Intracortical Infarcts in Small Vessel Disease

A Combined 7-T Postmortem MRI and Neuropathological Case Study in Cerebral Autosomal-Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy

Eric Jouvent, MD, PhD; Cyril Poupon, PhD; Françoise Gray, MD; Claire Paquet, MD; Jean-François Mangin, PhD; Denis Le Bihan, MD, PhD; Hugues Chabriat, MD, PhD

Background and Purpose—The purpose of this study was to report the detection of infarcts of the cerebral cortex in a patient with cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) using high-resolution postmortem 7-T MRI in association with pathological examination.

Methods—Whole brain high-resolution MRI data were obtained postmortem at 7 T in a 53-year-old patient with CADASIL. These MRI data were used to guide the neuropathological examination of the cortex.

Results—Combined with neuropathology, MRI allowed the delineation of intracortical infarcts confirmed by histological examination in this case. These lesions were not visible on the last in vivo MRI obtained at 1.5 T and were difficult to detect on neuropathological examination only.

Conclusions—Postmortem high-resolution MRI may help to detect intracortical infarcts in CADASIL and possibly in other small vessel diseases of the brain. (Stroke. 2011;42:e27-e30.)

Key Words: CADASIL ■ cerebral cortex ■ lacunar infarcts ■ MRI ■ neuropathology ■ small vessel disease

Some data suggest that the cerebral cortex may be involved in small vessel disease (SVD) of the brain related to age, hypertension, and diabetes.1,2 In those SVDs, like in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), microinfarcts and neuronal apoptosis have been described in postmortem pathological studies.3 Such microscopic structural changes might be extremely difficult to see in vivo because imaging techniques such as standard MRI (<3 T) do not provide adequate spatial resolution and ex vivo because microscopic examination cannot cover the whole brain so that small scattered lesions are easily missed. Ultra-high-field MRI, however, has the potential to offer adequate spatial resolution for the detection of very small focal ischemic or hemorrhagic lesions within the cerebral cortex in SVD. We report a postmortem MRI study performed in a patient with CADASIL on a 7-T MRI scanner in combination with subsequent pathological examination.

Methods

Patient

A 53-year-old patient had been followed in Lariboisière Hospital since 2006. He presented with a progressive cognitive decline at the age of 40 years. After his first stroke at age 50 years (left hemiparesis of acute onset), his Mini-Mental State Examination score was 21, his Mattis dementia rating scale 127, and his modified Rankin Scale score 1. The patient underwent 2 clinical MRI scans (in May 2006 and December 2008) after the genetic test of CADASIL had confirmed the presence of a typical mutation in the Notch3 gene. Compared with the whole cohort (288 patients), lesion loads on MRI were high (lacunar lesion volume, 3865 mm3 on first MRI and 4407 mm3 on second, above the 95th percentile; white matter lesion volume, 8473 mm3 on first MRI and 117 803 mm3 on second, above the 55th percentile; microhemorrhage number, 1 on first MRI, 2 on second, above the 70th percentile; Figure 1). After a second stroke at age 53 years, his clinical status rapidly worsened. The patient died 6 months later from complications of a urinary tract infection. The patient gave his informed consent to participate in this study, which was approved by a local ethics committee.

MRI and Image Processing and Analysis

The MRI protocol used in vivo has been previously reported.5 Postmortem MRI was performed using a 7-T clinical MRI scanner (Siemens, Erlangen, Germany) equipped with a head gradient insert coil (80 mT/m, 333 T/m/s) and an 8-channel head coil. The brain was contained in an hermetic plastic cylinder filled with a 10% buffered formalin solution and tightly maintained between hydrophilic textile sheets. High-resolution 3-dimensional T2* (HR-MRI) sequences were acquired through 3 contiguous slightly overlapping blocks; field of view=192 mm; 176 slices; slice thickness=300 μm; in-plane matrix 640×640 leading to an isotropic spatial resolution of
Low-resolution 2-dimensional T2* sequences were acquired through 7 contiguous slightly overlapping blocks, field of view 268 mm; 20 slices; slice thickness TH 700 μm; in-plane matrix 384×384; repetition time TR=900 ms; echo time TE1/TE2=13.7/29.9 ms; flip angle=65°; read bandwidth=70 Hz/pixel; averaging number of excitations=2, total scan time: 43 minutes.

**Pathological Examination**

Autopsy was performed 3 hours after death and was limited to the brain. MRI acquisitions were made after 1 month of 10% buffered formalin fixation followed by macroscopic examination on 1-cm thick coronal sections of the cerebral hemispheres and of the brain stem with the cerebellum perpendicular to its long axis. Those involving the cerebral hemispheres 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 of 3.0×2.0×0.5 cm were taken from the cerebral cortex with underlying white matter, basal ganglia, hypothalamus, midbrain, cerebellum, and brain stem. Additional samples were taken according to the results of HR-MRI. On selected 5-μm thick sections of samples from the frontal lobe at the level of the rostrum of corpus callosum (F1), occipital lobe at the level of the calcaneous sulcus, hippocampus, basal ganglia, thalamus, and cerebel- lum, immunohistochemistry was performed using an avidin–biotin complex, peroxidase-based method with antibodies raised against the protein β-amyloid Aβ (monoclonal β/A4 amyloid protein; Dako Cytomation, Glostrup, Denmark; 1/100), the phosphorylated tau protein (monoclonal mouse antihuman PHF-τ, clone AT8; Innogenetix, Gent, Belgium;1/20), and Notch3 (monoclonal antibodies raised against N3ECD kindly provided by Dr A. Joutel, INSERM U270, Faculté de Médecine Lariboisière, Paris, France; 1/10).

**Results**

HR-MRI revealed countless focal hypointensities scattered through the cortical mantle. The vast majority were hypointensities of linear shape with regular edges, a few hundred micrometers in diameter, crossing the cortical mantle on consecutive MRI slices. Those lesions actually corresponded to microvessels passing through the cortex. We also observed approximately 20 small hypointense foci of irregular shape and of signal intensity similar to that of white matter. Some of these lesions were round and did not reach the cortical mantle edges, whereas others were pyramidal with their bases lying on gray/white matter interface (Figure 2). Both lesions were present in all cerebral lobes, including the hippocampus (Figure 3). These lesions were more difficult to discern on low-resolution MRI (Figure 2). Histological examination showed that these lesions corresponded to intracortical infarcts. Few of these intracortical infarcts exhibited focal hemorrhages at their periphery, which were visible through small susceptibility artifacts on MRI (Figure 3). No “pure” microhemorrhage was detected within the cortex. Approximately 75% of these lesions were also seen on low-resolution MRI.

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**Figure 1.** MRI scans of our patient at 2 different visits in our center. From left to right: 3-dimensional T1-weighted, fluid-attenuated inversion recovery and gradient-echo (GRE) T2*. Compared with the whole cohort, this patient had high lesion loads.

**Figure 2.** Pathologically confirmed intracortical infarcts detected by HR-MRI. Round-shaped lesions confined to the cortical mantle (a, c, e and h), and pyramidal-shaped infacts with a large side on the gray/white border (d, f, g and i; note the hemosiderin border in g). These ischemic lesions were also visible on low-resolution MRI but more difficult to discern from the microvasculature (a’). Scale differs for each vignette; scaling factor is represented with black bars exactly corresponding to 10 voxels (3 mm). The extent of underlying white matter damage is not comparable to that noted in vivo on MRI scans. This may be related to the MRI sequences used in the present study or to formalin fixation.
Immunochemistry did not identify foci of amyloid angiopathy either near the intracortical infarcts or in the remaining material, but instead typical arteriolar changes of CADASIL containing Notch3-positive material. They were particularly conspicuous in leptomeningeal arterioles in which granular material was also present (Figure 4). In the neighborhood of the 2 types of intracortical infarcts, lumen narrowing and extent of vessel wall damage were similar. We did not find any thrombotic material within the vessel lumen in either case. We also observed diffuse Aβ plaques and occasional amyloid plaques in the absence of overt tau pathology throughout the cerebral cortex. These changes differed from the typical Alzheimer disease changes as already reported in previous CADASIL cases.

**Discussion**

To our knowledge, this is the first description of intracortical infarcts in CADASIL as well as in SVD related to age, hypertension, and diabetes detected postmortem using both whole brain 7-T MRI and pathological examination. The use of HR-MRI improved the detection of intracortical infarcts at pathological examination because their small number and wide scatter through the whole cerebral cortex would render their detection difficult based on pathological examination only.

There is accumulating evidence suggesting a possible role of cortical microinfarcts (detectable only by histology) on cognitive function in different populations, but the clinical impact of such lesions is still unclear, whereas it is largely recognized in cerebral amyloid angiopathy, another type of SVD. The present results further support the importance of cortical lesions that are not visualized on conventional MRI in SVD.

At 7 T, most lesions detected with HR-MRI were also visible using low-resolution MRI, although they were more difficult to differentiate from microvessels. This suggests that 7-T MRI could be used also in vivo to detect intracortical infarcts within the cortex. We identified 2 subtypes of intracortical infarcts. Whether these different subtypes are related to distinct underlying mechanisms is unknown. The different diameter or length of the very short penetrating arteries supplying the corresponding areas may explain this finding.

**Figure 3.** Comparison between HR-MRI and neuropathology. Top: occipital pyramidal-shaped intracortical infarcts (left, MRI; right, hematoxylin and eosin coloration); Bottom: intracortical infarcts within CA1 (left, MRI; right, glial fibrillary acidic protein immunochemistry, showing reactive gliosis around a cystic cavity). For MRI samples, black bar corresponding exactly to 10 voxels (3 mm) is shown for comparison.

**Figure 4.** Alterations of leptomeningeal arterioles in our case. Left, Hematoxylin and eosin staining reveals vessel wall alterations in leptomeningeal arterioles. Right, Immunostaining with Notch3 antibodies reveals positive staining of the vessel wall in leptomeningeal arterioles.
This study has several limitations. The data were obtained in a single case but our choice was to exclude sliced material or tissue fixed for years with formalin due to potential heterogeneous consequences on the MR signal. The lack of a control sample also limits the interpretation of the results. The role of hypertension or of another SVD in this case cannot be totally excluded, although this appears unlikely because typical vascular alterations of CADASIL were detected in the close vicinity of intracortical infarcts in the absence of amyloid angiopathy. Moreover, the patient had no history of hypertension or diabetes, his blood pressure was normal at inclusion, and glycohemoglobin was 4.7%.

So far, the presence of lacunar lesions in deep brain structures has led to the hypothesis that the length of penetrating arterioles was a key determinant in the occurrence of ischemic lesions in SVD related to hypertension and diabetes. The presence of intracortical infarcts in a genetic model of SVD may indicate that the central role played by the length of arterioles was overestimated in SVD because lesions occurring in deep structures are easier to detect than few lesions scattered over the whole cortical mantle. Further systematic studies are thus needed to search for the presence of intracortical infarcts in different types of SVD. The results of this study suggest that high-resolution MRI can help their detection in postmortem cerebral tissue. Whether optimized in vivo sequences can be used to detect these lesions will also need further investigations.

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Disclosures
None.

References
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小血管病における皮質内梗塞
— 死後の 7-T MRI と神経病理学的検査を併用した、皮質下梗塞および白質脳症を伴う常染色体優性遺伝性脳動脈線の症例研究

Intracortical Infarcts in Small Vessel Disease — A Combined 7-T Postmortem MRI and Neuropathological Case Study in Cerebral Autosomal-Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy

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背景および目的: 本研究の目的は、死後の 7-T 高分解能 MRI と病理学的検査により検索された、皮質下梗塞および白質脳症を伴う常染色体優性遺伝性脳動脈線 (CADASIL) の一例における脳血管病変を検討することであった。

方法: 53 歳の CADASIL 症候群の死後に 7-T MRI を実施し、脳全体の高分解能 MRI データを収集した。この MRI データを船形として、皮質の神経病理学的検査を実施した。

結果: 本症例では両検査を併用することで、MRI により皮質内梗塞を描出し、病理学的検査でそれを確認することもできた。生前最終に実施した 1.5-T MRI では、これら梗塞は描出できず、また神経病理学的検査のみでの検出は困難であった。

結論: 死後の高分解能 MRI は、CADASIL を含む脳小血管病の皮質内梗塞を検出するのに役立つと考えられる。

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図 1

高分解能 MRI 所見と神経病理学的な所見の比較: 上: 細胞内を形成した微弱な皮質内梗塞 (左: MRI)、右: ヘマトキシリンエオジン染色。下: CA1 の変形、左: MRI、右: グリア線維結合蛋白による免疫染色。変形の進展に伴うグリア線維の改変が観察される。
微小梗塞の病理、認知症、認知機能系
Microinfarct Pathology, Dementia, and Cognitive Systems

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背景および目的：認知症および認知機能欠損における微小梗塞の頻度は明らかではないが、本研究では地域社会における高齢者を対象に、微小梗塞と認知症、認知機能系の関連を検討した。

方法：Religious Orders Studyの被験者425例で、年1回の随時で、9種類の神経心理学的検査、認知症評価、脳SCAT検査、判定試験、閃僥試験を実施した。神経心理学的検査では、皮質および皮質下領域から採取した6μm切片をヘマトキシリン・エオジンで染色し、慢性微小梗塞の有無、部位を確認した。多変量回帰分析を実施し、死亡、卒中、内頸動脈硬化、アルツハイマー病の病期、レジドール症状について修正を行った。

結果：425例中129例（30%）に微小梗塞が認められた（皮質：54例、皮質下：85例、複数例：49例）。微小梗塞が認められた129例のうち58例（45%）には認知機能域のオッズが高く（OR = 1.77, 95% CI: 1.07～2.92）。特に皮質下に複数の微小梗塞を有する場合の値は高かった。また、微小梗塞は認知機能の平均的低下をも関連しており（偏定着 = 0.287, SE = 0.113, p = 0.012）、特に皮質下に複数の微小梗塞を有する場合にその傾向が顕著であった。微小梗塞とエピソード記憶（または出来事記憶）低下（偏定着 = 0.279, SE = 0.138, p = 0.044）、意図記憶低下（偏定着 = 0.291, SE = 0.120, p = 0.003）、記憶速度低下（偏定着 = 0.400, SE = 0.117, p < 0.001）の間には、明確な関連がみられた。さらに、単一、複数、皮質の微小梗塞の増加とともに、記憶域低下および認知機能の低下の間にも関連が認められた（いずれも p < 0.028）。内頸動脈硬化とアルツハイマー病の病程はこれらの関連に影響を及ぼしていない（いずれも p > 0.154）。

結論：微小梗塞は易感性にみられ、特に、皮質下に複数の微小梗塞を有する者は認知症のオッズが高い。微小梗塞は認知機能低下をも関連しており、特に記憶速度、意味記憶、エピソード記憶と強く関連している。

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