Prediction of Longitudinal Brain Atrophy in Multiple Sclerosis by Gray Matter Magnetic Resonance Imaging T2 Hypointensity

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Background: Gray matter magnetic resonance imaging T2 hypointensity, a marker of iron deposition, is associated with clinical impairment and brain atrophy in cross-sectional studies of multiple sclerosis. Treatment with intramuscular interferon beta-1a limits brain atrophy in the second year of treatment.

Objective: To test whether T2 hypointensity predicts brain atrophy and whether interferon affects this relationship.

Design: Post hoc analysis.

Setting: A multicenter treatment trial conducted at tertiary care comprehensive multiple sclerosis centers.

Patients: Patients with multiple sclerosis who took part in a 2-year clinical trial in which they received intramuscular interferon beta-1a (30 µg/wk) or placebo.

Main Outcome Measures: Deep gray matter T2 hypointensity, brain parenchymal fraction (BPF), and total T2, gadolinium-enhancing, and T1 lesion volumes.

Results: T2 hypointensity in various gray matter areas correlated with baseline BPF ($r=0.19$-$0.39$; $P=.001$-$0.03$). In placebo-treated patients (n=68), baseline T2 hypointensity predicted the change in BPF in the first year and throughout 2 years ($r=0.26$-$0.42$; $P<.001$-$0.03$). T2 hypointensity was chosen in regression modeling as the best predictor of BPF change at the 1-year ($R^2=0.23$; $P=.002$) and 2-year ($R^2=0.33$; $P<.001$) time points after accounting for all magnetic resonance imaging variables. In the interferon group (n=65), no relationship existed between baseline T2 hypointensity and BPF change.

Conclusions: Gray matter T2 hypointensity predicts the progression of brain atrophy in placebo- but not interferon beta-1a–treated patients. This predictive effect is seen as early as the first year. We hypothesize that interferon beta may exert its effect on brain atrophy in part by reducing a cascade of events that involve iron deposition as a mediator of neurotoxicity or as a disease epiphenomenon.

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Brain atrophy develops early in multiple sclerosis (MS), affects both gray and white matter, and is thought to represent neurodegeneration.1,4 Gray matter involvement shown by imaging12 and pathologic2 studies may occur by both remote effects of white matter injury (diaschisis) and direct effects of the disease on gray matter.3-12 The clinical relevance of brain atrophy11,14 is suggested by its better correlation with physical disability13,15 and physical disability than conventional magnetic resonance imaging (MRI) lesions. Because disease-modifying treatments have shown only partial effects on atrophy,1,2,21-24 there is a need to uncover the mechanisms of atrophy and develop more effective neuroprotective treatments.25 T2 hypointensity in the deep gray matter is thought to represent iron deposition in MS.26-31 Brain iron accumulation has been detected histologically, and iron metabolism is abnormal in these patients.26,32-37 Gray matter T2 hypointensity correlates with brain atrophy35 and physical disability in cross-sectional studies. We tested 3 hypotheses in this study of patients with relapsing-remitting MS: (1) baseline gray matter T2 hypointensity predicts the rate of whole-brain atrophy throughout 2 years; (2) interferon affects the relationship between T2 hypointensity and atrophy; and (3) conventional MRI can detect progressive T2 hypointensity.
intramuscular interferon beta-1a (Avonex, Biogen Idec, Cambridge, Mass) or placebo have been previously published. Inclusion required clinically definite, active relapsing-remitting MS. Of 301 patients enrolled in the study, 172 completed 2 years of follow-up. Sixteen had baseline MRI that was incompatible with the present study (corrupt or missing sequences). Images from the remaining 156 patients (n=78 per group) (Table 1) were analyzed for gray matter T2 hypointensity throughout 2 years. However, images from 23 of those patients could not be analyzed for the 1-year or 2-year conventional MRI data, and the patients were removed from the component of the study in which T2 hypointensity was compared with other MRI variables (Table 2). The smaller final cohort (n=68 placebo; n=65 interferon beta-1a) had baseline clinical and MRI characteristics similar to the larger cohort.

**IMAGING**

Subjects underwent yearly brain 1.5-T conventional spin-echo MRI with a 3-mm interleaved axial series using dual-echo T2-weighted (repetition time = 2000 milliseconds, echo times = 30 and 90 milliseconds) and precontrast and postcontrast T1-weighted (repetition time=600 milliseconds, echo time=20 milliseconds) images. Gray matter intensities were measured from T2-weighted (echo time=90 milliseconds) images on the baseline, 1-year, and 2-year studies using Java Image (version 1.0; Xinapse Systems, Northants, England). A region of interest (ROI) template (Figure 1) measured mean intensity of each structure normalized to the cerebrospinal fluid. The mean intraobserver and interobserver coefficients of variation for measurement of the ROI template were 3.2% and 5.4%, respectively. The ROI template was used to assess the gray matter intensity in the caudate, dentate, globus pallidus, putamen, red nucleus, thalamus, and brain parenchymal fraction across the duration of the study. Baseline characteristics of the subjects divided by treatment group are presented in Table 1. Correlations between baseline MRI variables and brain atrophy are presented in Table 2.

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**Table 1. Baseline Characteristics of Subjects Divided by Treatment Group**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo Group</th>
<th>Interferon Beta-1a Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>34.9 (6.8)</td>
<td>37.2 (7.0)</td>
<td>.04</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>5.8 (5.3)</td>
<td>6.5 (5.7)</td>
<td>.40</td>
</tr>
<tr>
<td>Prestudy annual exacerbation rate</td>
<td>1.2 (0.55)</td>
<td>1.1 (0.47)</td>
<td>.42</td>
</tr>
<tr>
<td>Kurtzke Expanded Disability Status Scale score</td>
<td>2.4 (0.88)</td>
<td>2.2 (0.78)</td>
<td>.28</td>
</tr>
<tr>
<td>Bilateral caudate T2 intensity</td>
<td>0.57 (0.04)</td>
<td>0.56 (0.06)</td>
<td>.21</td>
</tr>
<tr>
<td>Left dentate T2 intensity</td>
<td>0.40 (0.06)</td>
<td>0.40 (0.07)</td>
<td>.79</td>
</tr>
<tr>
<td>Right dentate T2 intensity</td>
<td>0.39 (0.06)</td>
<td>0.39 (0.06)</td>
<td>.89</td>
</tr>
<tr>
<td>Left globus pallidus T2 intensity</td>
<td>0.38 (0.04)</td>
<td>0.37 (0.05)</td>
<td>.61</td>
</tr>
<tr>
<td>Right globus pallidus T2 intensity</td>
<td>0.38 (0.04)</td>
<td>0.39 (0.06)</td>
<td>.52</td>
</tr>
<tr>
<td>Left putamen T2 intensity</td>
<td>0.49 (0.04)</td>
<td>0.48 (0.06)</td>
<td>.69</td>
</tr>
<tr>
<td>Right putamen T2 intensity</td>
<td>0.50 (0.05)</td>
<td>0.49 (0.06)</td>
<td>.46</td>
</tr>
<tr>
<td>Bilateral red nucleus T2 intensity</td>
<td>0.40 (0.04)</td>
<td>0.40 (0.05)</td>
<td>.88</td>
</tr>
<tr>
<td>Bilateral thalamus T2 intensity</td>
<td>0.52 (0.04)</td>
<td>0.52 (0.05)</td>
<td>.95</td>
</tr>
<tr>
<td>T2 hyperintense lesion volume, mm³</td>
<td>16,498 (15,377)</td>
<td>13,985 (15,359)</td>
<td>.31</td>
</tr>
<tr>
<td>T1 hypointense lesion volume, mm³</td>
<td>1765 (2391)</td>
<td>1288 (1614)</td>
<td>.16</td>
</tr>
<tr>
<td>Gadolinium-enhancing lesion volume, mm³</td>
<td>233 (459)</td>
<td>228 (556)</td>
<td>.94</td>
</tr>
<tr>
<td>Brain parenchymal fraction</td>
<td>0.83 (0.02)</td>
<td>0.83 (0.02)</td>
<td>.96</td>
</tr>
</tbody>
</table>

*Data are given as mean (SD).

**Table 2. Correlations Between Baseline MRI Variables and Brain Atrophy**

<table>
<thead>
<tr>
<th>Baseline MRI Variable</th>
<th>BPF at Baseline in All Subjects (n = 133)</th>
<th>1-Year BPF Percentage Change</th>
<th>2-Year BPF Percentage Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 68)</td>
<td>Placebo Group</td>
<td>Interferon Beta-1a Group (n = 65)</td>
<td>Placebo Group (n = 68)</td>
</tr>
<tr>
<td>r</td>
<td>P Value</td>
<td>r</td>
<td>P Value</td>
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<tr>
<td>Caudate (bilateral) T2 intensity</td>
<td>0.30 &lt;.001</td>
<td>0.36 .003</td>
<td>0.08 .51</td>
</tr>
<tr>
<td>Dentate (left) T2 intensity</td>
<td>0.03 .75</td>
<td>-0.04 .74</td>
<td>0.04 .75</td>
</tr>
<tr>
<td>Dentate (right) T2 intensity</td>
<td>0.08 .36</td>
<td>0.01 .93</td>
<td>0.05 .72</td>
</tr>
<tr>
<td>Globus pallidus (left) T2 intensity</td>
<td>0.39 &lt;.001</td>
<td>0.42 &lt;.001</td>
<td>0.00 .98</td>
</tr>
<tr>
<td>Globus pallidus (right) T2 intensity</td>
<td>0.34 &lt;.001</td>
<td>0.21 .08</td>
<td>0.02 .86</td>
</tr>
<tr>
<td>Putamen (left) T2 intensity</td>
<td>0.27 .002</td>
<td>0.28 .02</td>
<td>0.09 .49</td>
</tr>
<tr>
<td>Putamen (right) T2 intensity</td>
<td>0.30 &lt;.001</td>
<td>0.19 .13</td>
<td>0.07 .60</td>
</tr>
<tr>
<td>Red nucleus (mean) T2 intensity</td>
<td>0.28 .001</td>
<td>0.07 .58</td>
<td>0.01 .96</td>
</tr>
<tr>
<td>Thalamus (mean) T2 intensity</td>
<td>0.19 .03</td>
<td>0.37 .002</td>
<td>0.13 .28</td>
</tr>
<tr>
<td>BPF</td>
<td>. . .</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Gadolinium-enhancing lesion volume</td>
<td>0.03 .75</td>
<td>-0.03 .84</td>
<td>-0.57 &lt;.001</td>
</tr>
<tr>
<td>T1 hypointense lesion volume</td>
<td>-0.41 &lt;.001</td>
<td>-0.17 .18</td>
<td>-0.61 &lt;.001</td>
</tr>
<tr>
<td>T2 hyperintense lesion volume</td>
<td>-0.40 &lt;.001</td>
<td>-0.19 .12</td>
<td>-0.62 &lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: BPF, brain parenchymal fraction; MRI, magnetic resonance imaging.
ing ROI T2 intensity (on 10 randomly chosen images) were 0.3% (range, 0.1%-0.6%) and 1.3% (range, 0.1%-2.9%), respectively. Follow-up studies were coregistered to their baseline images in 50 randomly chosen subjects (25 from each treatment group) to test the effects of intrasubject differences in head position on the change in T2 intensity. Whole-brain atrophy (brain parenchymal fraction [BPF]) and T2 hyperintense, and gadolinium-enhancing lesion volumes were obtained.

STATISTICAL ANALYSES

If left-right or anterior-posterior parity was established by a paired-samples t test, intrasubject T2 intensities were collapsed within structures. Between-group and longitudinal differences (paired-samples t test) and Pearson correlations were evaluated. P<.05 was considered statistically significant. A forward stepwise multiple regression analysis evaluated how baseline variables predicted BPF change. P≤.15 was required for a variable to be included, and P<.05 was required for a variable to be retained in the final model.

RESULTS

Left vs right intrasubject baseline-normalized T2 intensities were similar in the caudate (P =.67) and red nucleus (P =.84) and in thalamic ROIs (P =.54) but not in the dentate (P =.04), putamen (P <.001), and globus pallidus (P <.001). The 2-year change in ROI T2 intensity in the placebo group was a mean of +0.20% (range, −1.72% [decrease over time] to +2.06% [increase over time]; P =.06-.96). In the interferon group, the change was a mean of +3.33% (range, +2.27% to +4.37%; P =.21-.49). There were no differences between treatment groups (P =.22-.73). Image coregistration had no effect on the ability to detect ROI T2-intensity change (P =.08-.87). The BPF and T2 intensities were unrelated to age (P =.07-.76).

Baseline T2 hypointensity was associated with baseline whole-brain atrophy (lower BPF; Table 2). In the placebo group, baseline T2 hypointensities, but not MRI lesions and BPF, were associated with the percentage of

Figure 1. Placement and shape of regions of interest (ROIs) used to measure T2 intensities. CSF indicates cerebrospinal fluid; PU, putamen; AT, anterior thalamus; PT, posterior thalamus; GP, globus pallidus; HC, head of caudate; RN, red nucleus; and DT, dentate nucleus. The ROIs 5 mm in diameter were placed in the HC, AT, and PT. The ROIs 2 mm in diameter were placed in the RN. The PU was traced manually, and the GP and DT were traced by an edge-finding seed-growing technique. Care was taken to avoid inclusion of small hyperintensities, such as lesions or perivascular spaces, within the ROIs.
decrease in BPF during the next 1 year or during the full 2 years (Table 2 and Figure 2). All baseline MRI variables were entered into regression analysis to determine the best predictors of 1-year or 2-year BPF change. In the placebo group, the best MRI predictors of change in BPF in the first year were T2 hypointensity in the thalamus and dentate nucleus ($R^2 = 0.23; P = .002$). In the interferon group, T2 hyperintense and gadolinium-enhancing lesion volumes ($R^2 = 0.38; P = .006$) were the best predictors of change in BPF. In the placebo group, T2 hypointensity in the thalamus was the best baseline predictor of BPF change during 2 years, with T2 hypointensity in the left putamen and red nucleus adding predictive value in the final model ($R^2 = 0.33; P < .001$). In the interferon group, baseline T2 hyperintense lesion volume was the best predictor of BPF change during 2 years, with gadolinium-enhancing lesion volumes also chosen in the final model ($R^2 = 0.38; P < .001$). Thus, the relationship between T2 hypointensity and progressive brain atrophy emerged in the first year. Interferon affected the relationship between early tissue changes and subsequent brain atrophy, beginning in the first year.

In patients receiving placebo, baseline T2 hypointensity in the deep gray nuclei predicted the progression of whole-brain atrophy throughout 2 years. T2 hypointensity was selected over the other MRI measures as most strongly predictive of the progression of brain atrophy. These findings suggest a longitudinal relationship between early iron deposition and subsequent tissue loss. However, the strength of the relationship between T2 hypointensity and progressive brain atrophy was only moderate, indicating that other factors were involved.

T2 hypointensity in the deep gray matter most likely represents pathologic iron deposition, as has been confirmed pathologically in normal aging and neurodegenerative disorders. Emerging data implicate abnormal iron metabolism as one of the pathophysiologic mechanisms of MS. Ferritin levels are elevated in patients with chronic progressive MS. Heme oxygenase 1 is upregulated and is related to increased tissue iron in MS. Nonheme iron that acts as a catalyst in oxidation reactions may be abnormally sequestered in deep gray tissue and thus may contribute to secondary tissue damage caused by lipid peroxidation. We speculate that the association between T2 hypointensity and brain atrophy indicates a role for pathologic iron deposition as either a mediator or a disease epiphenomenon.

In patients treated with interferon beta-1a, the relationship between baseline T2 hypointensity and progressive atrophy was disrupted. Moreover, a striking finding was that interferon treatment led to an unmasking of the relationship between all conventional MRI lesion measures at baseline and subsequent whole-brain atrophy. This effect appeared in the first year, indicating that interferon may alter the neurodegenerative process earlier than previously thought. We do not understand the reasons for this treatment-associated change in predictive relationships between early MRI findings and subsequent brain atrophy. One explanation may relate to the multifactorial nature of the atrophy process, in which a drug therapy may have a targeted effect on one aspect of the pathogenic cascade but not another. For example, interferon beta is known to limit the formation of new lesions but may not affect the evolution of established lesions.

Interferon beta-1a may affect brain atrophy by modifying systemic inflammatory mediators related to oxidative stress. Heme oxygenase 1 expression and mitochondrial iron deposition precipitated by inflammatory cytokines are attenuated by interferon beta. However, it may be difficult to extrapolate in vitro data of interferon effects to patients with MS because little if any evidence shows that interferon enters the central nervous system. Another possible mechanism is interferon's ability to stabilize the blood-brain barrier, limiting entry of inflammatory cells and possibly the influx of iron. Such limitations of iron accumulation or oxidative stress may help to keep the oxidative load in the brain below a critical threshold level for neurotoxicity. Accordingly, the predisposition of patients with a heavy oxidative burden (T2 hypointensity at baseline) to more severe atrophy would
be interrupted early in the disease process by interferon beta-1a. Although we explore the possibility that interferon limits the aspect of brain atrophy that is related to iron deposition in gray matter, such contentions are purely speculative and require further study.

A potentially confounding factor is the reduction of atrophy in the second year in the interferon group, leading to a restricted range of change in BPF and thus a decreased correlation with baseline T2 hypointensity due to a statistical phenomenon independent of any effect of therapy. This effect is unlikely given its appearance during the first year, in which the rate of brain atrophy was unaffected by interferon. Other possible causes for the lack of relationship between T2 hypointensity and subsequent brain atrophy in the interferon group include undetermined direct effects of interferon on gray matter or a chance occurrence. Further studies are warranted to confirm and extend our findings.

T2 hypointensity was asymmetric in some ROIs, in agreement with previous studies that showed laterality of MS gray matter involvement. The asymmetry may relate to normal brain structural variability, a nonrandom part of the MS disease process, or an artifact of technique or small sample size.

The thalamus, where T2 hypointensity was most significantly predictive of the progression of atrophy, has been implicated as a site of involvement in MS. Although pathologic iron deposition and neurodegeneration are most likely global processes, it is logical for the thalamus to be linked to whole-brain atrophy owing to its extensive reciprocal cortical and subcortical connections.

A longitudinal change in T2 intensity of gray matter nuclei was not detected in this study. It is likely that T2 intensity relative to cerebrospinal fluid on standard T2-weighted images is insensitive and unreliable to measure short-term changes in tissue iron. We were limited in this post hoc analysis because the original MRI protocol was not optimized for iron detection. This limitation should be addressed in future studies by iron-sensitive MRI. Another limitation was that clinical correlations were not investigated; however, previous work has linked T2 hypointensity of deep gray matter to physical disability and disease course. Rudick et al examined this cohort for effects of corticosteroid treatment and found no effect on the rate of atrophy. The level of disease severity and prevalence of corticosteroid use were similar in both treatment groups; therefore, if the effect is present, it is likely to affect both groups.

This study links T2 hypointensity and brain atrophy in relapsing-remitting MS. Although we describe what could be a causal relationship between iron deposition and neurodegeneration, iron deposition or some other factor that causes T2 hypointensity may be an epiphenomenon. Future studies should specifically localize and quantify tissue iron and further investigate a possible neurotoxic role.

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