

Short communication

A CADASIL case with normal skin biopsy and without mutations in exons 3 and 4 of the *Notch3* gene[☆]

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Abstract

The diagnosis of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is usually confirmed by genetic testing or skin biopsy. We here report the case of a 69-year-old woman with recurrent transient ischemic attacks (TIAs) and strokes, seizures, and dementia without any mutations in exons 3 and 4 of the *Notch3* gene and with a normal skin biopsy, but who showed characteristic CADASIL abnormalities on brain pathological examination. Our findings suggest that negative results in these two tests do not exclude the disease and a leptomeningeal biopsy or a second skin biopsy should be considered in such cases. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an adult-onset hereditary disorder characterized by recurrent transient ischemic attacks (TIAs) and strokes, migraine, seizures, and dementia [1]. The pathological hallmark of this disease is the presence of characteristic granular osmiophilic material (GOM) within the basal membrane of brain vascular smooth muscle cells; these vascular changes were also later reported in nerve, striated muscle, and skin [2–4]. In 1997, mutations within the *Notch3* gene were identified in these patients and the diagnosis of CADASIL can now be confirmed by genetic testing [5]. In cases in which no mutations are found, ultrastructural examination of a skin biopsy should be considered [6].

We here report the case of a woman who presented recurrent TIAs and strokes, seizures, and dementia in whom the skin biopsy was normal and DNA analysis failed to detect mutations in the exons 3 and 4 of the *Notch3* gene. However, the diagnosis of CADASIL was

established by postmortem cerebral examination. This case shows that standard tests for CADASIL can give false-negative results and that further investigations are warranted in some patients.

2. Case presentation

In May 1995, a 69-year-old female patient suddenly developed right hemiparesis and hypoesthesia, recovering in about 10 min. Four years previously, she had suffered a similar episode. The patient had bilateral glaucoma. Thirteen years previously, laminectomy was performed because of disc herniation. Amigdalectomy and surgery for lower member varices had been performed several years previously. There was no history of hypertension, diabetes, hypercholesterolemia, cardiac disease, migraine, cigarette smoking, or alcohol consumption. Her father had died at the age of 55 due to pneumonia following a stroke, her mother at the age of 93 from unknown causes, and her brother at the age of 66 from colon carcinoma. She had one daughter with a normal MRI who, from the age of 14 years, had suffered from seizures that were controlled with phenobarbital. There was no other family history of cerebrovascular diseases, seizures, or migraine. Neurological examination revealed only mild brachial right side weakness. Routine hematological and biochemical tests, clotting

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function tests, lipid metabolism, electrocardiogram, echocardiogram, chest radiograph, extracranial Doppler ultrasonogram of the carotid and vertebral arteries, MRI angiography, and transcranial Doppler were normal. MRI revealed diffuse periventricular white matter abnormalities. In July 1997, she had a right-sided partial motor seizure with secondary generalization and was admitted to our hospital. An electroencephalogram revealed generalized low amplitude slowing with frontal intermittent rhythmic delta activity predominantly in the left fronto-parieto-occipital areas. Blood and urine amino acids, serum lactate, pyruvate, arylsulfatase, betagalactosidase, protein electrophoresis, immunoglobulin, cortisol, ceruloplasmin, carnitine, protein C, protein S, antithrombin III, vitamin B12, and thyroid hormone values were normal. DNA analysis for mitochondrialopathies and tests for striated muscle antibodies, anticardiolipin antibodies, antinuclear antibodies,

and rheumatoid factor were negative. Low levels of vitamin B6 ($0.3 \mu\text{g}/100 \text{ ml}$, normal range 3–83) and folic acid ($4 \text{ nmol}/\text{l}$, normal range 7–39) were found. Homocysteine levels were high ($29.6 \mu\text{mol}/\text{l}$, normal range 5–15) and vitamins were administered. Examination of the cerebrospinal fluid showed $2471 \text{ erythrocytes}/\text{mm}^3$ and $340 \text{ mg}/\text{l}$ of protein, and serologic tests for herpes simplex, syphilis, and Lyme disease were negative. SPECT Tc99 showed hypoperfusion in the frontal and temporal lobes bilaterally and in the left parietal lobe. Nerve conduction studies and muscle biopsy results were normal. Genomic DNA was extracted from peripheral blood leukocytes for analysis for mutations in exons 3 and 4 of the *Notch3* gene. Electron microscopy of the skin biopsy was normal. Cognitive deterioration progressed. In October 1997, neurological examination showed a lethargic, disoriented patient. Gait and constructive apraxia were noted.

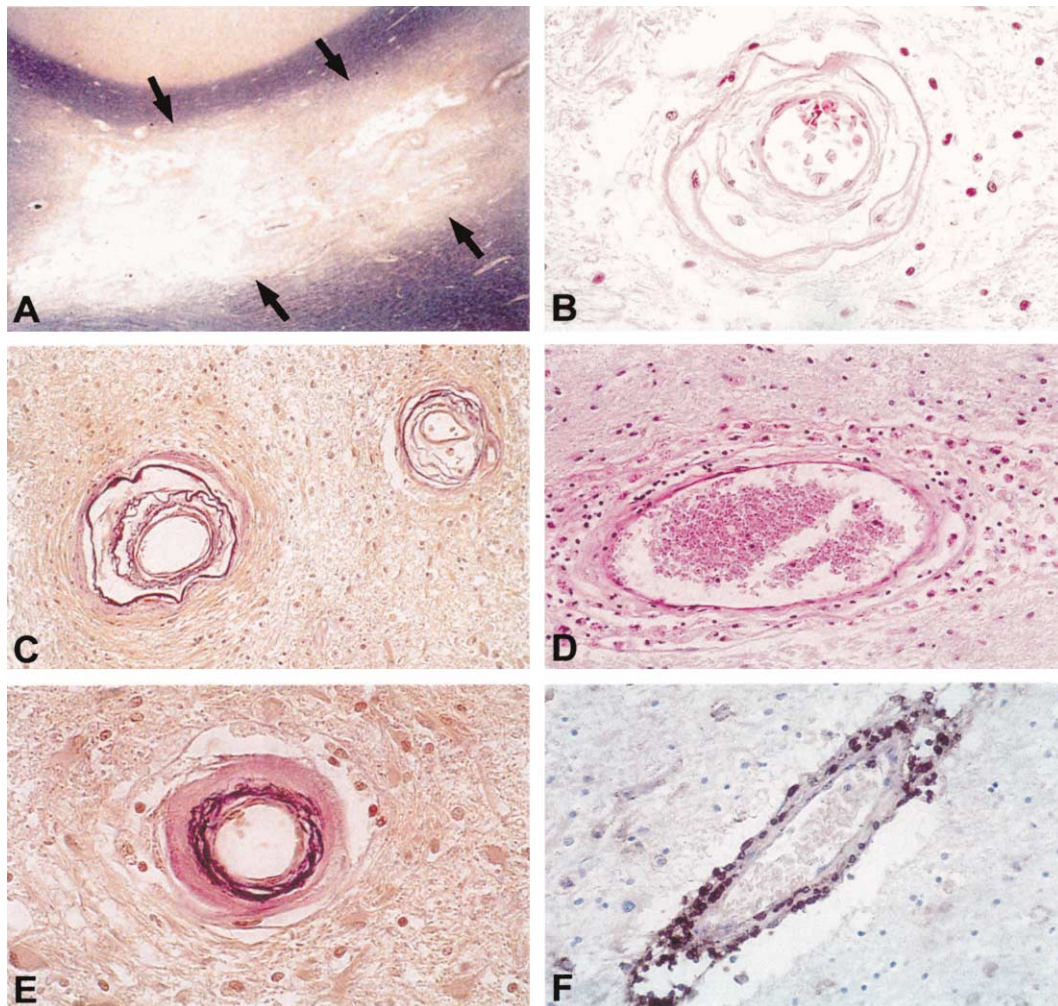


Fig. 1. Illustration of the characteristic white matter and vascular changes in our CADASIL case. (A) Small lacunar infarcts (arrows) in the parietal white matter. Paraffin section from the parietal region, stained for myelin. Loyez stain ($\times 20$). (B) Thickening and fibrosis of the wall of a small deep-penetrating artery. HE stain ($\times 300$). (C) Thickening, reduplication, and fragmentation of the internal elastic lamina of two small arteries. VGE ($\times 150$). (D) Perivascular cuff of macrophages around a small cerebral vessel. PAS ($\times 300$). (E) Concentric thickening of the arterial wall showing marked fibrosis. VGE ($\times 200$). (F) Microphotograph showing a perivascular inflammatory infiltrate. The inflammatory cells are positive for leucommun. Some of the immunoreactive cells are infiltrating the vessel wall ($\times 200$).

Deep-tendon reflexes were brisk. Hypertonia, bilateral grasp reflexes, and primitive reflexes were observed. Plantar responses were flexor. She became bedridden and died in March 1998 of pneumonia.

3. Materials and methods

The brain was fixed in 10% formalin and 19 blocks were taken from different regions of the cerebral cortex, white matter, basal ganglia, thalamus, brainstem, and cerebellum. After embedding in paraffin, 7- μ m-thick sections were prepared and stained using the haematoxylin–eosin (HE), van Gieson–elastin (VGE), Loyez, periodic acid–Schiff (PAS), toluidine blue, Congo Red, thioflavin S, Oil red O, and Sudan black techniques. The avidin–biotin–peroxidase technique was used to study the binding of monoclonal antibodies specific for vimentin (DAKO, M725; diluted 1:50), human leukocyte common antigen (DAKO, M701; diluted 1:200), α -smooth muscle actin (Sigma, A-2547; diluted 1:1000), CD 68 (DAKO, M814; diluted 1:100), and T cells (DAKO, M742; diluted 1:100). Polyclonal antibodies specific for ubiquitin (DAKO, Z0458; diluted 1:300) or GFAP (DAKO, Z 334; diluted 1:500) were also used. Small samples taken from the deep white matter and small fragments of the leptomeningeal arteries were processed for ultrastructural analysis; the ultrathin sections were contrasted with uranyl acetate and lead citrate and examined using a Philips CM-10 electron microscope.

Direct sequencing of DNA extracted from leukocytes while the patient was alive was performed. Exons 3 and 4

were amplified by using polymerase chain reaction with the following sets of primers: forward primer N1F, 5'-TGTGCTGCCCAACCAAGCGA-3', and reverse primer N1R, 5'-ACTGACCACACCCCGACTA-3', and forward primer N2AF, 5'-TAGTCGGGGGTGTGGTCAGT-3', and reverse primer N3AR, 5'-CCTCTGACTCTCCTGAGTAG-3', for exons 3 and 4, respectively. Amplicons were sequenced in both directions by means of dye-labeled terminators on an ABI377 DNA sequence apparatus (Perkin-Elmer, Applied Biosystems, Paris, France) [7].

4. Results

4.1. Neuropathology

The weight of the brain was 1030 g. Gross examination revealed moderate atherosclerosis of the large cerebral arteries. On coronal brain slices, multiple, sometimes confluent, small, granular, grayish, lacunar infarcts were seen in the deep white matter of both hemispheres. Some lacunar infarcts were also found in the basal ganglia and thalamus. The brainstem, cerebellum, and spinal cord were spared.

Histological analysis showed multiple lacunar infarcts in the deep white matter (Fig. 1A) associated with diffuse pallor of the myelin. Vascular changes consisted of thickening and fibrosis of the wall of small- and medium-sized leptomeningeal arteries and arterioles penetrating the white matter and basal ganglia (Fig. 1B). Fragmentation of the elastic lamina of some arteries was also present (Fig. 1B), but the most consistent change was destruction of the

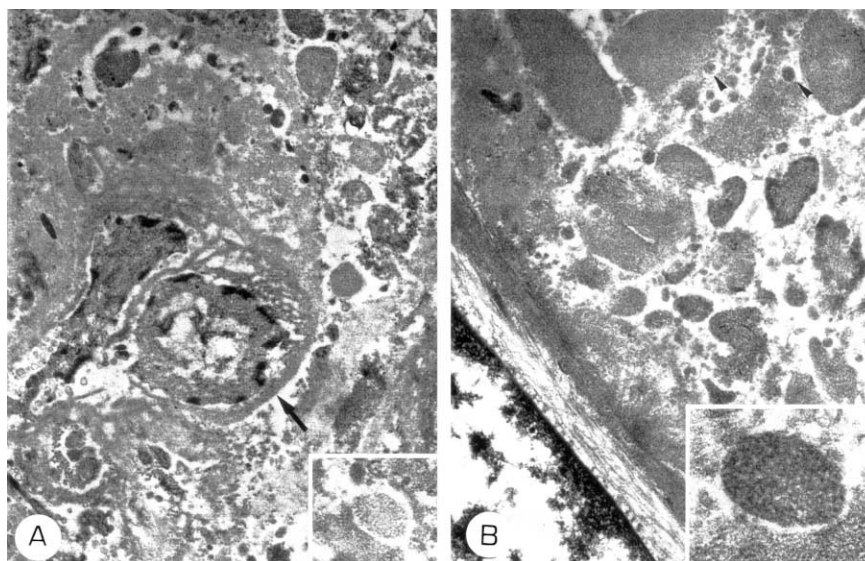


Fig. 2. Illustration of the accumulation of GOM in the leptomeningeal arteries. (A) Electron micrograph ($\times 12,000$) showing smooth muscle cells, in the tunica media of the affected vessel wall, surrounded by thickened basal membrane-like material (arrow) and numerous GOM. (B) Higher magnification view of the granular, electron-dense material in the media (arrowhead), associated with cell debris ($\times 24,000$). (Insets in C and D) High magnification of GOM deposit pushing away the basal membrane-like material that accumulated around smooth muscle cells. Magnification of insets: $\times 24,000$ and $\times 32,000$, respectively.

muscular layer, which had a smudged granular appearance and showed marked loss of smooth muscle cells. The remaining muscle cells were clear and ballooned. The eosinophilic granular material, deposited in the muscular layer, was slightly positive with PAS, VGE, and Sudan black stains and negative for amyloid (Congo Red and thioflavin S). Smooth muscle cell immunoreactivity was severely reduced in the muscular layer due to the severe muscle cell loss. The granular material of the media was immunopositive for vimentin and fibronectin. Some perivascular cuffs of inflammatory cells, mainly T lymphocytes, were present (Fig. 1F). No senile plaques were found in the cerebral cortex and no beta-amyloid deposits were seen in the leptomeningeal and cortical arteries. A few neurofibrillary tangles were found in hippocampus and entorhinal cortex.

Ultrastructural analysis of the brain showed marked destruction of smooth muscle cells with an accumulation of GOM in the muscular layer (Fig. 2). These extracellular granular deposits were numerous, especially around smooth muscle cells, and sometimes embedded in small vacuoles in the basal lamina-like material. The small arteries of the white matter showed severe destruction, sometimes with complete disappearance of the tunica media, which was replaced by cell debris and macrophages containing electron-dense granular material; in some areas, smooth muscle cells and GOMs were no longer recognizable.

4.2. Molecular genetics

There were no mutations in exons 3 and 4 of the *Notch3* gene.

5. Discussion

The diagnosis of CADASIL was established in our patient by characteristic changes on brain pathological examination. Both skin biopsy and standard genetic testing failed to reveal the characteristic changes of CADASIL. The clinical presentation of our patient, with recurrent TIA and subcortical strokes, seizures, and dementia, was suggestive of CADASIL. Although the age of onset of CADASIL in our patient is later than often reported, it falls within the reported range of 27–65 years [5]. Moreover, subtle clinical findings may have been present for years before the patient sought medical attention.

Data concerning the sensitivity and specificity of tests for CADASIL are still lacking. In 50 unrelated patients strongly suspected of having CADASIL, no mutation of the *Notch3* gene was seen in 5 [5]. In two studies, GOM was found in the skin biopsy of all CADASIL patients (8 and 14 patients, respectively) [8,9] and in none of the control group [8], and skin biopsy has been suggested as the method of first choice for the diagnosis of CADASIL [9]. However, a CADASIL case with GOM in the

meningeal vessels, but without changes in the skin and striated muscle arteries, has been reported [10]. The same authors reported a further case with a clinical and family history highly suggestive of CADASIL, but with a negative skin biopsy. Unfortunately, genetic analysis was not performed on these two patients.

The family history of our patient is inconclusive. Her father died aged 55 after a stroke. Her daughter has epilepsy and had a normal MRI at the age of 42; since seizures are rarely the initial symptom of CADASIL and MRI penetrance of CADASIL is almost complete between 30 and 40 years of age [11], the diagnosis of CADASIL is unlikely. A sporadic case has been reported [7]. However, the small number of family members and the lack of a satisfactory family history do not allow us to clearly define our case as sporadic.

It could be argued that abnormalities in the skin biopsy could be focal [12], and, therefore, false-negative biopsies could result from the use of insufficient or inadequate material. However, the existence of negative skin biopsies in CADASIL has been confirmed by a recent genetically confirmed Canadian CADASIL family, in which skin biopsy was negative in all eight members affected [13]. It could also be claimed that the our genetic screening was incomplete, since, in some CADASIL patients, the mutation is not located on exons 3 or 4 but on one of the 21 remaining exons of *Notch3* gene. However, screening for the additional 21 exons is very time consuming and rarely performed. Moreover, in some patients, genetic testing may fail to show changes, even after complete screening.

Although negative genetic testing or negative skin biopsies have already been reported in CADASIL patients, we were unable to find a case in which both tests were negative. Our findings therefore suggest that skin biopsy and DNA examination do not detect all affected patients. Negative results do not exclude the disease, and biopsy of leptomeningeal arteries or a second skin biopsy should be performed in these cases.

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