Neuropathological Correlates of Temporal Pole White Matter Hyperintensities in CADASIL

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Background and Purpose—White matter (WM) hyperintensities on MRI or leukoaraiosis is characteristic of stroke syndromes. Increased MRI signals in the anterior temporal pole are suggested to be diagnostic for cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), with 90% sensitivity and 100% specificity. The structural correlates of these specific WM hyperintensities seen on T2-weighted and FLAIR sequences in the temporal pole of CADASIL are unclear. We assessed pathological changes in postmortem tissue from the temporal pole to reveal the cause of CADASIL-specific WM hyperintensities.

Methods—A combination of tinctorial and immunostaining approaches and in vitro imaging methods were used to quantify the extent of perivascular space (PVS), arteriosclerosis determined as the sclerotic index, WM myelination as the myelin index, and damage within the WM as accumulated degraded myelin basic protein in samples of the anterior temporal pole from 9 CADASIL and 8 sporadic subcortical ischemic vascular dementia cases, and 5 similarly aged (young) and 5 older controls. Luxol fast blue-stained serial sections from a CADASIL case were also used to reconstruct the temporal pole, which was then compared to the MR images.

Results—Luxol fast blue sections used to reconstruct the temporal pole revealed an abundance of enlarged PVS in the WM that topographically appeared as indistinct opaque regions. The mean and total areas of the PVS per WM area (%PVS) were significantly greater in CADASIL compared to the controls. The myelin index was severely reduced in CADASIL in relation to the subcortical ischemic vascular dementia and control sample that was consistent with increased immunoreactivity of degraded myelin basic protein, indicating myelin degeneration. Cerebral microvessels associated with the PVS exhibited a 4.5-fold greater number of basophilic (hyalinized) vessels and a 57% increase in the sclerotic index values in CADASIL subjects compared to young controls. A significant correlation between the quantity of hyalinized vessels and sclerotic index values was also apparent (P<0.05).

Conclusions—Our findings suggest that MRI hyperintensities in the temporal pole of CADASIL patients are explained by enlarged PVS and degeneration of myelin accompanied by lack of drainage of the interstitial fluid rather than lacunar infarcts. Consistent with the lack of MR hypersignals in the temporal pole of older subcortical ischemic vascular dementia subjects, our observations imply greater progression of pathological changes in CADASIL patients. (Stroke. 2009;40:00-00.)

Key Words: cognitive impairment CADASIL dementia stroke subcortical ischemic vascular dementia

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is likely the most common form of all hereditary small vessel diseases of the brain, which leads to cognitive decline and dementia.1 CADASIL is caused by mutations within the epidermal growth factor-like repeat domain of the NOTCH3 gene located on chromosome 19p13. More than 150 mutations have been described, with nearly all involving either a gain or loss of cysteine residue. Although the pathological mechanisms remain unclear, the gene defects are linked to degeneration of vascular smooth muscle cells both in peripheral organs and the brain, leading to multiple small infarcts in the white matter (WM), deep gray matter, and the pons.2 The distinct arteriopathy in CADASIL, which involves thickening of vessel wall, loss of vascular smooth muscle cells, and endothelial cell abnormalities3 ultimately affects cerebral perfusion,4 as demonstrated by neuroimaging tracer studies.5,6 MRI has enabled characterization of the burden of leukoaraiosis and lacunar infarction in stroke syndromes. In CADASIL,7,8 T2-weighted hyperintensities in the deep WM, internal and external capsules, and especially in the temporal pole are almost selective, with a suggested 90%

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sensitivity and 100% specificity. Curiously, patients often present the temporal pole hyperintensities seen on T2-weighted and FLAIR MR sequences even in their early 20s, whereas major ischemic events typically begin at approximately age 50. Chabriat et al have reported that dilated perivascular spaces (PVS) in CADASIL patients were located in the lentiform nuclei (94%) and subcortical WM of the temporal lobes (66%). PVS around small perforating arteries are pial-lined, interstitial fluid-filled spaces, and are readily seen to be enlarged in the WM of elderly subjects. PVS play an important role in lymphatic drainage from the brain. Similar to the small infarcts or lacunes, they may be detected as hypointense (T1-weighted) and hyperintense (T2-weighted) areas in MR images, and thus clustered PVS could be mistaken as a lacune or cavitated infarct. The pathological correlates of these radiological findings are unknown. We performed a postmortem study to quantify the degrees and extent of perivascular space and arteriopathic changes within the temporal pole WM of CADASIL subjects compared to young and older controls, and those with subcortical ischemic vascular dementia (SIVD).

### Materials and Methods

**Subjects**

Samples of temporal pole were collected from 9 CADASIL cases, 5 controls of similar age (young), and 5 older controls. To prove disease- and age-specific changes, the study also included samples from 8 SIVD subjects (Table). Formalin-fixed blocks of the right or left temporal pole representing Brodmann areas 20 to 22 were sampled coronally from the tip of the pole and anterior to the accumbens nucleus. The samples were acquired from the Newcastle Brain Tissue Resource Centre, Newcastle General Hospital, and 2 other sources: the Neuropathology Department, Frenchay Hospital, Bristol, and the Institute of Psychiatry, London (courtesy of Dr Claire Troakes and Dr Safa Al Sarraj). Available case notes and...
radiological reports indicated that CADASIL subjects showed extensive WM changes consistent with SIVD of the brain and met the minimum criteria for cognitive impairment used in our poststroke study. None of the controls, young or older group, had neurological or pathological evidence for cerebrovascular disease or neurodegenerative disorder. Clinical and pathological features were defined according to established criteria. CADASIL diagnosis was confirmed by direct DNA sequencing for NOTCH3 gene mutations. The Table shows the demographics, duration of disease, relevant risk factors, and the genetic disposition of the CADASIL cases and data summaries of the SIVD group and controls.

Histopathology and Immunohistochemistry
Paraffin-embedded temporal pole blocks were serially cut into 10-μm-thick sections. Sections were stained with Luxol fast blue (LFB), cresyl fast violet, and hematoxylin and eosin (H&E). For immunohistochemistry, tissue sections were processed essentially as described previously. To reveal changes in vascular components within microvessels, adjacent sections were immunostained with antibodies to α-smooth muscle actin (Dako) and medin (courtesy of Dr P. Naslund, Karolinska Institute, Sweden) for vascular smooth muscle cells, collagen IV (Sigma) for the basement membrane, and the glucose transporter 1 (GLUT1; Chemicon) for the endothelium. There were no apparent relationships between the density of immunostaining and length of fixation or postmortem interval from death to fixation of tissue between the groups. Variation in the LFB-cresyl fast violet, LFB only, and H&E staining, and immunohistochemistry was minimized by processing sets of sections on the same occasion including the reference specimens, by the same technician. All of the histopathologic analyses were performed on coded specimens.

Temporal Pole Reconstruction
To simulate MR images in vitro, serial sections of 10-μm thickness were cut from ~1-mm coronal block of the temporal pole from 1 of the CADASIL cases (CAD6; Table). One section at every 50 μm, for a total of 20 sections, were stained with LFB and then their digital images were precisely overlaid as a stacking column using Adobe Photoshop. The resultant composite 2-dimensional image was compared to the MR scan of the temporal pole. In addition, a sample of the temporal pole from another CADASIL case (CAD3; Table) was cut in the sagittal plane of the cerebral hemispheres and stained with H&E and α-smooth muscle actin to follow the longitudinal course of the distorted perforator arterioles.

Arteriosclerotic Changes, Perivascular Space, and In Vitro Digital Imaging
To determine the extent of small vessel arteriopathic and PVS changes, serial sections from each sample of similar size (Table) were stained with H&E and LFB-cresyl fast violet for the sclerotic index and PVS analysis, respectively. H&E-stained sections were viewed at 6.3X magnification and images of arterial/arteriolar vessels (>50 μm) and PVS were randomly taken. We measured the outer and inner diameters (exterior and interior diameters) of at least 30 arterial vessels per sample, of which exterior diameters were 50 to 200 μm (Figure 1). The sclerotic index and area of each vessel (V) were used to calculate the sclerotic index and area of vessel (V). Tissue section stained with H&E from CAD3. Antibodies to epileptogenic peptide or degraded myelin basic protein (dMBP; Chemicon) and amyloid precursor protein (APP; 22C11; Chemicon) were used to quantify the damaged area in WM. Images of 15 to 20 randomly selected WM ROI were taken and the percentages of immunostained area were measured, which were averaged to yield mean percentage area containing dMBP (%dMBP) and APP (%APP) for each sample.

Statistical Analysis
Statistical analysis was performed using SPSS version15. The statistical tests include Mann–Whitney U test, 1-way ANOVA, and post hoc (Tukey) comparisons and nonparametric Spearman (rho) correlation.

Results

Gross Pathological Changes in WM of Temporal Pole
Gross examination of the temporal pole sections showed clearly visible PVS (Figures 1, 2) in the WM of the majority of the CADASIL cases. Macroimages of sections stained with LFB showed that in 8 of 9 CADASIL cases, PVS were visible, accompanied by high degree of WM pallor or rarefaction (Figure 2). However, similar PVS within the WM were not evident in the SIVD cases or the controls (data not shown). Reconstructed 2-dimensional image simulated as
MR scan of temporal pole (Figure 2) revealed variable regions of low intensity created by the PVS when sections were stacked together; these collectively represented the hyperintensities seen in MRI (Figure 2A,B,D). The temporal pole sections cut in the sagittal plane of the brain along the axis of the vessel length also confirmed the existence of PVS along distorted penetrating vessels (not shown). One of the cases with the p.Arg558Cys mutation apparently did not reveal bilateral WM hyperintensities on MRI in the anterior temporal lobe (CAD4; Table) but showed T2-weighted signal changes in subcortical structures including the internal and temporal lobe (CAD4; Table) but showed T2-weighted signal changes in subcortical structures including the internal and external capsules. This case was, however, retained in the morphological analyses.

Enlarged PVS in WM

The total area (mm²) of PVS was significantly greater in CADASIL samples compared to the other groups (n=290 vessels) was significantly increased compared to similar age (young) controls (n=120); 60.0% (SD=21.2) in CADASIL and 13.3% (5.5) in the controls (P<0.001; Mann–Whitney U test). As expected, sclerotic index was significantly increased, by 57%, in the CADASIL patients compared to young controls (0.47 vs 0.30; P<0.001; Mann–Whitney U test; Figure 3C; Table). We also observed that there was a significant correlation between the number of hyalinized vessels and sclerotic index values (P<0.05). Again, the CAD4 case had the lower sclerotic index (0.38) compared to the other CADASIL cases. There was no apparent relationship between duration of disease and the number of hyalinized vessels or sclerotic index values.

Myelin and Axonal Damage in WM of Temporal Pole

MI analysis revealed that the myelin was severely affected in CADASIL, with the mean MI value as 24.3 in comparison to the other samples (SIVD, 34.6; young controls, 45.0; older controls, 43.6; Figure 4A). A significant difference was detected between CADASIL and young controls (P=0.028), whereas the difference between CADASIL vs SIVD and older controls failed to reach significance (P=0.093 and P=0.072, respectively). Low MI values were consistent with increased dMBP immunoreactivity in CADASIL (Figures 4, 5). We noted dMBP immunoreactivity in the myelinated nerve fibers and the soma of oligodendrocytes in patients with CADASIL and SIVD (Figure 5A–5C) that was also consistent with the regions of pallor seen with LFB (Figure 2). However, in CADASIL cases (Figure 5A,B), the immunoreactivity for dMBP was more intense in the soma of oligodendrocytes than in the myelin sheaths surrounding the axon (Figure 5B, inset), and appeared to be different from that
in SIVD, a pattern previously seen in acute phase of cerebral hemorrhage. The young or older controls generally lacked dMBP-positive cell bodies and fibers (Figure 5D). Quantitative analysis confirmed that the %dMBP was significantly greater in CADASIL compared to young controls \((P<0.026)\), and in SIVD it was greater than in older controls \((P<0.0038)\;\text{Figure 4B}\). In addition to myelin degradation, we also noted abundant deformed axons as well as punctate deposits of APP immunoreactivity in CADASIL cases (Figure 5E). Compared to the young controls, the mean %APP was 60-fold greater in CADASIL samples \((P<0.05)\).

**Discussion**

Our main findings suggest that WM hyperintensities in the temporal pole best relate to the numerous fluid-filled PVS or increased number of PVS rather than the presence of lacunar infarcts. A previous report had indicated aging-related subcortical MRI changes are associated with arteriosclerosis and enlarged PVS. Our data are consistent with the report but suggest that dilated PVS with the accompanying vascular changes are more profound in CADASIL. We suggest enlarged PVS are evident more so in the temporal pole because of its unique convolutional structure and vascularization by the branches of the anterior temporal artery. Because the resolution of MRI is currently not sufficiently high to distinguish single large fluid-filled space \((\geq 2 \text{ mm})\) from clustered smaller spaces, we suggest increased PVS collectively reflect WM hypeintensities on MRI.

The increased PVS may only partially specify the almost confluent hypersignals on T2-weighted and FLAIR MRI in CADASIL. We also demonstrated quantitative WM changes as indicated by decreased MI and increased accumulation of dMBP in CADASIL cases compared to young controls or older SIVD cases. Our data are consistent with the previous findings from diffusion tensor imaging studies showing increased diffusivity and decreased anisotropy, indicating WM disintegration or rarefaction may progress rapidly over relatively short periods of time and affect executive function.

Other factors that may contribute to the integrity of the WM include breakdown or structural changes within the WM caused by changes in the microvasculature including breach of the blood–brain barrier. It is likely that the increased burden and insufficiency in the drainage of the interstitial fluid and degraded protein products out to the lymphatic systems also play a role in PVS enlargement not only in the temporal pole but several subcortical structures. Thus, the evident myelin depletion, edema between myelin tracts, and subsequent axonal disruption, together with increased PVS, likely intensify the strength of the hyperintensities in the temporal poles. Although there were greater WM changes in SIVD, we did not observe significant differences in PVS size between SIVD and older controls. This lends support to the notion that PVS enlargement in CADASIL results from a
unique mechanism or accelerated degenerative WM pathology not clearly apparent in SIVD.

Various studies have reported progressive vascular abnormalities in CADASIL,9,29 that are apparent in skin even at early age (20–30 years old).3,29–32 When present in brain such vascular abnormalities, also indicated by the sclerotic index, no doubt contribute to the reduced cerebral blood flow and cerebral blood volume to induce WM hypoperfusion and progressively affect cognitive function in CADASIL.33–36 Endothelial cell abnormalities and blood–brain barrier dysfunction may further contribute to WM damage. Studies by Adler et al37 indicated that blood–brain barrier disruption can cause osmotic demyelination, and result in increased permeability of the vessel wall and mobilization of inflammatory factors, such as macrophages, lymphocytes, and complement, which may also cause myelin damage.38 Although incontrovertible evidence for an inflammatory response has not yet been reported,39 involvement of these factors in CADASIL pathology requires further assessment.

Is there functional significance of the dilated or enlarged PVS in CADASIL? Although we found no clear relationship between the %PVS and disease duration in CADASIL subjects, previous studies have indicated that size of dilated PVS in other regions of the brain correlates with cognitive impairment.12,40–42 MacLullich et al42 showed that increased enlarged PVS in the basal ganglia and centrum semiovale were correlated with worsening cognitive function, particularly verbal memory in healthy elderly men.

One of the potential limitations of our study was that we focused on 1 region relative to the WM hyperintensities and PVS, which have been described in other subcortical structures in CADASIL.21 Despite this caveat, we believe to have adequately evaluated the temporal pole of the cases and similar age controls by assessing large numbers of cerebral microvessels and PVS. We observed that the CADASIL case with the p.Arg558Cys (in exon 11) change lacked enlarged PVS consistent with the apparent absence of WM hyperintensities in temporal pole. Other mutations in exon 11 reported so far did reveal hyperintensities in the temporal pole, suggesting either this mutation is distinct, or more likely, NOTCH3 mutation alone does not lead to the WM changes. Whereas genotype–phenotype relationships in CADASIL are not widely established,43,44 meta-analysis involving a large number of cases may tease out such outlier cases. Our individual case analysis revealed wide variation in PVS pathology similar to MR hyperintense signals between CADASIL cases. This supports the concept that other factors like lifestyle may be involved in the cause of temporal pole hyperintensities on MRI in CADASIL.

In summary, our findings showed that the area and, by extension volume of PVS, in WM were significantly increased in temporal pole of CADASIL subjects. We suggest
that PVS resulting collectively from abnormalities within arterial walls and WM demyelination account for the morphological correlates of the temporal pole WM hyperintensities evident on MRI. Our observations are consistent with the previous findings of lack of WM hyperintense signals in the temporal pole of aging controls or sporadic SIVD cases, and accord with the rapid progression of vascular pathology in CADASIL.

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Disclosures
None.

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