White Matter Hyperintensities and Their Penumbra Lie Along a Continuum of Injury in the Aging Brain

Pauline Maillard, PhD; Evan Fletcher, PhD; Samuel N. Lockhart, BS; Alexandra E. Roach, MS; Bruce Reed, PhD; Dan Mungas, PhD; Charles DeCarli, MD; Owen T. Carmichael, PhD

Background and Purpose—Aging is accompanied by clinically silent cerebral white matter injury identified through white matter hyperintensities (WMHs) on fluid-attenuated inversion recovery (FLAIR) and diffusion tensor imaging (DTI). The temporal course of FLAIR and diffusion tensor imaging changes within WMHs and their less-injured periphery (ie, their penumbra), however, has not been fully studied. We used longitudinal diffusion tensor imaging and FLAIR to explore these changes.

Methods—One hundred fifteen participants, aged 73.7±6.7 years, received clinical evaluations and MRIs on 2 dates. WMHs and fractional anisotropy (FA) maps were produced from FLAIR and diffusion tensor imaging and coregistered to a standardized space. Each distinct WMH was categorized as growing, stagnant, or noncontiguous incident. The penumbra of each WMH was similarly categorized as corresponding to a stagnant, growing, or noncontiguous incident WMH. Linear mixed-effect models were used to assess whether FA and FLAIR measurements changed between baseline and follow-up and differed between tissue categories.

Results—Baseline FA differed significantly by tissue category, with the following ordering of categories from highest to lowest FA: penumbra of noncontiguous incident, then stagnant, then growing WMHs; noncontiguous incident, then stagnant, then growing WMHs. Despite differences in baseline values, all tissue categories experienced declines in FA over time. Only noncontiguous incident WMHs showed significant FLAIR signal increases over time, and FLAIR signal significantly decreased in stagnant WMHs.

Conclusions—WMHs and their penumbra vary in severity and together span a continuous spectrum of white matter injury that worsens with time. FLAIR fails to capture this continuous injury process fully but does identify a subclass of lesions that seem to improve over time. (Stroke. 2014;45:00-00.)

Key Words: aging ■ diffusion tensor imaging ■ magnetic resonance imaging ■ neuroimaging

There is now a substantial body of evidence that clinically silent brain injury, including MRI infarction, white matter hyperintensities (WMH) on fluid-attenuated inversion recovery (FLAIR) MRI, subtle white matter (WM) injury apparent on diffusion tensor imaging (DTI), and tissue atrophy on T1-weighted MRI, accrues insidiously beginning in middle age and is associated with increased risk of clinically manifest neurological conditions late in life, including stroke, dementia, and cognitive decline. Therefore, preventing or slowing such silent brain injury has emerged as major goal of current research.

Modifying the course of WMH accrual has received particular attention because of evidence that lesions may arise from clinically silent cerebrovascular injury and, therefore, may be preventable or modifiable through treatment of vascular risk factors. We have previously used DTI to show that WMHs represent a core of severe WM injury surrounded by a region of WM that is more mildly injured. We introduced a new term: WMH penumbra to describe this specific WMH surrounding region. Although this term is not yet universally adopted and subsequent work uses different terminology for the same phenomenon, we continue to use the term here. This mildly injured periphery is differentially vulnerable to future conversion to WMH. Among community-dwelling elders with prevalent WMHs, such extensions of WMHs into adjacent tissue represent as much as 80% of the accrued WMH volume over time, and greater extension is associated with greater concurrent cognitive decline. These findings suggest that WMH penumbra may represent a clinically relevant target for interventions that arrest the progression of WMHs. However, any effort to arrest WMH accrual among individuals with prevalent WMHs depends critically on how the WM

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injury represented by WMHs and their penumbra evolves over time. Although penumbra is at increased risk of conversion to WMH, it is unclear whether penumbra actually differs from WMH in terms of their vulnerability to future injury: WM integrity within WMHs themselves may continue to decrease over time after they are identified as WMHs or they may have already reached effective floor levels of tissue integrity. It is also unclear how much heterogeneity there is among WMHs and penumbra in terms of such future changes. Better understanding of whether WMH and their penumbra truly differ in terms of prognosis or whether they simply represent different points on a continuum of degeneration severity could clarify the usefulness of separating WMH and penumbra into separate tissue categories and targeting one or the other for interventions.

To our knowledge, no studies to date have tracked the temporal course of WM integrity change within both WMHs and their penumbra to better understand their prognoses. To accomplish this, we used longitudinal FLAIR and DTI imaging in 115 cognitively normal individuals. In addition, based on our previous observation that appropriately normalized FLAIR signal intensities may provide a continuous measure of WM integrity similar to fractional anisotropy (FA),10 we tested whether FA and FLAIR have a complementary or redundant role in describing the WM degeneration process occurring within WMH and their penumbra.

**Methods**

**Sample**
The sample included 115 community-dwelling individuals who received comprehensive clinical evaluations according to standardized criteria at the Alzheimer’s Disease Center at the University of California, Davis. The present sample included only individuals who were classified as cognitively normal at both baseline and follow-up with no clinically significant cognitive impairment identified based on detailed medical history, neurological examination, and neuropsychological testing using the uniform data set battery. In addition, all subjects received a standardized MRI scan of the brain at 2 different dates (mean [SD] interscan interval: 3.3 [1.6] years). The institutional review boards at all participating institutions approved this study, and subjects gave written informed consent. The Table summarizes participant characteristics.

<table>
<thead>
<tr>
<th>Variables</th>
<th>All</th>
</tr>
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<tbody>
<tr>
<td>No. of subjects</td>
<td>115</td>
</tr>
<tr>
<td>Age, y</td>
<td>73.7±6.7</td>
</tr>
<tr>
<td>Education, y</td>
<td>12.8±4.7</td>
</tr>
<tr>
<td>No. (%) male</td>
<td>26 (30)</td>
</tr>
<tr>
<td>No. (%) with history of hypertension</td>
<td>76 (66.6)</td>
</tr>
<tr>
<td>No. (%) with history of diabetes mellitus</td>
<td>36 (31.6)</td>
</tr>
<tr>
<td>Interscan time interval, y</td>
<td>3.3±1.6</td>
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<tr>
<td>Baseline brain matter volume, cm³</td>
<td>896.5±99.9</td>
</tr>
<tr>
<td>Baseline white matter hyperintensivity volume, cm³</td>
<td>5.7±8.9</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD.

**Image Acquisition and Processing**
All brain imaging was performed at the University of California, Davis Imaging Research Center on a 1.5-T GE Signa Horizon LX Echospeed system. Three sequences were used: a 3-dimensional T1-weighted coronal spoiled gradient-recalled echo acquisition, a FLAIR sequence, and DTI using the diffusion tensor-weighted echo-planar sequence (DTI-EPI; Stanford University, CA). All image acquisition was performed according to methods previously reported.11,12

FA maps were calculated from baseline and follow-up DTI exams and warped to a DTI template. Segmentation of WMH was performed on baseline and follow-up FLAIR images in their native space by a semiautomated procedure previously described.12,13 FLAIR images were then linearly aligned to the corresponding T1-weighted scan using a previously described image registration method.11 The T1-weighted scan was then warped to a T1-weighted template defined in the same space as the DTI template,14 thus allowing FLAIR and T1 images, as well as WMH maps, to be placed in the same coordinate frame (Methods in the online-only Data Supplement).

**Normalizing FLAIR and FA Intensities**
Normalized FLAIR (nFL) maps were then created by normalizing the intensity distributions of all warped FLAIR images to account for the arbitrary absolute scaling of FLAIR intensities within each scan. Intensities were scaled to set the mean in image intensity within the cranial vault to fixed reference values, All FA values were normalized using a previously described method10 that indexes FA at each voxel against healthy young adult FA values at that voxel to account for intervoxel FA differences that are due solely to intervoxel differences in the intrinsic organization of underlying WM tracts.11 In such normalized FA (nFA) maps, values <1 indicate that the subject exhibits reduced FA compared with FA values exhibited by the young reference group at that location.

A 2-year follow-up nFA map was computed for each subject by interpolating FA values between baseline and follow-up nFA values at a 2-year follow-up time point. The same method was used to compute 2-year follow-up nFL maps.

**Categorization of WMHs**
A first objective of this study was to characterize the time course of nFA and nFL change within WMHs. To understand these changes and how they related to changes in the WMHs themselves, we first placed individual WMHs into categories according to whether or not the lesions grew over time. To do so, distinct WMHs were labeled on the baseline WMH maps in template space and corresponding WMHs were identified on follow-up WMH maps, noncontiguous incident WMHs were labeled on follow-up maps as well.1 Lesions ≤5 mm³ in volume were excluded from analysis. All other lesions were then placed into 3 categories: (1) baseline WMH that did not get larger over time were labeled stagnant WMH, (2) baseline WMH that grew larger over time were labeled growing WMH, and (3) WMH identified only on follow-up scans were labeled noncontiguous incident WMH (Figure 1).

**Categorization of WMH Penumbra**
The second objective was to characterize how nFA and nFL change within penumbra. Based on our previous findings,8 we categorized all WM voxels within 8 mm of a WMH as its penumbra; voxels within 8 mm of ≥1 WMHs were assigned to the nearest WMH. To better understand penumbra integrity changes and how they related to changes to the corresponding WMH, each penumbra was assigned to a category based on the category of the WMH including (1) the penumbra of stagnant WMH, (2) the penumbra of growing WMHs, and (3) the penumbra of noncontiguous incident WMH (Figure 1).

**Statistical Analysis**

**Descriptive Statistics of WMH and Penumbra Categories**
For each individual, overall WMH volumes were computed for the 3 WMH categories, log-transformed to normalize population variance, and compared using pair wise paired t test.
WMH Analysis

The first goal of this analysis was to characterize, at a lesion level, differences between baseline and follow-up nFA and nFL within the 3 WMH categories. To do so, we calculated mean nFA and mean nFL within each distinct lesion in baseline and follow-up images. We then conducted linear mixed-effects regression with lesion mean nFA or lesion mean nFL as the dependent variable. To determine whether nFA and nFL differed between WMH categories at baseline, we entered WMH category (stagnant, growing, and noncontiguous incident WMH) as a fixed effect. To determine whether nFA and nFL changed significantly over time regardless of WMH category, we entered session (baseline versus follow-up) as a fixed effect. To determine whether 2-year change in nFA and nFL differed between WMH categories, the interaction between WMH category and session was also included.

WMH Penumbra Analysis

The second goal of analysis was to characterize, at the level of individual penumbra, differences in baseline and follow-up nFA and nFL within the 3 penumbra categories (penumbra of stagnant, growing, and noncontiguous incident WMH). To do so, we calculated mean baseline and follow-up nFA and nFL within each distinct penumbra. We then conducted linear mixed-effects regression with mean penumbra nFA or nFL as the dependent variable. Analogous to the WMH analysis, we entered penumbra category as a fixed effect to identify differences in nFA and nFL between categories. We entered session as a fixed effect to identify changes in nFA and nFL over time across all categories, as well as the interaction of category and session.

Both models included age and hypertension as nuisance covariates because both have been reported to be associated with FA. Multiple measurements per subject generally result in the correlated errors that are explicitly forbidden by the assumptions of standard (between-subjects) AN(C)OVA and regression models. Mixed-effects models flexibly give correct estimates of fixed effects in the presence of the correlated errors that arise from a data hierarchy, by controlling for the effects of individual and eventual nested effects. Therefore, the models used in the present study also specified nested random effect of lesion ID (or lesion penumbra ID) within subject for the intercept, as well as the slope associated with Session effect. Statistical analyses were performed using R version 2.13.0 (R Development Core Team, 2009, Vienna, Austria).

Results

Descriptive Statistics of WMH

Analysis of the total volume of each WMH type within individual brains suggests interlesion heterogeneity and confirmation of previous findings related to longitudinal WMH change. The total volume of growing WMH (range: [3.4×10⁻³; 38.40]) was significantly larger (P<0.001, on log-transformed volumes) than the volume of noncontiguous incident WMH ([0.027; 1.14]). The volume of stagnant WMH ([6.7×10⁻³; 16.33]) was less than that of growing WMHs (P<0.001) but still substantial, suggesting that WMHs are heterogeneous in terms of change over time.

Baseline and Change in DTI and FLAIR Among WMH Categories

Diffusion Tensor Imaging

At baseline, mean nFA within WMHs that went on to subsequently grow over time was lower than that of WMHs that remained stagnant (mean±SD: 0.882±0.16 versus 0.897±0.16; P=0.043; Figure 2) and that of non-WMH voxels that became noncontiguous incident WMHs during the follow-up period (0.902±0.19; P<0.001). In contrast, nFA decreased significantly over time among all WMH categories (β=−0.012/y; P<0.001), and the amount of decrease did not significantly differ between categories (P=0.56). The mean nFA estimates at baseline and follow-up suggested that the 3 categories covered a continuum of values that differed at baseline according to category but not rate of change. For example, mean nFA of noncontiguous incident WMH at follow-up was similar to mean nFA of baseline stagnant WMH; similarly mean nFA of follow-up stagnant WMH values was similar to mean nFA of baseline growing WMH.

Fluid-Attenuated Inversion Recovery

At baseline, mean nFL within growing WMHs was greater than that of stagnant WMHs (mean±SD: 142.5±16.04 versus 118.8±13.74; P<0.001). However, among WMH categories, only noncontiguous incident WMH showed significant increases in nFL over time (β=8.16/y; P<0.001), whereas mean nFL within stagnant WMHs decreased significantly over time (β=−4.08/y; P<0.001). Growing WMH did not show significant changes in

Figure 1. Example of white matter hyperintensity (WMH) tissue categorization. Individual lesions are depicted in baseline and follow-up fluid-attenuated inversion recovery images, as well as the respective expanded views (first to fourth columns, respectively). The boundaries of growing and stagnant WMHs as they appear in baseline and follow-up images are shown in solid black. The boundaries of penumbras of baseline growing and stagnant lesions are shown in solid white on the baseline image and are superimposed on the follow-up image in dashed white. The boundaries of noncontiguous incident WMH and penumbra are shown in solid black and white on the follow-up image and are overlaid onto the baseline image in dashed-line contours.
Stroke June 2014

mean nFL over time (β=−0.01/y; P=0.99). Unlike mean nFA, mean nFL values at baseline and follow-up did not span a continuous range of values across WMH categories.

Baseline and Change in DTI and FLAIR Among Penumbra Categories

Diffusion Tensor Imaging

At baseline, nFA was significantly lower in the penumbra of growing WMHs compared with the penumbra of stagnant WMHs (mean±SD: 0.908±0.09 versus 0.916±0.09; P=0.046), which in turn was significantly lower than the nFA of voxels that became the penumbra of noncontiguous incident WMH (0.923±0.012; P=0.0203; Figure 2). Similar to the WMH categories, nFA within each penumbra category also declined significantly over time (β=−6.1×10−3/y; P=0.048), but rates of change were not significantly different across penumbra categories (P=0.36). Similar to the WMH categories, baseline and follow-up mean nFA values suggested that the 3 penumbra categories spanned a continuum of values. For example, mean nFA among voxels that became penumbra of noncontiguous incident WMHs was not significantly different from the baseline mean nFA of stagnant WMH penumbra, and the follow-up mean nFA of stagnant WMH penumbra was not significantly different to the baseline mean nFA of the penumbra of growing WMHs. The follow-up mean nFA of the penumbra of growing WMHs, in turn, was similar to the baseline mean nFA of voxels that went on to become WMHs between baseline and follow-up.

Fluid-Attenuated Inversion Recovery

At baseline, nFL was significantly greater in the penumbra of growing WMHs compared with that of the penumbra of stagnant WMHs (mean±SD: 108.23±7.5 versus 106.78±7.9; P<0.001; Figure 2). However, only nFL in the penumbra of growing WMHs and voxels that became the penumbra of noncontiguous incident WMH experienced a significant increase in nFL over time (β=0.53/y, P=0.0044 and β=1.43/y, P<0.001).

Discussion

This study had 4 key findings. First, WMHs, their penumbra, and normal WM all showed similar decline in WM integrity over time as measured by DTI. Second, WMHs and penumbra were heterogeneous with respect to the severity of WM injury they represented, with differing categories of novel, stagnant, and growing lesions spanning a continuous spectrum of injury severity as measured by DTI. Third, FLAIR and DTI differed with respect to signal changes over time: unlike DTI, only tissue that became noncontiguous incident WMHs experienced major FLAIR changes over time, and there were major gaps in FLAIR signal values between tissue categories. Fourth, the class of WMHs that did not grow over time had FLAIR signal values that actually improved (decreased) over time. We discuss the significance of each finding in turn.

Similarity of WM Integrity Changes in WMHs, Penumbra, and Normal WM

In aging, WMHs are usually conceptualized as loci where the WM has been irrevocably injured. More recently, we and others have introduced the concept of WMH penumbra as areas surrounding WMHs whose WM integrity is more subtly reduced and that are more vulnerable than other healthy WM to convert to WMHs over time. These 2 concepts led us to hypothesize that WMH penumbra are high-priority targets for intervention because they are potentially amenable to treatment, whereas WMHs are not. This hypothesis is especially relevant given that the time course of development of WMHs may be modifiable through treatment of vascular risk factors.15-17 However, the findings of this study suggest that
this hypothesis is flawed: not only do WMHs have measurable losses of WM integrity over time but these losses are also similar in magnitude to those of both penumbra and healthy WM. The implication of this finding is that all WM, including WMHs, may be viewed as vulnerable to integrity loss over time and potentially viable targets for intervention strategies aimed at slowing or halting such losses.

**Heterogeneity of Diffusion MRI Properties Within WMHs and Penumbra**

The spatial distribution and overall burden of WMHs, as well as the relationship of WMH burden to vascular risk factors and genetics, are known to be heterogeneous. In addition, the signal properties of certain MRI sequence types, including DTI, FLAIR, and magnetization transfer imaging, within WMHs have also been shown to be heterogeneous, reflecting heterogeneity in the pathological substrate. For example, differences in MRI signals have been reported between periventricular and deep WMH and between confluent and punctuated lesions. Magnetic resonance spectroscopy studies have similarly shown such signal heterogeneity. Our study confirms and extends these findings in 2 ways. First, we characterized the previously reported heterogeneity of WMHs on diffusion MRI as graded differences in WM integrity between growing, stagnant, and noncontiguous incident lesions. In addition, we showed that for FLAIR, such signal heterogeneity extended longitudinally, whereas for diffusion MRI, it did not: the rate of change in FLAIR signal properties varied by lesion type, whereas all lesion types showed similar rates of change in the diffusion MRI signal. Because greater MRI signal severity within WMHs may have clinical implications for cognitive impairment, there is an ongoing need to continue to characterize WMH heterogeneity both in terms of pathogenesis and in terms of severity of tissue damage. The present study suggests that characterizing the severity of WM injury by separately quantifying the burden of noncontiguous incident, stagnant, and growing lesions, as well as their corresponding penumbra may help to achieve this goal.

**FLAIR and DTI Provide Differing Information**

There are now 2 substantial, almost completely separate bodies of work on the DTI and FLAIR properties of the aging brain. It has never been clear whether the 2 techniques provide essentially the same or differing information about the WM, both because FLAIR studies dichotomize the image signal into hyperintense and normointense categories rather than examine the signal as a continuous entity and because collection of DTI and FLAIR is rare. Our findings suggest that DTI captures a continuously evolving, almost linear, process of WM change in the aging brain. Conversely, we found that FLAIR is more dichotomous; the most healthy and the most severe tissue categories had categorically low and high signal values respectively, as well as minimal changes over time. The implication of this finding is that studies wishing to quantify the full range of WM changes, including the earliest declines from peak adult WM integrity values in middle age or earlier, should rely on DTI rather than FLAIR to do so.

**Improvement in Stagnant Lesions**

The discovery of stagnant lesions that seem to improve slightly over the study interval was a unique finding of our study. Resolving inflammation is one plausible mechanism that could explain the FLAIR signal decreases seen in stagnant lesions: degenerating myelin (myelin debris) may trigger the recruitment of glia to clear the debris; the high concentration of lipid-protein–rich myelin debris would be expected to cause an elevated FLAIR signal, which then resolves after debris clearance. However, the true mechanism underlying such improvement is currently unknown and warrants further study.

**Limitations**

There are limitations to our findings. First, we only measured DTI and FLAIR at 2 time points and are, therefore, unable to assess whether tissue may go through a series of categorical changes, such as changes from normal WM to penumbra and onward to WMH as would be suggested by our model of a continuum of WM injury. Second, we excluded small lesions (<5 mm³) from the analysis. Third, WMH categories (growing, stable, or noncontiguous incident) were determined in template space, which may have led, because of interpolation bias produced during registration steps, to miscategorization. To assess this issue, we also performed WMH categorization in FLAIR native space, and the results did not significantly differ from those presented in the present article. Finally, we did not include the anatomic territory of WMH occurrence in the analyses. Any of these additional data items have the potential to more carefully characterize FLAIR changes over time and how these relate to DTI changes.

**Conclusions**

The present study provides in vivo evidence that age-related WM lesions and their penumbra can be graded by WM injury severity, change similarly over time, and lie along a continuum of WM injury. We also found that FLAIR and DTI have differing measurement properties that may make one or the other technique more appropriate to deploy depending on the scientific question.

**Sources of Funding**

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**Disclosures**

None.

**References**

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Data Supplement (unedited) at:
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SUPPLEMENTAL MATERIAL

White Matter Hyperintensities and their Penumbra Lie Along a Continuum of Injury In The Aging Brain

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Supplemental Methods

Image acquisition and processing
All brain imaging was performed at the University of California, Davis Imaging Research Center on a 1.5-T GE Signa Horizon LX Echospeed system. Three sequences were used: a 3-dimensional T1-weighted coronal spoiled gradient-recalled echo acquisition, a FLAIR sequence, and DTI using the diffusion tensor weighted echo-planar sequence (DTI-EPI; Stanford University, Stanford, CA). All image acquisition was performed according to previously reported method\textsuperscript{1,2}.

Diffusion weighted images were generated using gradients applied in six directions given by \((G_x,G_y,G_z) = (1,1,0), (1,-1,0), (1,0,1), (1, 0, -1), (0,1,1) (0,1,-1)\) with total gradient diffusion sensitivity measured at \(b=1000\) s/mm\(^2\). To improve spatial registration prior to calculation of the maps, an image distortion correction (“unwarping”) scheme based on an earlier scheme by Haselgrove\textsuperscript{3} was applied to the images. FA was calculated at each image from the 3 eigenvalues of the diffusing tensor (see \textsuperscript{4} for a detailed description). FA maps were calculated from baseline and follow-up DTI exams. A DTI template with a 1.5 x 1.5 x 1.5mm\(^3\) voxel size was computed from the study sample DTI images. Starting with the FA map of a reference study subject as an initial template, we iteratively performed the following steps: 1. warping skull-stripped FA maps to the template using linear and non linear transformations and 2. averaging resulting warped FA maps together and using this average as the template for the next iteration. All baseline and follow-up FA maps were finally warped to the resulting DTI template. All procedures described above were performed using FSL software tools\textsuperscript{4} (http://www.fmrib.ox.ac.uk/fsl/index.html).

Segmentation of WMH was performed by a semiautomated procedure using a set of in-house computer algorithms and programs previously described\textsuperscript{5}. In this procedure, FLAIR image voxel intensities are corrected for bias on a slice by slice basis. Corrected intensities, modeled as a Gaussian distribution, exceeding the mean value plus 3.5 standard deviations are labeled as WMH in each slice. Non-brain tissues were removed from the T1-weighted scan, and baseline and follow-up FLAIR images were linearly aligned to the corresponding T1-weighted scan using a previously described image registration method\textsuperscript{1}. The T1-weighted scan was then warped to a T1-weighted template defined in the same space as the DTI template\textsuperscript{6}, thus allowing FLAIR and T1 images as well as WMH maps to be placed in the same coordinate frame.
REFERENCES


