ORIGINAL ARTICLE

Apoptosis in Cerebral Autosomal-Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy

Francoise Gray, MD, PhD, Marc Polivka, MD, Anand Viswanathan, MD, PhD, Marie Baudrimont, MD, PhD, Marie-Germaine Bousser, MD, and Hugues Chabriat, MD, PhD

Abstract
To test the hypothesis that an apoptotic process plays a role in the pathogenesis of cerebral lesions in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), we examined samples from frontal, temporal, insular, and occipital regions, basal ganglia, and cerebellum from 4 patients with CADASIL, 2 withBinswanger disease, and 3 controls. Apoptotic cells were identified using in situ end labeling and activated caspase 3 immunostaining. Immunolabeling for Notch3, the β-amyloid protein precursor, and phosphorylated neurofilament protein was performed on successive sections. Apoptosis of vascular cells was markedly increased in status cribrosus in CADASIL, both in basal ganglia and subcortical white matter, suggesting that concomitantly with Notch3 deposition it may play a causative role in the dilatation of Virchow-Robin spaces. Neuronal apoptosis was found in CADASIL, mostly in cortical layers 3 and 5. Its severity correlated semiquantitatively with the extent of ischemic lesions and axonal damage in the underlying white matter. It was more severe in demented patients. Only occasional apoptotic neurons were found in the Binswanger cases and none in the controls. This supports the view that neuronal apoptosis may contribute to cortical atrophy and cognitive impairment in patients with CADASIL and that it may, at least partly, result from axonal damage in the underlying white matter.

Key Words: Apoptosis, CADASIL, Cortical atrophy, Lacunar infarct, Status cribrosus (état criblé), Subcortical white matter, White matter damage.

INTRODUCTION
Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary arteriopathy (1) caused by mutations of the NOTCH3 gene mapped on chromosome 19 (2, 3). It is characterized by specific vascular lesions predominantly involving the white matter and basal ganglia (4–7), including pathognomonic electron-dense osmiophilic granular material in the media of small arteries and capillaries associated with degenerating smooth muscle cells (8) and accumulation of the ectodomain of Notch3 receptor in vessel walls, identifiable by immunohistochemistry (9). CADASIL is regarded as a model of subcortical ischemic vascular dementia related to small vessel disease (10).

The classical neuropathologic changes reported in CADASIL, whether ischemic (subcortical lacunar infarcts or leukoencephalopathy) or hemorrhagic (microbleeding), clearly result from structural mural changes. However, additional lesions have been described in which the pathogenic mechanisms are not so clear.

Dilatation of Virchow-Robin spaces forming status cribrosus (also termed état criblé or type III lacunes) (11, 12) is frequent in CADASIL and is mostly located in the basal ganglia and subcortical white matter (13). It does not seem to be directly related to the ischemic or hemorrhagic lesions (13).

In CADASIL, progressive cortical atrophy associated with cognitive decline has been found by imaging (14), supporting a single case report of loss of cortical neurons in the absence of typical ischemic changes (15). The mechanism of neuronal damage in this scenario is unclear.

Previous studies indicates that apoptotic pathways play an important role in delayed brain injury after ischemic infarction. Whereas necrotic cell death occurs at the core of an infarct, apoptotic cell death predominates in peri-infarct areas (16). We postulated that apoptosis might be important in the pathophysiology of neuronal loss seen at a distance from subcortical ischemic lesions in CADASIL. Experimentally, a role for Notch3 receptor signaling in the regulation of vascular smooth muscle cell apoptosis has been demonstrated in vitro (17, 18). Although the pathogenetic mechanism linking NOTCH3 mutations and the vascular changes in CADASIL remains unclear, a defect in Notch3 signaling system is likely, which might facilitate apoptosis of vascular cells (7, 19).

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MATERIALS AND METHODS

To test the hypothesis that apoptosis participates in cerebral lesions in CADASIL, we examined brain samples from 4 patients with CADASIL, with characteristic mutations of the NOTCH3 gene. The clinical and imaging data of these patients have been reported previously (20–22). Two patients who died from subcortical arteriosclerotic encephalopathy (Binswanger disease) with comparable cognitive impairment and white matter changes and 3 individuals who died suddenly without neurologic disease (1 motor vehicle accident death and 2 gunshot-related deaths) were investigated according to the same protocol and served as controls. Epidemiologic, clinical and genetic data of these 9 cases are summarized in Table 1.

Gross examination of the brains was performed after 1 month of 10% buffered formalin fixation. Large slices of the cerebral hemispheres and of the brainstem/cerebellum at the level of the dentate nuclei were embedded in paraffin and 15-μm-thick sections were stained by hematoxylin and eosin and cresyl violet combined with Luxol fast blue (Klüver and Barrera stain). Smaller blocks were taken from the cerebral cortex with underlying white matter, deep gray nuclei, midbrain, cerebellum, and brainstem; 5-μm-thick sections were stained with hematoxylin and eosin, Masson’s trichrome, periodic acid-Schiff, and Bodian silver impregnation combined with Luxol fast blue.

On selected 5-μm-thick sections of samples from the frontal lobe at the level of the rostrum of corpus callosum (F1), hippocampus, temporal lobe at the level of T1, insula, occipital lobe at the level of the calcarine sulcus, lenticular nuclei, thalamus and cerebellum, immunohistochemistry was performed using an avidin-biotin complex (ABC) peroxidase-based method with the following antibodies (Abs): a monoclonal Ab raised against the precursor of the protein β-amyloid (anti-Alzheimer precursor protein A41, 1:200; Boehringer Mannheim, Philadelphia, PA) to identify axonal damage (23); a monoclonal Ab raised against phosphorylated neurofilament protein (monoclonal mouse anti-human neurofilament protein, clone 2F11, 1:50; Dako, Glostrup, Denmark); a polyclonal Ab raised against activated caspase3 (affinity-purified rabbit anti-human/mouse caspase3 active, 1:1,000; R&D Systems Europe, Lille, France) to identify apoptotic cells; and a monoclonal Ab raised against N3ECD (kindly provided by Dr. A. Joutel, INSERM U270, Faculté de Médecine Lariboisière, Paris, France) to demonstrate Notch3 deposition (9). Controls included the omission of the primary antibody and simultaneous staining of known positive or normal material for all techniques.

The presence of apoptotic cells was also looked for using in situ end labeling (ISEL) to identify internucleosomal DNA fragmentation. This was performed with the ApopTag kit (Q Biogene; MP Biomédical, Illkirch, France) according to the manufacturer’s recommendations and modified using an alkaline phosphatase technique to avoid false positivity related to lipofuscin, as described previously (24). Endothelial cells, which have a rapid turnover and often undergo apoptosis, served as an internal positive control (25).

As previously reported (20), semiquantitative evaluation was performed by 2 independent neuropathologists (FG, MB) blinded to the clinical data. The intensity of Notch3 and β-amyloid precursor protein (β-APP) expression was scored as follows: 0 = absent, 1 = mild, 2 = marked, and 3 = intense. Apoptosis of endothelial cells was evaluated by ISEL and scored as follows: 1 = normal physiologic, when 1 positive endothelial cell per vessel section was occasionally found; 2 = increased, when almost every vessel section showed at least 1 apoptotic endothelial cell; and 3 = intense, when each vessel section showed 1 or usually several apoptotic endothelial cells.

The severity of neuronal apoptosis in the cerebral cortex was also evaluated by ISEL and scored as follows: 0 = no apoptotic cells; 1 = occasional isolated apoptotic neurons; 2 = occasional groups of apoptotic neurons; and 3 = frequent apoptotic neurons.

RESULTS

Apoptotic cells were identified using both activated caspase 3 immunostaining and ISEL in all cases with CADASIL and Binswanger disease and only seldom in the “normal” controls. Caspase3 immunopositive cells were always positively stained by ISEL on successive sections, but ISEL-positive cells were much more numerous; therefore, we chose ISEL for semiquantitative evaluation. Caspase3 immunopositive cells were usually normal in appearance (Fig. 1F), consistent with caspase3 activation being an early phenomenon preceding DNA strand breakage and terminal nuclear and cytoplasmic changes (26); however, one cannot exclude the possibility that other

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TABLE 1. Epidemiologic, Clinical, and Genetic Data in 4 Patients with CADASIL, 2 Patients With Binswanger Disease, and 3 Normal Controls

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>Cognitive Impairment: MMS Score</th>
<th>Disease Duration (years)</th>
<th>Notch3 Mutation</th>
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<tr>
<td>CADASIL</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>52/M</td>
<td>+++ not available</td>
<td>13</td>
<td>Exon 3, nt 406</td>
</tr>
<tr>
<td>2</td>
<td>65/M</td>
<td>+ 25/30</td>
<td>10</td>
<td>Exon 3, nt 406</td>
</tr>
<tr>
<td>3</td>
<td>70/F</td>
<td>– 29/30</td>
<td>24</td>
<td>Exon 4, nt 583</td>
</tr>
<tr>
<td>4</td>
<td>69/F</td>
<td>++ 13/30</td>
<td>21</td>
<td>Exon 3, nt 291</td>
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<tr>
<td>Binswanger disease</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>65/M</td>
<td>+++ not available</td>
<td>10</td>
<td></td>
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<tr>
<td>2</td>
<td>82/F</td>
<td>++ 14/30</td>
<td>6</td>
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<td>Controls</td>
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</tr>
<tr>
<td>1</td>
<td>56/M</td>
<td>– not available</td>
<td>—</td>
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<tr>
<td>2</td>
<td>43/M</td>
<td>– not available</td>
<td>—</td>
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<tr>
<td>3</td>
<td>40/M</td>
<td>– not available</td>
<td>—</td>
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</table>

CADASIL, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; MMS, Mini-Mental State; nt, nucleotide.

–, no cognitive impairment; +, mild cognitive impairment; ++, marked cognitive impairment; ++++, dementia.
caspases, other signals, and even other pathways can be involved in the apoptotic process. Some ISEL-positive cells demonstrated characteristic apoptotic morphologic appearances with shrunken cytoplasm and pyknotic nuclei (Fig. 1D, E), consistent with evidence that DNA breakage, while preceding terminal morphologic changes, persists in the later stages of apoptosis (27).

Apoptosis of astrocytes and oligodendrocytes was a frequent finding in the white matter of both the CADASIL andBinswanger disease cases. It was not seen in the normal controls. Apoptosis of white matter glial cells was particularly severe in the more affected regions in the frontal and occipital lobes and seen to a lesser extent in the brainstem and least often in the cerebellar white matter. It was mostly found in the subcortical white matter, always at a distance from lacunar or more extensive infarcts, within peripheral edematous changes (Fig. 1A, B). Apoptotic glial cells were not found in the deep white matter.
where myelin pallor predominated and was associated with severe arteriolar changes with luminal stenosis, including lipohyalinosis, in the Binswanger disease cases (28, 29) and Notch3 deposition with fibrosis (30) in the CADASIL cases.

Apoptosis of macrophages/microglial cells was not uncommon in pathologic areas. Reactive macrophages/microglial cells expressing activated caspase3 or positively stained by ISEL were frequent in destructive lesions, in the white matter and in small cortical infarcts or around ischemic neurons, both in CADASIL and Binswanger disease cases. They were not found in unaffected regions or in the 3 normal controls.

Apoptosis of vascular cells was markedly increased in patients with CADASIL. This finding was particularly obvious in areas of status cribrosus in the lenticular nuclei and thalamus (Fig. 2A–C). The majority of apoptotic vascular cells were endothelial, but a number of recognizable pericytes were also stained by ISEL (Fig. 2D, E). In those areas with “état criblé,” at least 1 vascular cell (score 2) and, more often, several vascular cells (score 3) per vessel section were positively stained by ISEL. The Binswanger

![Figure 2](https://academic.oup.com/jnen/article-abstract/66/7/597/2916833/600)
disease cases showed milder status cribrosus in the lenticular nuclei; status cribrosus was discrete in the thalamus of 1 patient and absent in the other. In those areas only occasional (score 1) apoptotic vascular cells were observed (Fig. 2F).

Dilatation of the perivascular spaces in the subcortical white matter extending to the cerebral cortex (Fig. 3H) was found only in CADASIL patients, predominantly in the temporal lobe. It was particularly striking and visible on gross examination in Case 3 (Fig. 3B), confirming MRI data (Fig. 3A), and also involved the frontoorbital (Fig. 3B) and insular regions in this patient. Within the type III lacunes, in the cerebral cortex and underlying subcortical white matter, the vessel walls were not markedly thickened (Fig. 3C, G, H), and Notch3 deposition was the main vascular alteration. In those areas, at least 1 endothelial
cell or pericyte (score 2) and, more often, several vascular cells (score 3) per vessel section were positively stained by ISEL. Some larger arterioles showed slight adventitial fibrosis and characteristic granular deposits (Fig. 3D) and Notch3 positivity (Fig. 3E) in the absence of significant stenosis. In those vessels, vascular apoptosis involved not only endothelial cells and pericytes, but also a large number of smooth muscle cells (Fig. 3F, G). In contrast, in the deeper white matter, dilatation of the perivascular space was moderate or absent, and most small arterioles showed marked fibrous thickening and luminal stenosis. In those areas, the number of smooth muscle cells was markedly reduced and only a few endothelial cells were positively stained by ISEL (score 1).

Apoptosis of neurons was a consistent finding in the CADASIL cases (20). In the cerebral cortex it predominantly involved layers 3 and 5 as demonstrated by ISEL (Figs. 1C–E, 4B) and expression of activated caspase3 (Fig. 1F); it was more severe in the frontal and occipital lobes (Fig. 4B) where subcortical white matter ischemic changes predominated (Fig. 4A). In those areas there were increased numbers of neurons expressing phosphorylated neurofilament protein (NF) particularly in layers 3 and 5 (Fig. 4D). Neuronal apoptosis was minimal in Ammon’s horn where the underlying white matter was usually devoid of ischemic lesions and only occasional neurons expressing NF were present. Semiquantitative evaluation showed that the severity of neuronal apoptosis in layers 3 and 5 of the cerebral cortex correlated closely with the degree of axonal damage in subcortical white matter (Fig. 4C). Apoptotic neurons were not localized to the rare cortical microinfarcts, and the degree of apoptosis was not related to that of Notch3 deposition. As previously described, the degree of cortical apoptosis appeared to be related to cognitive impairment (20). Apoptosis of nerve cells was milder in the basal ganglia (lenticular nuclei and thalamus) despite frequent lacunar infarcts and constant status cribriform in these nuclei. Axonal damage was not uncommon in those areas but was usually found a distance away in the internal capsule. Neuronal apoptosis was rare or absent in the cerebellar cortex, where associated subcortical lesions were rare.

In the Binswanger disease cases with comparable myelin loss (Fig. 4E) and axonal damage (Fig. 4F) in subcortical white matter, only occasional isolated apoptotic neurons were found in layers 3 and 5 of the frontal and occipital cortex (Fig. 4G). Interestingly, in those regions the number of neurons expressing NF in layers 3 and 5 was comparable to that in CADASIL in the same areas (Fig. 4H). A few single apoptotic neurons were present in the hippocampus of the oldest case and none in the cerebellum. In the normal controls, a single apoptotic neuron was found in the hippocampus in 1 case. No neuron expressed NF. The most significant results involving apoptosis of nerve cells and endothelial cells are summarized in Table 2.

**DISCUSSION**

Using activated caspase 3 immunostaining and ISEL, we identified apoptotic neurons, astrocytes, oligodendrocytes, and microglial and vascular cells in 4 unrelated cases of CADASIL with different mutations of the NOTCH3 gene. It is now well established that once the apoptotic process is initiated, particularly in neurons, the cell is generally cleared in a matter of hours (31); therefore, the relatively large number of apoptotic cells we found in our cases is noteworthy. Interpretation of apoptosis in postmortem studies is an issue deserving further comment. Initiation of apoptosis is a 2-step process (25). The first step (known as priming) designates which cells will undergo programmed cell death. In the second step (known as triggering), primed cells undergo irreversible fragmentation of DNA, which then leads to cell death (25, 32). Triggering events only initiate DNA fragmentation in primed cells (25, 33) and are unlikely to cause apoptosis on their own, so although they can be regarded as amplifying factors, they do not preclude reliable identification of apoptosis. The lack of cortical apoptosis observed in our 3 normal controls further supports this view.

The presence of apoptotic glial cells in the peripheral edematous white matter, away from ischemic foci in patients with both CADASIL and Binswanger disease, was not
unexpected. Programmed cell death occurs after brain ischemia (34). Both animal experiments (35) and neuropathologic studies in humans (16) have shown that apoptosis predominated in the penumbral area, whereas necrosis occurred in the center of the infarct. The degree of glial apoptosis was similar in patients with CADASIL and Binswanger disease. This suggests that both types of arteriopathic leukencephalopathies may result from similar mechanisms, including chronic ischemia in the terminal distribution territories of deep white matter vessels (30, 36) and edema due to disorders of the blood-brain barrier in these vessels (37).

The positive microglial staining is also a nonspecific finding reported in different disorders (24, 38). It was comparable in CADASIL and Binswanger disease cases, and its interpretation is not straightforward. ISEL is not absolutely specific for double-stranded DNA breaks and can also detect single-stranded breaks in cell multiplication (27).

Apoptosis of microglial cells can be considered a physiologic mechanism whereby the brain rids itself of cells that have proliferated after ischemic brain injury and the same mechanism probably also operates for astrocytes (39).

Apoptosis of nerve cells and excessive apoptosis of vascular cells in areas with status cribrosus seem to be original findings more specific for CADASIL. Dilatation of the perivascular spaces is frequent in CADASIL and has been recognized since the initial postmortem case report of the disease (4). An original type of subcortical lacunar lesion in the anterior temporal lobe was found on MRI by van den Boom et al (40) in 59% of patients with CADASIL and was absent in controls. Microscopic examination in 1 case showed dilatation of the perivascular space of the perforating arteries at the junction of gray and white matter, reminiscent of the laminar lacunar lesions between the cortical ribbon and the subcortical white matter described by Ruchoux et al (8). Increased frequency of dilated Virchow-Robin spaces in CADASIL has also been confirmed in a prospective MRI study of 50 CADASIL patients, 1 with pathologic postmortem examination (our Case 1) (13). The authors concluded that status cribrosus due to dilatation of the perivascular spaces was a feature of CADASIL and was mostly located in the putamen and temporal white matter. Our pathologic findings tend to confirm these data and show that status cribrosus in CADASIL is even more diffuse, involving the entire striatum (Fig. 2A, B), the globus pallidus and the thalamus (Fig. 2C).

Involvement of the subcortical white matter, particularly in the temporal lobe, extending in Case 3 to the frontoorbital region (Fig. 3B) and insula (Fig. 3A), was a constant finding in our CADASIL cases and was not observed in the Binswanger disease patients, supporting the view that this localisation may be specific for CADASIL (40).

The etiopathologic mechanisms underlying status cribrosus, état criblé, or type III lacunes (11, 12) are probably multiple and not entirely clear. It is generally accepted that this change reflects an alteration of the blood-brain barrier with increased permeability of the vessel walls resulting from various types of small vessel disease. It is a classical complication of hypertensive small vessel disease (41) and was present in our 2 Binswanger disease cases. It was also a striking feature in a recently described rare hereditary condition characterized by retinal arteriolar tortuosity and leukencephalopathy (42).

In our CADASIL cases, dilatation of the perivascular spaces was not marked in the deep white matter where fibrosis and stenosis of arterioles is severe. It predominated in the gray matter and directly underlying subcortical white matter where the vascular changes were less severe, including adventitial fibrosis and Notch3 deposition in the absence of definite stenosis (30, 43). These findings are in line with the lack of correlation between the extent of dilatation of perivascular spaces and the severity of ischemic or hemorrhagic lesions in CADASIL (13). In contrast, there was a striking increase of vascular cell apoptosis in areas of status cribrosus. This suggests that vascular cell apoptosis, particularly of endothelial cells, together with accumulation of the ectodomain of Notch3 receptor in vessel walls participates in the alteration of the blood-brain barrier underlying the dilatation of perivascular spaces in CADASIL. Both types of vascular changes may result from the same cause. Some in vitro and animal experiments suggest that the Notch3 signaling system may protect vascular cells from apoptosis (17, 18) and among several hypotheses, the possibility has been raised that

FIGURE 4. Neuronal apoptosis in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (A–D) and Binswanger disease (E–H) cases. (A) CADASIL Case 2. Coronal section of the left cerebral hemisphere at the level of the calcarine gyrus: myelin stain shows myelin loss in the deep white matter (Klüver and Barrera stain). (B) Occipital cortex in the same case: numerous nerve cells in the third layer were positively stained by in situ end labeling (ISEL) (ApopTag; original magnification: 300×). (C) Corticosubcortical junction in the occipital cortex in the same case: β-amyloid precursor protein (APP) immunostaining showed axonal damage in the white matter. A few nerve cells with shrunken cytoplasm and pyknotic nucleus were present in the overlying cortex (avidin-biotin complex [ABC] peroxidase; original magnification: 100×). (D) Occipital cortex in the same case: phosphorylated neurofilament protein (NF) immunostaining showed that numerous nerve cells in layers 3 and 5 expressed NF (ABC peroxidase; original magnification: 100×). (E) Binswanger disease Case 2. Coronal section of the left cerebral hemisphere at the level of the calcarine gyrus: myelin stain showed myelin loss in the white matter with relative sparing of the optic radiations and white matter of the gyri (Klüver and Barrera stain). (F) Occipital cortex in the same case: 1 neuron in the third layer was positively stained by ISEL (ApopTag; original magnification: 200×). (G) Corticosubcortical junction in the occipital cortex in the same case: APP immunostaining showed axonal damage in the white matter (ABC peroxidase; original magnification: 100×). (H) Occipital cortex in the same case: immunostaining showed that numerous nerve cells in layers 3 and 5 expressed NF (ABC peroxidase; original magnification: 100×).
Semiquantitative evaluation of neuronal apoptosis, axonal damage in the subcortical white matter, and expression of Notch3 in the cerebral cortex of the frontal lobe, occipital lobe, and hippocampus of 4 CADASIL patients, 2 Binswanger disease patients, and 3 controls.

<table>
<thead>
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<th>Region</th>
<th>Neuronal Apoptosis</th>
<th>Axonal Damage</th>
<th>Notch3 Expression</th>
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</thead>
<tbody>
<tr>
<td>Ammon’s Horn</td>
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<td></td>
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<tr>
<td>Lenticular Nuclei</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
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<td></td>
<td></td>
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<tr>
<td>Subcortical WM</td>
<td></td>
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<tr>
<td>Frontal Lobe</td>
<td></td>
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<tr>
<td>Occipital Lobe</td>
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<tr>
<td>Hippocampus</td>
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<table>
<thead>
<tr>
<th>Region</th>
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<th>Binswanger disease</th>
<th>Controls</th>
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<tr>
<td>Axonal Damage TUNEL</td>
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<td>Notch3 Expression ICC</td>
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The widespread neuronal apoptosis in the cerebral cortex in CADASIL, a model of pure vascular dementia, was also an original finding. In a previous study we found that neuronal apoptosis correlated with cognitive impairment (20). Cortical apoptosis predominated in layers 3 and 5 of the cerebral cortex, which contain large numbers of pyramidal neurons with high levels of acetylcholinesterase activity (44); this finding is consistent with the significant decrease of acetylcholinesterase activity reported in a CADASIL patient with dementia (15). The hippocampal areas were relatively spared (15), in keeping with the regional pattern of apoptosis observed in our study. It is also consistent with the preservation of memory-encoding processes, even in the late stages of CADASIL, in patients with dementia (10, 45).

Unlike apoptosis of glial cells, neuronal apoptosis seemed to be neither topographically nor quantitatively related to ischemia. Indeed, neuronal apoptosis was not found within or close to the occasional cortical micro-infarcts present in the patients. It was not seen in the hippocampus despite the presence of some ischemic neurons. In our patients, semiquantitative evaluation showed that the number of apoptotic neurons in cortical layers 3 and 5 correlated well with the extent of white matter lesions and the intensity of axonal damage in subcortical white matter. This finding suggests that apoptosis of cortical neurons in CADASIL may be due to axonal damage in the underlying white matter through deafferentation (46) or retrograde neuronal degeneration (47, 48). The observation of an increased number of neurons expressing NF in layers 3 and 5 of the cortex overlying white matter with severe axonal damage, in the areas with marked neuronal apoptosis, tends to support that view. Indeed neurofilament phosphorylation in neuronal perikarya has been shown to occur after axotomy (49, 50). A secondary mechanism might be more common than previously thought; it has been proposed that cognitive impairment associated with subcortical ischemic vascular disease may result from loss of cortical gray matter (51). The observation of apoptotic neurons in layers 3 and 5 of the cortex in our cases with Binswanger disease tends to support that view. However, the smaller number of apoptotic neurons in the Binswanger disease cases who showed comparable axonal lesions in the subcortical white matter and a comparable number of NF-expressing neurons in the overlying cortex suggests that in CADASIL other factors operate, such as impairment of Notch3 signaling. Although Notch3 expression is limited to vascular smooth muscle cells in adult human tissues (9), it is more widely expressed in the developing CNS in which it has been shown that loss of Notch3 activity leads to increased neuronal apoptosis (52). One can speculate that in pathologic circumstances that may cause neuronal apoptosis (i.e. axonal damage) in adults, impairment of Notch3 signaling system may have an amplifying or aggravative role.
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