Cerebral Microbleeds in CADASIL
A Gradient-Echo Magnetic Resonance Imaging and Autopsy Study

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Background and Purpose—An increased frequency of clinically silent microbleeds (MB) has recently been observed in patients with sporadic small-vessel disease related to vascular amyloid deposition or hypertension. In this study, we searched for cerebral MBs in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a unique type of small-vessel disease caused by mutations in the Notch3 gene. Our purposes were (1) to determine the frequency, extent, and pattern of MBs in CADASIL; (2) to analyze the relationship between MBs and T2-hyperintense lesions; and (3) to evaluate the histopathology of brain tissue affected by MBs.

Methods—Gradient-echo, T2/PD-weighted dual-echo, and T1-weighted MRI scans of the brain were obtained from 16 consecutive CADASIL subjects and 16 age-matched control subjects. T2-lesion volume measurements were made with a semiautomated segmentation technique based on local thresholding. Postmortem examinations were performed on the brains of 7 additional CADASIL subjects.

Results—Focal areas of signal loss on gradient-echo images suggesting past MBs were found in 11 CADASIL individuals (69%) and no control subjects (P<0.001). The average number of MBs was 5.9±7.3 (range, 0 to 22) in individual CADASIL patients. MBs were associated with age (r=0.71, P=0.002) and total lesion volume (r=0.75, P=0.001). However, after correction for age, the correlation with lesion volume was no longer significant. MBs were located simultaneously in various parts of the brain with a preference for cortical-subcortical regions (38%), white matter (20%), thalamus (13%), and brainstem (14%). Eighty-two percent of the MBs were located outside areas appearing hyperintense on T2-weighted images. Postmortem examination revealed focal accumulations of hemosiderin-containing macrophages in 6 of the 7 brains (86%). They were always found outside ischemic lesions.

Conclusions—This study shows a high frequency and multiplicity of MBs in individuals with CADASIL. Our results suggest that MBs and ischemic lesions are largely independent manifestations of the underlying angiopathy. The pattern of MBs shows a significant overlap with that reported in other types of small-vessel disease. (Stroke. 2002;33:67-71.)

Key Words: angiopathy • CADASIL • echo-planar imaging • intracerebral hemorrhage

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary disease of small blood vessels caused by mutations in the Notch3 gene.1,2 Clinical manifestations include recurrent ischemic episodes, cognitive deficits, and migraine mostly with aura, as well as psychiatric disorders.3,4 There have been single reports of intracerebral hemorrhages (ICHs),5,6 but the meaning of these observations remains unclear given the absence of ICH in large patient series.3,4

MRIs show lacunar infarcts and less well demarcated subcortical T2 hyperintensities that may show different degrees of hypointensity on T1-weighted scans.7-9 These alterations are caused by a unique type of nonarteriosclerotic, amyloid-negative angiopathy involving small arteries and capillaries primarily in the brain but also in other organs.10 Ultrastructural examination reveals characteristic granular osmiophilic material within the vascular basal lamina, which is often seen in close association with vascular smooth muscle cells. Vascular smooth muscle cells have been shown to degenerate and eventually disappear.11 Together, these changes contribute to progressive disintegration of the vascular wall.

Recent MRI studies of patients with other types of small-vessel disease (SVD) such as cerebral amyloid angiopathy and hypertensive SVD have called attention to a high frequency of petechial hemorrhages (microbleeds [MBs]) in these conditions.12-17 MBs have further been observed in asymptomatic elderly individuals.18 They were found to be associated with age, hypertension, and extensive white matter changes,18,19 which are risk factors or indicators of SVD. MBs have thus been connected to structural alterations of small blood vessels. From these findings, we hypothesized...
that the angiopathy in CADASIL may similarly predispose to MBs.

At the site of an ICH, hemosiderin remains stored in macrophages. T2*-weighted gradient-echo (GE) pulse sequences enhance the magnetic susceptibility (and resultant signal dropout) caused by the deposition of chronic blood products in tissues, thus increasing the sensitivity for hemorrhages.20 Because hemosiderin remains stored at the site of a hemorrhage for many years, GE MRI is suited to assess the overall pattern of past hemorrhages.13,16

In the present study, we obtained GE and dual-echo MRI scans (1) to determine the frequency, extent, and pattern of MBs in CADASIL and (2) to analyze the relationship between MBs and T2-hypointense lesions. Postmortem examinations were performed on the brains of 7 additional CADASIL patients to investigate the histopathology of brain tissue affected by MBs.

Patients and Methods

Study Population

We studied 16 consecutive subjects with CADASIL (6 men, 10 women; mean age, 49.5±10.7 years; range, 29 to 64 years; mean disease duration, 12.6±11.5 years; range, 0 to 45 years) from 13 families. All but 1 individual had developed ≥1 of the following manifestations: migraine with aura (n=8 patients), transient ischemic attacks and/or stroke (n=13), dementia (n=1), or depression (n=3). In all cases, the diagnosis had been confirmed either by skin biopsy (n=10)21–23 or by demonstration of a Notch3 mutation (n=14); R90C (n=4), C93F, R110C, R133C, R169C (n=2), R182C (n=2), C194F, or delD239-D253 (n=2).24–25

Control subjects consisted of 16 age-matched healthy subjects (5 men, 11 women; mean age, 49.7±9.4 years; range, 32 to 63 years). Informed consent was obtained from all subjects before study initiation. None of the 32 subjects had taken oral anticoagulants in the past. Moderately elevated blood pressure levels (systolic pressure, between 140 and 160 mm Hg) were found in 2 CADASIL patients and 1 control subject. There was no history of head trauma, intracranial tumors, coagulopathy, or ICH in any of the subjects.

MRI Study

MRI scans were obtained from all subjects with the same 1.5-T system (Magnetom Vision, Siemens). Twenty-four contiguous axial 5-mm-thick slices (no interslice gap) were obtained with the following pulse sequences: (1) T2/PD-weighted dual-echo fast spin echo (repetition time [TR], 3030 ms; echo time [TE], 16/98 ms; field of view [FOV], 250 mm; matrix, 192×256) and (2) T1-weighted spin echo (TR, 768 ms; TE, 14 ms; FOV, 250 mm; matrix 192×256). Twenty-two axial contiguous 5-mm-thick slices (interslice gap, 1.5 mm) were obtained with a T2*-weighted GE sequence (TR, 1000 ms; TE, 22 ms; flip angle, 40°; FOV, 210 mm; matrix, 224×256). T2*-weighted GE images were also obtained in the coronal plane (23 contiguous 4-mm-thick slices without interslice gap). The axial slices were positioned on a plane that joined the most inferointerolateral and infero-posterior parts of the corpus callosum.

Images were jointly analyzed by 3 raters (T.A.Y., M.H., M.D.). Focal areas of prominent signal loss on T2*-weighted images were analyzed. MBs were defined as homogenous rounded lesions with a diameter >2 mm.14,15,16 Areas of symmetric hypointensity of the globus pallidus, likely to represent calcifications or nonhemorrhagic iron deposition, were disregarded.18,26 Vascular flow void artifacts were excluded by tracing areas of local signal loss on adjacent slices. The number of MBs was separately assessed for each of the following locations: cortical (ie, strictly limited to the cortex), cortical-subcortical, white matter, basal ganglia (caudate nucleus, putamen, globus pallidus), thalamus, brainstem (midbrain, pons, medulla oblongata), and cerebellum (cortical-subcortical, white matter, deep nuclei). MBs were further classified according to their spatial relationship to T2-hypointense lesions as follows: within lesions (ie, surrounded by a rim of T2-hypointense signal), adjacent to lesions (<3 mm apart), or at distance from lesions (≥3 mm) appearing hypointense on T2/ PD-weighted images. Any abnormalities present on the MRI scans were identified consensually by the 3 observers. Lesion volume measurements were performed with a semiautomated segmentation technique based on local thresholding.27

Autopsy Study

Postmortem examinations were done on the brains of 7 additional CADASIL cases (5 men, 2 women) who had come to autopsy between 1986 and 1998. None of them had previously been investigated by GE MRI, thus precluding a correlation study between neuroimaging and postmortem findings. In 6 of them, mutational screening of the Notch3 gene revealed a deleterious mutation (R110C, R117F, R133C, C174Y, C185R). Age at death ranged from 28 to 64 years (mean, 52.3±13.2 years). The postmortem delay was 5 to 48 hours. Brains were fixed in an unbuffered 7% formaldehyde solution for ≥2 weeks and were cut into 1-cm coronal (6 cases) or horizontal (1 case) sections. The brainstem was cut into horizontal sections. Large hemispheric tissue blocks were taken from the frontal lobe at the plane of the tip of the anterior horn, precentral region with temporal lobe at the plane of the striatum, postcentral region at the plane of the geniculate bodies, and occipital lobe adjacent to the posterior horn. Additional tissue was taken from optic nerves and chiasma, geniculate bodies, cortex, midbrain, pons, medulla, and cerebellum.

The tissue was embedded in paraffin wax and cut into 10-μm sections for histology. Each tissue block was processed for the following staining methods (1 section each): hematoxylin and eosin, cresyl violet, Klüver-Barrera myelin stain, Bodian silver impregnation for axons, van Gieson’s stain for elastic fibers, Perls’ reaction for hemosiderin, periodic acid-Schiff reaction, and congo red (for amyloid).

MBs were defined as focal accumulations of hemosiderin-containing macrophages17 visible on Perls’ reaction stains. Hemosiderin deposits not contained within macrophages and limited to the basal ganglia, likely to represent nonhemorrhagic iron deposition, were disregarded.28

Statistical Analysis

Statistical evaluation was performed with the Statistical Analysis System version 8.01 for Windows (SAS Institute). Differences between CADASIL individuals and control subjects were investigated with Fisher’s exact test. Correlations between neuroimaging variables and age were calculated with the Spearman rank correlation coefficient. To correct for multiple testing, a value of P≤0.01 was considered significant.

Results

MRI Study

All CADASIL individuals showed hypertensive T2-weighted abnormalities on brain MRI. The volume of lesions ranged from 51.7 to 214.1 mL (mean, 110.8±51.6 mL) and correlated significantly with age (r=0.74, P<0.001). Single white matter lesions were found in 12 control subjects (mean volume, 0.2±0.2 mL; range, 0 to 0.8 mL).

GE T2*-weighted MRIs revealed MBs in 11 CADASIL individuals (69%) and no control subjects (P<0.001) (Figure 1). The total number of MBs in CADASIL individuals was 94 (mean, 5.9±7.3; men, 6.7±8.1; women, 5.4±7.1). The number of MBs detected in CADASIL individuals correlated with age (r=0.71, P=0.002) and volume of lesions (r=0.75, P=0.001). However, after correction for age, the correlation

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with lesion volume was no longer significant (partial correlation coefficient $= 0.48$, $P = 0.07$).

The number of MBs found in individual CADASIL patients ranged from 0 to 22. In most cases, MBs were noticed simultaneously in various parts of the brain (Figure 1). The distribution of MBs was as follows (in descending order): cortical-subcortical, 36 (38%); white matter, 19 (20%); brainstem, 13 (14%: pons, 9; medulla, 3; midbrain, 1); thalamus, 12 (13%); basal ganglia, 7 (8%); cerebellum, 4 (4%: white matter, 3; cortical-subcortical, 1; deep nuclei, 0); and cortical, 3 (3%). The size of the MBs ranged from 2 to 10 mm, with most (90%, n = 85) ranging from 2 to 5 mm.

Seventy-seven of the MBs (82%) were located outside areas appearing hyperintense on T2/PD-weighted images (Figure 1). More specifically, 47 MBs (50%) were located at a distance from ($\geq 3$ mm) and 30 MBs (32%) were located adjacent to (<3 mm) such areas. Seventeen MBs (18%) were found within areas appearing hyperintense on dual-echo scans.

**Autopsy Study**

All 7 autopsy cases showed lacunar infarcts and diffuse white matter changes, with the latter consisting of various degrees of demyelination, axonal loss, gliosis, and enlargement of the extracellular spaces. In all cases, electron microscopy revealed characteristic granular osmiophilic material within the basal lamina of small blood vessels.

Focal accumulations of hemosiderin-containing macrophages were found in all but 1 of the 7 brains (86%) examined. The single patient without past hemorrhages was younger (age, 28 years) than those with such findings (mean age, 56.5 ± 8.2 years; range, 48 to 65 years). A total of 7 past hemorrhages were found in the following locations: white matter (n = 2), basal ganglia (n = 1), pons (n = 2), and optic nerve (n = 2). Their size ranged from 0.2 to 1.0 mm, and they were seen only at the microscopic level. In all cases, siderophages were found in the vicinity of small blood vessels (diameter, 100 to 300 $\mu$m), which showed degenerative changes in their wall but were surrounded by intact-appearing tissue with no evidence of ischemic damage (Figure 2). In none of the cases was there evidence of vascular amyloid by congo red staining. In addition, there was no evidence of vascular malformations or parenchymal calcifications.

**Discussion**

This study shows a high frequency and multiplicity of homogenous rounded foci of prominent signal loss on GE T2*-weighted cranial MRIs of CADASIL individuals. Previous studies correlating MRI with histopathological findings
have shown that such signal abnormalities represent hemosiderin deposits after previous MBs.\textsuperscript{14,17} We cannot exclude other potential causes of focal T2-signal loss such as parenchymal calcifications or vascular malformations in our MRI study. However, no such changes were found in our autopsy material, whereas a high frequency of MBs in CADASIL is demonstrated by our finding of petechial hemorrhages in 6 of 7 autopsy cases. Because MBs are small and inconspicuous in routinely stained histological sections, they may have been missed in previous autopsy studies.

Our findings confirm and extend previous studies indicating a strong association between MBs and SVD.\textsuperscript{14,16,17} Vascular abnormalities in CADASIL involve virtually all layers of the vascular wall.\textsuperscript{10,11,29,30} Moreover, some authors have described Charcot-Bouchard aneurysms.\textsuperscript{31} It is conceivable that these changes may affect the vulnerability of blood vessels in CADASIL.

MBs were found to be scattered throughout the brain without a clear predilection for one specific structure. This agrees with the generalized nature of the underlying angiopathy. Most MBs were found to be located outside T2 hypersignals. Similarly, on histopathological analysis, hemosiderin-containing macrophages were found within largely intact brain tissue. These findings suggest that most petechial hemorrhages occur independently from ischemic lesions in CADASIL. Our observations are in some contrast with findings in sporadic SVD. Tanaka et al\textsuperscript{14} found MBs to be commonly surrounded by T2-hyperintense areas. On histopathological analysis, they found foci of old hemorrhages to be regularly associated with gliosis and ischemic lesions.\textsuperscript{14} However, in another study, the association between hemosiderin deposits and tissue necrosis was less consistent.\textsuperscript{17} In our CADASIL population, only a small proportion of MBs were found to be located within areas appearing hyperintense on T2-weighted scans. Given the large volume of T2 hypersignals, the observed colocalization could be coincidental. Alternatively, it could indicate some direct relationship between ischemic lesions and petechial hemorrhages in a subset of MBs. Experimental and clinical studies have shown secondary alterations in vascular permeability and vessel wall integrity after the occlusion of large arteries.\textsuperscript{32–34} However, whether such secondary changes occur in SVD is still unknown.

In this study, we found a significant correlation between age and number of MBs. Similarly, there was a strong correlation between age and volume of lesions appearing hyperintense on dual-echo scans, as expected from previous studies.\textsuperscript{35,36} In contrast, there was no significant correlation between the number of MBs and volume of lesions when age is introduced as a covariate. These findings are in keeping with the chronic nature of the underlying angiopathy and its proposed role in causing 2 largely independent complications: ischemic lesions and petechial hemorrhages.

The clinical implications of MBs in CADASIL are unknown. From large patient series, there is no evidence of an increased frequency of hemorrhagic stroke.\textsuperscript{3,4} However, there have been 2 reports on normotensive CADASIL patients who died after an ICH.\textsuperscript{5,6} None of them had been investigated for the presence of MBs. Quite relevantly, 1 of the patients had been treated with oral anticoagulants. Thus, it is premature to conclude that there is an increased frequency of ICH or a predictive potential of MBs in CADASIL. However, in view of the known risk for anticoagulant-related ICH in patients with extensive white matter lesions,\textsuperscript{37} particular caution must be advised in giving oral anticoagulants to CADASIL patients.

It has been suggested that particular angiopathies are associated with specific patterns of MBs. Thus, for example, there is evidence of a high frequency and preferential occurrence of cortico-subcortical MBs in cerebral amyloid angiopathy.\textsuperscript{12} The results of the present study and those of others\textsuperscript{15,16,18} indicate a considerable overlap between the distribution of MBs seen in different conditions. Consequently, the diagnostic importance of particular MB patterns remains questionable, whereas the presence of MBs appears to be a valuable indicator for SVD.

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