Clinical Severity in CADASIL Related to Ultrastructural Damage in White Matter

In Vivo Study With Diffusion Tensor MRI

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Background and Purpose—CADASIL is a newly recognized cause of subcortical ischemic strokes that progressively leads to dementia associated with pseudobulbar palsy and severe motor disability. This deleterious progression and the severity of clinical presentation are widely variable among affected subjects. The exact role played by MRI white-matter abnormalities, a hallmark of the disease, in the severity of the clinical phenotype remains poorly understood.

Methods—To address this issue, we used diffusion tensor imaging (DTI), a new MRI technique highly sensitive to white-matter microstructural changes, in 16 symptomatic patients and 10 age-matched controls. Mean diffusivity and anisotropy of diffusion were measured within hyperintensities identified on T2-weighted images (T2WI) and outside these lesions on 4 slices at the level of centrum semiovale.

Results—We found a 60% increase of water mean diffusivity and a parallel loss of diffusion anisotropy in hyperintensities identified on T2WI. The same pattern of diffusion changes, but of lesser intensity, was found in the normal-appearing white matter on T2WI. Mean diffusivity in regions with increased signal on T2WI was higher in patients with severe clinical disability compared with those with no or mild deficit (1.33±0.11 versus 1.13±0.11 10⁻³ mm²/s, P<0.01). Furthermore, diffusion measured within T2 hyperintensities correlated with both the Mini-Mental State Examination and Rankin scale scores. In patients with a severe clinical status, the increase of water diffusion in these regions exceeded 70% in comparison with values obtained in the normal white matter in control subjects.

Conclusions—These results indicate that DTI is able to detect important ultrastructural changes in regions with increased signal on T2WI and within the normal-appearing white matter in CADASIL. The diffusion changes might be related to both neuronal loss and demyelination. The degree of the underlying ultrastructural alterations is related to the severity of the clinical status with a possible threshold level of white-matter damage above which severe neurological impairment may occur in this disease. DTI appears to be a promising technique for monitoring disease progression in CADASIL. (Stroke. 1999;30:2637-2643.)

Key Words: dementia ■ diffusion ■ leukoencephalopathy ■ magnetic resonance imaging ■ white matter

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a small-artery disease secondary to mutations of Notch 3 gene on chromosome 19.¹² The disease usually causes completed subcortical strokes and/or transient ischemic attacks during midadulthood, and it leads to dementia with pseudobulbar palsy and severe functional disability.³⁴ The exact determinants of this clinical progression remain unknown. The severity of the clinical presentation and the rapidity of progression are remarkably variable between individuals of identical age who share the same gene mutation.⁵⁻⁸ In some cases, a regularly progressive worsening can even occur in the absence of ischemic events.³⁵⁻⁸ A hallmark of CADASIL is the presence of increased signal intensities (hyperintensities) on T2-weighted MR images (T2WI) in the white matter.⁸⁹ The extent of white-matter signal abnormalities (WMA) on T2WI differs greatly among affected subjects, and it increases with age, as does the risk of dementia.¹⁰ However, although all demented CADASIL subjects appear to have confluent and diffuse WMA, some asymptomatic or mildly affected subjects harbor similar lesions.¹⁰ Therefore, the extent of WMA observed on T2WI does not account for the phenotypic severity in CADASIL. In addition, the microstructural tissular alterations underlying these signal abnormalities and their exact contribution to the clinical phenotype remain uncertain.

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To further investigate the white-matter abnormalities in CADASIL and their clinical correlates we used the diffusion tensor imaging (DTI) technique,\textsuperscript{11,12} a new MRI method sensitive to the microstructural integrity of cerebral white matter.\textsuperscript{13} Diffusion, which corresponds to the random motion of water molecules, is modified by the presence of structural components of the tissue such as membranes, organelles, and macromolecules.\textsuperscript{14} In a random microstructure such as gray matter, diffusion of water is isotropic, ie, identical in every direction in space. This is not the case in the white matter, in which diffusion is higher along the white-matter fibers than across them.\textsuperscript{12,15} This anisotropy may be related to the packing density of white-matter tracts and/or to the degree of myelination.\textsuperscript{16,17} DTI is based on the evaluation of diffusion in at least 6 noncolinear directions in space.\textsuperscript{18} This neuroimaging method provides a quantitative measurement of water diffusion and anisotropy in white matter that is independent of the subject orientation in the magnet. In this study, using DTI, we obtained an orientationally averaged measure of water diffusion (mean diffusivity [MD]) and the degree of diffusion anisotropy [volume ratio (VR)] =0–1 (anisotropic–isotropic)].\textsuperscript{19} in symptomatic patients with the mutated Notch 3 gene and in healthy subjects. To investigate the role of the microstructural changes in white matter on the clinical presentation of the disease, we assessed MD and VR within and outside T2 signal abnormalities distinct from typical infarcts in the white matter.

### Subjects and Methods

#### Subjects

Sixteen symptomatic patients with CADASIL\textsuperscript{4} were selected. All had a deleterious mutation of the Notch 3 gene\textsuperscript{20} at different positions of the gene: 622 (n=4), 475 (n=4), 406 (n=3), 499 (n=1), 659 (n=1), 1261 (n=1), and 3031 (n=1).\textsuperscript{20} They had a detailed neurological examination during the 2 hours preceding the MRI examination, including a brief evaluation of the cognitive deficit with the Mini-Mental State Examination (MMSE)\textsuperscript{21,22} and the degree of handicap with the Rankin scale.\textsuperscript{23} Patients were considered demented if they satisfied the DSM-IV\textsuperscript{24} criteria of dementia and had an MMSE score of <25. The minimal score in all these tests was given to only 1 patient who was at the most severe stage of the disease, totally bedridden, tetraparetic, incontinent, and unable to communicate. Patients were separated in 2 groups according to the severity of the clinical presentation (mild or severe), based on the presence of dementia and/or a Rankin scale score of >2 at the time of examination.

To obtain an aged-matched control group, 10 healthy subjects aged >40 years were selected from our 20 volunteers investigated during the same period with the same technique. All these subjects fulfilled the following criteria: (1) no familial vascular disorder, (2) no history of neurological disorder, (3) normal neurological and general examination, (4) MMSE score >28, and (5) normal T1- and T2-weighted MRI (T1WI and T2WI).

Informed consent was obtained from each subject or from a closer relative if they were too severely disabled to give a written consent. This study was approved by an independent ethics committee.

#### MRI

T1- and diffusion-weighted images were acquired with a 1.5-T Signa Horizon Echospeed MRI system (Signa General Electric Medical Systems) equipped with gradients hardware allowing up to 23 mT/m. A standard quadrature head coil was used for RF transmission and reception of the NMR signal. Reduction of subject head motion was achieved by placing pillows on either side of the subject’s head and positioning a fixed strip around the forehead.

T1-weighted images were acquired first in the axial plane using a spoiled gradient echo sequence (124 slices 1.2 mm thick, TR=10.3 ms, TE=2.1 ms, TI=600 ms) and 24×24 cm field of view. Acquisition time was 7 minutes 38 seconds.

Diffusion-weighted images were acquired with echo-planar imaging in the axial plane at 26 slice location, 5 mm thick. For each slice location, a T2-weighted image with no diffusion sensitization, followed by 11 b values (incrementing linearly to a maximum value of 1000 s/mm²) were obtained in 6 non-colinear directions (x, y, z, x-y, x-z, y-z). The image resolution was 128×128, field of view 24×24 cm, TE=96.4 ms, TR=3300 ms. The total acquisition time of DTI was 8 minutes 12 seconds.

The diffusion tensor parameters were calculated on a pixel by pixel basis as described by Basser et al.\textsuperscript{18} The mean diffusivity (MD) and the volume-ratio (VR) were calculated as described by Basser et al\textsuperscript{18} and Pierpaoli et al, respectively.\textsuperscript{19}

#### Regions of Interest

In order to define regions of interest, the volume of T1WI was resliced to match that of DTI without correction for contour differences related to susceptibility artifacts of DTI. The analysis was performed in the white matter of the centrum semiovale using the 4 consecutive axial planes located just above the lateral ventricles. This brain area was chosen to reduce the regional variability of the measurement of diffusion anisotropy\textsuperscript{12} and because it is consistently affected in CADASIL.\textsuperscript{10,25,26}

Regions of interest were delineated on T1WI and T2WI in each of these 4 planes using a software dedicated for segmentation.\textsuperscript{27} The same examiner (H.C.) delineated regions of hypointensity on T1WI (T1 WMA) and increased signals on T2WI (T2 WMA) acquired during DTI without diffusion sensitization. MD and VR within T2 WMA were averaged across the 4 planes after excluding the areas corresponding to typical infarcts as delineated on T1WI.

In addition, square regions of interest (total surface 11.2 to 16.8 cm\textsuperscript{2}) were positioned several millimeters outside these signal abnormalities on the same planes and then grouped to calculate the diffusion parameters within the normal-appearing white matter (NAWM) on T2WI.

In controls, 4 large regions of interest (2 on the right and 2 on the left side, total surface 29.6 cm\textsuperscript{2}) were positioned in the centrum semiovale on each plane and grouped for calculation of MD and VR.

#### Statistical Analysis

A 1-way ANOVA was used to analyze the differences between the DTI parameters obtained in patients and control subjects. An ANCOVA was used to investigate the DTI parameters of patients related to the clinical severity using 1 within-subjects factor (region: T2 WMA or NAWM), 1 between-subjects factor (group: mild or severe clinical impairment) and age as a covariate. The Fisher test of protected least significant difference was used for post hoc analysis of multiple comparisons.

Correlations between the different DTI parameters were investigated with simple regression analysis. The relationships between clinical scales and DTI parameters were investigated with the Spearman rank correlation score.

Standard tests (Student unpaired t test and χ\textsuperscript{2} test) were used for other comparisons.

Values of P<0.05 were considered statistically significant. Data are presented as mean±SD. The statistical analysis was performed with use of the Statview software (Abacus Concepts, Inc).

#### Results

#### Clinical Data

The mean age of the 16 patients was 59.3±7.6 years (median 61.5, range 43 to 68 years), which did not differ significantly from that of our control subjects (54.6±7.2 years). All patients were symptomatic and had a history of migraine with...
T1WI and T2WI

All patients had widespread T2 hyperintensities in the white matter and basal ganglia. Thirteen had T1 hypointensities (small infarcts). One patient had T1 hypointensities only in the white matter, another only in the basal ganglia, and the 11 remaining patients in both areas.

All 7 patients with a severe clinical impairment had lesions typical of small infarcts (diagnosed on both T1WI and T2WI) in the white matter (100%) and basal ganglia (100%). In contrast, only 5 of 9 of those with a mild clinical impairment had lesions in both. The cumulative frequency of patients with severe or mild clinical deficit was constant when the increase in mean diffusivity was suggestive of small infarcts in the white matter (P<0.005).

MD and VR calculated within T2 WMA in all patients were highly correlated (r=0.50, P=0.0002). Although less strong, a significant correlation was found between the same parameters measured in the NAWM (r=0.45, P=0.02). MD measured within WMA in patients with severe clinical disability was higher than the corresponding values obtained in patients having no or only mild clinical deficit. The difference with values calculated in the normal white matter of controls was significant for all diffusion parameters calculated in T2 WMA and in the NAWM.

Comparison Between Patients With Mild or Severe Clinical Impairment

Regional Effect

MD within T2 WMA differed from MD measured in the normal white matter (P<0.0001) in the complete patient group. This was also the case for VR (P<0.0001). These differences were significant in each group of patients.

Group Effect

Only MD calculated in T2 WMA significantly differed between patients with severe (1.28±0.11 10⁻³ mm²/s) and those with mild clinical impairment (1.16±0.13 10⁻³ mm²/s, P<0.05) (Figures 1 and 3).

The interaction between the clinical severity and the regional difference was also statistically significant for MD (P=0.02) but not for VR (P=0.3), which indicates that the highest increase of MD in the WMA is associated with severe clinical impairment.

The cumulative frequency of patients with severe or mild clinical status according to the increase of MD in WMA is shown in Figure 4. A severe clinical impairment was observed when the increase in mean diffusivity was >70%. It was constant when the MD increase was >80%.

Correlations With Clinical Scales

There was a strong correlation between both MD and VR measured within T2 WMA and the MMSE score (MD:...
There was also a strong correlation between these parameters and the Rankin score (MD: \( r = -0.74, P = 0.004 \); VR: \( r = -0.813, P = 0.003 \)). The increase of VR measured in the NAWM was correlated to the Rankin score (\( r = -0.59, P = 0.02 \)) but not to the MMSE score.

**Discussion**

This study shows major diffusion changes in the white matter of patients with CADASIL that are closely related to clinical impairment. Our results indicate a 60% increase in MD of water in regions of hyperintensities on T2WI. Changes in the extracellular space are considered the major source of diffusion changes in brain.\(^{28}\) In contrast to acute ischemia causing a massive reduction in diffusion associated with cytotoxic edema,\(^{28,29}\) the increased diffusion observed in CADASIL is probably related to the expansion of the extracellular space within the white matter.\(^{30,31}\) This expansion might be caused by the major loss of white-matter structural components (astrocytes, oligodendrocytes, axons, and myelin), a nonspecific feature of the disease.\(^{32-36}\) This is consistent with postmortem findings in CADASIL. Histological studies in patients deceased after a long course of the disease showed a marked white-matter rarefaction with increase of the extracellular space.\(^{34,35}\) This is also frequently reported in other vascular diseases associated with “leukoaraiosis.”\(^{37-39}\)

In the present study, we also found a loss of anisotropy within T2 WMA. The orientated axonal membranes are reported to be the major determinants of anisotropic water diffusion in the white matter.\(^{16,17}\) The exact contribution of myelin sheaths in the preferential orientation of water diffusion in white matter remains disputed,\(^{40,41}\) but anisotropic water diffusion has been observed in nonmyelinated fibers.\(^{16}\) Jones et al\(^ {31}\) suggested that both gliosis and axonal loss might cause this reduction of anisotropy in patients with “ischemic leukoaraiosis.” However, it is our view that the strong correlation between MD and VR in WMA in their study, identical to that in ours, is consistent with an expansion of the extracellular space and is related mainly to the loss of highly ordered axons in WMA. Significant glial proliferation would be expected to result in a reduction in anisotropy, but with a relative preservation of MD reflecting the dense structural organization of the glia. Therefore, we think that the loss of axons, myelin, or both along orientated white-matter tracts is the main cause of the diffusion changes in T2 WMA observed in CADASIL. This partial loss of structural components of white matter has been previously reported as “incomplete infarction” underlying WMA in ischemic leukoaraiosis.\(^ {42}\)

Another relevant finding is that water diffusivity was increased and anisotropy decreased in the NAWM, which suggests that the cerebral ultrastructure is also damaged outside the abnormal regions, as seen on T2WI. Such diffusion changes have been similarly reported in multiple sclerosis at distance from T2 hyperintensities.\(^ {43}\) Interestingly, Narayanan et al\(^ {44}\) found a loss of N-acetyl aspartate in the NAWM of multiple sclerosis patients in support for axonal damage, or loss, along pathways traversing inflammatory lesions. In the present study, the high correlation between the
values of diffusion measured in the NAWM and those found inside T2 WMA suggests that the corresponding underlying ultrastructural modifications are related. Changes in the NAWM might be due to axonal loss and/or demyelination secondary to the severe white-matter lesions inside WMA. Another hypothesis might be that the changes in diffusion and anisotropy in these different areas correspond to the gradual severity of microstructural changes in the white matter. This interpretation is in agreement with pathological data that shows a progressive severity of histological changes from the subcortical to the deep white matter, the most frequent location of infarcts and T2 signal abnormalities in CADASIL.45 In one postmortem study, demyelination alone was predominant within the superficial white matter, whereas both demyelination and neuronal loss associated with small infarcts were observed in the deep white matter.33 We may speculate that the moderate increase of diffusion in the NAWM is related mainly to myelin loss, whereas the severe increase observed inside T2 WMA mainly reflects neuronal loss in CADASIL. Experimental models of chronic ischemia show that oligodendrocytes are particularly sensitive to hypoperfusion and that damage to myelin precedes that to axons in the white matter.46 Actually, whether diffusion changes in the NAWM in CADASIL correspond primarily to direct consequences of chronic ischemia or to Wallerian degeneration secondary to small deep infarcts remains unknown. However, the increased diffusion in the NAWM also observed in the patients who had no white-matter infarcts on T1WI suggests that these ultrastructural changes may not be purely a secondary phenomenon. Further investigations in subjects having the mutated gene before the appearance of WMA and prospective studies will probably help to settle this issue.

Figure 3. T1WI, T2WI, MD and 1-VR maps obtained in 1 patient with a mild clinical disability and in 1 patient with a severe clinical impairment (dementia and Rankin score >2). Pixels with values of MD above $1.8 \times 10^{-2}$ mm$^2$/s corresponding to values close to those measured in the CSF have been removed (black appearance of CSF and infarcts). The values of MD measured in the centrum semi-ovale were higher in the demented subject. The maximal increase was observed in the deepest white matter in close proximity to infarctions. (VR varies from 0 to 1. The presentation of the 1-VR map is chosen only to present high values of anisotropy in white and to keep the black background.)

Figure 4. Cumulative frequency of mild or severe clinical presentation according to the increase of MD calculated in T2 WMA. A severe clinical impairment was observed when this increase was >70%. Above 80%, all patients presented with a severe disability, which explains the constant elevation of the corresponding cumulative frequency after this limit.

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An important finding is that the clinical severity and the diffusion parameters measured in the white matter were strongly related in our patients. The MD increase was higher within T2 hyperintensities in patients with severe functional disability and/or dementia. Moreover, both MD and VR obtained inside WMA and MD calculated in the NAWM were correlated to both the MMSE and Rankin scores. The difference in MD within T2 WMA was observed after correction for age in our analysis, which suggests that factors other than age determine the relationship between the clinical severity and the diffusion changes in WMA in CADASIL.

These results emphasize that the degree of MD increase within WMA might play an important role in the clinical phenotypic variability of CADASIL. We observed that patients with an averaged MD in T2 WMA >80% were all severely disabled. This suggests a possible threshold effect for the increase of diffusion above which severe neurological impairment may occur during the course of CADASIL. Consequently, the microstructural tissue changes causing the most severe increase of diffusion in the white matter might be related to the clinical status in CADASIL. Recently, Yousry et al.26 reported that the amount of T1 lesions presumably related to small infarcts was related to the clinical status in CADASIL. Our results emphasize that the ultrastructural alterations outside the white-matter infarcts but within WMA may be also related to the clinical severity of the disease. The possible threshold effect of white-matter ultrastructural alterations causing a severe clinical status is in accordance with the concept of 2 main pathophysiological steps in the clinical progression of demyelinating disorders: a silent stage of cumulative axonal degeneration with acute clinical events of complete or good recovery followed by a stage of progressive and irreversible neurological disability when the neuronal loss has exceeded a certain level.47 A prospective DTI study is necessary to evaluate whether the measurement of diffusion within the white matter has a prognostic value in CADASIL. In addition, it is noteworthy that in this study we aimed, by means of global scales such as the MMSE and Rankin scale, to investigate whether diffusion parameters were associated with dementia and severe motor disability. Further investigations are needed to determine whether focal diffusion changes in WMA or in the NAWM are associated with focal or subtle cognitive or functional alterations.

In conclusion, major modifications of water diffusion that are associated with focal or subtle cognitive or functional alterations in the pathophysiology of CADASIL.48,49 This in vivo, quantitative tool may provide a putative marker for monitoring disease progression in CADASIL and for future therapeutic trials in disorders associated with “leukoaraiosis.”

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