to premature death about 20 years after the initial clinical onset.^{5–7} Magnetic resonance imaging (MRI) of the diseased brain shows various degrees of diffuse hyper-intensity within the periventricular and subcortical white matter, along with lacunar infarctions in the thalamus, basal ganglia, and brain stem.^{8–10} Pathologic degeneration and loss of smooth muscle cells (SMCs) within the small arteries of the brain are characteristic features and the ultra-structure of the brain arteries shows granular osmiophilic material (GOM) surrounding the vascular smooth muscle cells.^{11,12} Similar changes can be found in the arteries of various other organs such as skeletal muscle, nerve vessels, and in the skin,¹³ providing evidence that CADASIL is indeed a systemic arteriopathy. The deposition of GOM is highly specific to CADASIL and Ruchoux et al¹³ proposed the use of skin biopsy for the diagnosis of CADASIL. Electron microscopic study of skin vessels is now widely accepted as part of the evaluation for CADASIL.^{14–16} Although the use of skin biopsies in this disease is well known among neurologists, dermatologists are not familiar with this disease and only a few reports have appeared in the dermatological journals.^{17–19} Here, we report the ultrastructural changes in 2 Japanese cases of CADASIL and review the current literature pertaining to this disease.

CASE REPORT

Case 1

A 39-year-old Japanese female visited Mito Red Cross Hospital complaining of dizziness, dysarthria, disturbance in gait, and chronic headaches for the past year. Her family history was unremarkable. A MRI brain scan revealed heterogenous hyperintensities on the T2-weighted images in the white matter of both cerebral hemispheres. These resembled confluent images of multiple lacunar infarctions. Over the course of the next 4 months, she suffered from 2 episodes of right hemiparesis, and each time MRI revealed the presence of a new lacunar infarct in the left cerebral hemisphere. Due to her apparent lack of stroke risk factors, her relatively young age, and the presence of a preceding chronic headache together with the characteristic MRI images, CADASIL was the suspected diagnosis. A biopsy was taken from normal-appearing skin on her buttock and processed for electron microscopy (EM). Small arteries in the deep

From the *Department of Dermatology, Keio University School of Medicine, Tokyo, Japan; †Department of Neurology, Keio University School of Medicine, Tokyo, Japan; and ‡Department of Neurology, Mito Red Cross Hospital, Ibaraki, Japan.

Reprints: Akira Ishiko, Department of Dermatology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan (e-mail: ishiko@sc.itc.keio.ac.jp).

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dermis showed no major structural cellular degeneration under low-power observation (Fig. 1A); however, there were enlarged areas between the endothelium and the SMCs and between the adjacent SMCs due to a loss of cell junctions. Deposits of granular osmiophilic dense material (GOM) were abundant near shallow folds on the SMC plasma membrane (Fig. 1B). GOM consisted of aggregates of electron-dense granules, and the density of these granules was greater nearer the plasma membrane and the granules became less electron-dense further from the plasma membrane. The GOM was 200 to 800 nm in diameter but there were electron lucent spaces, 10 to 30 nm in width, between the plasma membrane of the SMCs and most of the GOM. Some GOM was present continuous with the plasma membrane and to cytoplasmic electron-dense material in the SMC (Fig. 1C). GOM was also seen around venule pericytes in the deep dermis, but the amount of GOM was significantly less, compared with that in the arteries. These EM findings are diagnostic for CADASIL.

Case 2

A 42-year-old Japanese male with an unremarkable family history was admitted to the neurology department suffering from tinnitus and dizziness for one and a half years, with MRI findings indicating multiple infarcts and leukoencephalopathy. The patient has had no other symptoms. CADASIL was suspected from MRI scans and a biopsy was taken from normal-appearing skin on the upper arm and processed for EM. SMCs from small arteries in the deep dermis

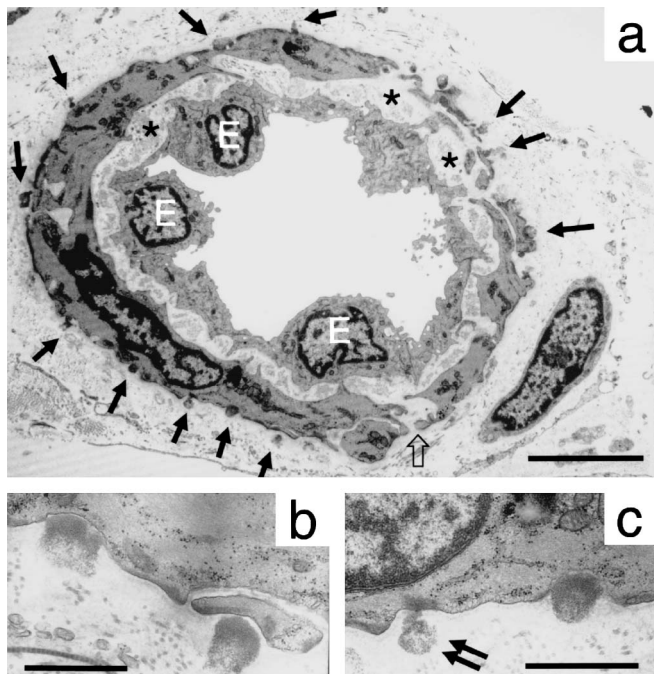


FIGURE 1. Electron microscopy of an arteriole in the deep dermis of case 1. A, There were increased spaces between endothelial cells (E) and SMCs (asterisk) and between the adjacent SMCs due to a loss of junctions (open arrows). There was abundant GOM (arrows) adjacent to plasma membrane indentations of SMC along the dermal wall of the vessels. Bar = 5 μ m. B, Higher magnification of the typical GOM deposits. C, Sometimes GOM was seen continuous with an intracytoplasmic electron-dense material (double arrows).

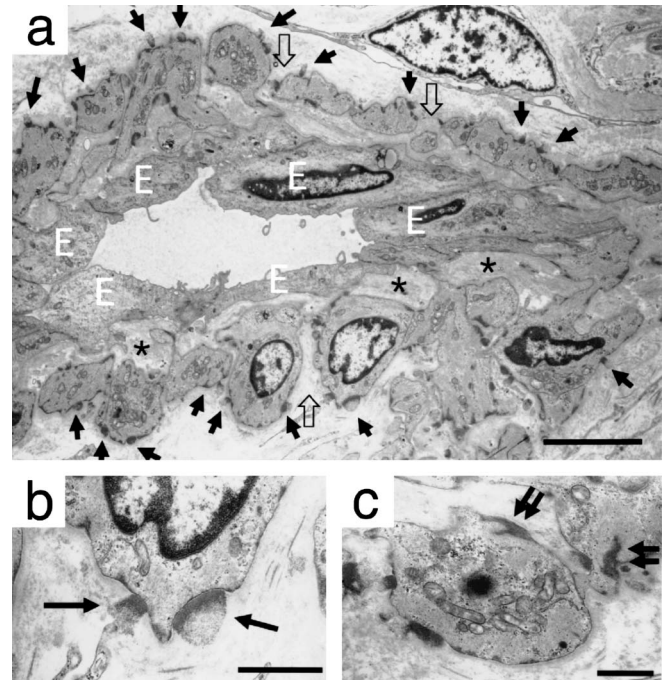


FIGURE 2. Electron microscopy of an arteriole in the deep dermis from case 2. A, The arteriole shown here is larger than that in Fig. 1. The dermal side or outer plasma membrane of the vessel is irregular and shows indentations. There are increases in the spaces between endothelial cells (E) and SMCs (asterisk) and between two adjacent SMCs (open arrow). There was much GOM (arrows) along the SMC vessel plasma membrane. Bar = 5 μ m. B, Higher magnification of the typical GOM. C, GOM was occasionally seen as a continuous intracytoplasmic electron-dense line of material (double arrows).

showed a typical, uneven outline and abundant GOM was found in these SMC indentations along the plasma membrane (Fig. 2A, B). There were increases in the spaces between the endothelium and the SMCs and between adjacent SMCs due to a loss of junctions. The amount and morphology of the GOM was similar to case 1, although this case exhibited milder clinical symptoms. Electron-dense material continuous with the plasma membrane was also seen within the cytoplasm of the SMCs (Fig. 2C).

DISCUSSION

The diagnosis of CADASIL in these 2 cases was based on electron microscopic study of the skin and MRI scan findings together with the clinical symptoms. According to Dichgans' review of 102 cases,²⁰ recurrent ischemic episodes (transient ischemic attack) were the most frequent clinical presentations identified in 71% of cases. In addition, cognitive deficits (48%), dementia (28%) were frequently accompanied by a disturbance in gait (90%), urinary incontinence (86%), and pseudobulbar palsy (52%) in CADASIL patients. Migraine headaches (38%), psychiatric disturbances (30%), and adjustment disorders (24%) were also present in some cases.²⁰ Our cases showed no family history of cerebral angiopathy or dementia, and were thought to represent sporadic cases of

CADASIL. This disease is caused by mutations in the Notch3 gene, mapped on chromosome 19,³ and the presence of such de novo mutations in the Notch 3 gene in CADASIL has previously been demonstrated. Thus, cases of leukoencephalopathy with an unremarkable family history might not have been diagnosed properly so that the disease may be more frequent than anticipated.²¹

Notch3 belongs to a family of highly conserved Notch/LIN-12 receptors involved in cell fate determination,³ and its expression is restricted to vascular SMCs in adult human tissues.²² It encodes a protein of 2321 amino acids that contains 34 tandem EGF-like repeats. Interestingly, all CADASIL mutations ultimately result in the addition or the loss of a cysteine residue within one of the EGF domains that invariably contains 6 cysteine residues. More than 400 affected families as well as sporadic cases have so far been identified.²¹

Granular osmiophilic dense material can be found in the arteries of heart, kidney, small and large intestine, liver, skeletal muscles, and skin^{13,14} and a skin biopsy is an easy and effective method for detecting GOM in the diagnosis of CADASIL. The specificity of GOM in the dermal arteries for the diagnosis of CADASIL approaches 100% in several studies. All 14 specimens taken from affected individuals including 2 asymptomatic carriers of *Notch3* mutations showed vascular GOM deposits.²³ One member of a suspected CADASIL family, who suffered from a vascular leukoencephalopathy but was negative for GOM in a skin biopsy, was later proven not to carry any *Notch3* mutation.²⁴ GOM can be detected in the skin at such an early stage that the only reported clinical symptom may be a migraine with a normal MRI.²⁵ Dermal blood vessel morphology including the presence of GOM correlated well with changes in the brain arterioles, suggesting that dermal vessels can be used to check the condition of brain vessels in future therapeutic trials with CADASIL patients.¹⁶ Thus, a skin biopsy might not only be useful in diagnosing the disease but also in estimating the disease activity.

Recent reports have highlighted the relationship between the Notch3 mutations, SMC ultrastructure, and the pathogenesis of vascular degeneration. Under normal circumstances Notch3 undergoes proteolytic cleavage leading to a 210-kDa extracellular fragment and a 97-kDa intracellular fragment in normal vascular SMCs. However, in CADASIL vasculature, the 210-kDa extracellular fragments accumulate within the vascular SMCs, in the close vicinity to the plasma membrane and GOM.²² Therefore, CADASIL mutations may impair the clearance of the Notch3 ectodomain from the cell surface. Transgenic mice expressing mutant Notch3 have recently demonstrated similar vascular changes to human CADASIL patients. GOM accumulation and degeneration of vascular SMCs were both observed in the mutant mice providing evidence that the Notch3 mutation can have significant effects on GOM deposition.²⁶

Thus the detection of GOM in the vascular SMC is a diagnosis hallmark of CADASIL. It is important for dermatologists to be aware of this disease, and the role of a skin biopsy with electron microscopic studies in its evaluation.

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