Highlights

- Compared to healthy controls, patients with MS showed extensive regional deep and cortical gray matter atrophy. Cortical atrophy was particularly present in frontal and temporal lobes.

- Pathology in anatomically connected white matter tracts statistically explained both regional deep and cortical gray matter atrophy in relapsing-remitting patients, but only deep gray matter atrophy in patients with secondary-progressive (SP) MS.

- The weaker relationship between regional gray matter atrophy and anatomically connected white matter pathology in patients with SPMS suggests a more independent neurodegenerative process in these patients.
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Unraveling the relationship between regional gray matter atrophy and pathology in connected white matter tracts in long-standing multiple sclerosis

In Human Brain Mapping. 2015 May;36(5):1796–1807

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Abstract

Introduction
Gray matter (GM) atrophy is common in multiple sclerosis (MS), but the relationship with white matter (WM) pathology is largely unknown. Some studies found a co-occurrence in specific systems, but a regional analysis across the brain in different clinical phenotypes is necessary to further understand the disease mechanism underlying GM atrophy in MS. Therefore we investigated the association between regional GM atrophy and pathology in anatomically connected WM tracts.

Materials and methods
Conventional and diffusion tensor imaging was performed at 3T in 208 patients with long-standing MS and 60 healthy controls. Deep and cortical GM regions were segmented and quantified, and both lesion volumes and average normal-appearing WM fractional anisotropy of their associated tracts were derived using an atlas obtained by probabilistic tractography in the controls. Linear regression was then performed to quantify the amount of regional GM atrophy that can be explained by WM pathology in the connected tract.

Results
MS patients showed extensive deep and cortical GM atrophy. Cortical atrophy was particularly present in frontal and temporal regions. Pathology in connected WM tracts statistically explained both regional deep and cortical GM atrophy in relapsing-remitting (RR) patients, but only deep GM atrophy in secondary-progressive (SP) patients.

Conclusion
In RRMS patients, both deep and cortical GM atrophy were associated with pathology in connected WM tracts. In SPMS patients, only regional deep GM atrophy could be explained by pathology in connected WM tracts. This suggests that in SPMS patients cortical GM atrophy and WM damage are (at least partly) independent disease processes.
Introduction

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the central nervous system. Although focal white matter (WM) lesions are still the most important MRI characteristic used today in MS diagnosis (1) and clinical trials (2–4), it is now recognized that gray matter (GM) atrophy is an essential component of the disease (5,6). GM atrophy has shown to be present early in the disease, across all different MS disease types, and to be associated with physical disability, cognitive decline and disease duration (7–15). Although the exact mechanism underlying GM atrophy is unknown, several hypotheses have been postulated (16). They include primary damage of the GM, such as neuronal and axonal loss (17); but also secondary damage due to axonal transection by WM lesions (18), or gradually increasing damage due to network degeneration (i.e., gradually increasing effects of local WM and GM pathology throughout the brain).

Several magnetic resonance imaging (MRI) studies investigated the presumed relationship between GM atrophy and WM pathology. Most of these reported associations between GM loss and increased lesion load (8,10,13–15,19–22), and some additionally reported associations between atrophy and normal-appearing tissue damage (23). Previous work in our own group indicated that, in patients with long-standing MS, whole-brain and deep GM atrophy are particularly associated with WM atrophy and lesion volume, while cortical atrophy is associated with normal-appearing white matter integrity loss (24). To explore the spatial relationship between GM atrophy and WM pathology, some authors investigated whether regional WM pathology is related to GM atrophy in specific regions. Using voxelwise statistics, they found that regional GM atrophy can be better explained by WM pathology in co-localized areas compared to whole-brain measures (25–27). Only a few studies used a direct analysis of structural connectivity (using diffusion tensor imaging; DTI) to assess the association between WM pathology and GM atrophy in connected areas (28,29). Those studies focused mostly on specific GM regions or WM tracts in early MS patients and found a stronger relation between GM atrophy and WM lesions in connected regions. To gain more insight in the relationship between GM atrophy and WM pathology, an analysis of these mechanisms across the entire brain and in the different disease types is necessary, but to our knowledge, this has not yet been performed.

Therefore, in the present study, we aimed to assess the association between regional GM atrophy and pathology in connected WM tracts on a whole-brain level. In our unique large cohort of long-standing MS patients (24), we quantified regional atrophy and used a custom-made atlas of typical regional connectivity to quantify the amount of WM pathology in
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connected tracts. Linear regression was performed to quantify the amount of regional GM atrophy that can be explained by WM pathology in the connected tracts.

**Materials and methods**

**Participants**

The institutional ethics review board approved the study protocol and all subjects gave written informed consent prior to participation. All patients were diagnosed with clinically definite MS (1), and were recruited from the clinical MS database in our MS center if they had a disease duration of at least 10 years. Disease severity of the patients was measured on the day of scanning using the Expanded Disability Status Scale (EDSS) (30). To allow for comparison of the imaging measures in the MS patients with control values, healthy control subjects were recruited via advertisements in the hospital and from non-related family and friends of the patients. A more detailed description of the cohort, recruitment and eligibility procedure can be found elsewhere (24); it should be noted that the participants and imaging data used in the current study overlapped with that previous study.

**Magnetic resonance imaging**

Scanning was performed on a 3T whole body scanner (GE Signa HDxt, Milwaukee, WI, USA) using an eight-channel phased-array head coil. The protocol included a 3D T1-weighted fast spoiled gradient recalled echo (FSPGR) sequence (repetition time (TR) 7.8 ms, echo time (TE) 3 ms, inversion time (TI) 450 ms, flip angle 12º, sagittal 1.0 mm slices, 0.94 × 0.94 mm² in-plane resolution) for volumetric measurements and a 3D fluid attenuated inversion recovery sequence (FLAIR; TR 8000 ms, TE 125 ms, TI 2350 ms, sagittal 1.2 mm slices, 0.98 × 0.98 mm² in-plane resolution) for lesion detection. Furthermore, 2D echo-planar DTI (TR 13000 ms, TE 86 ms, 2.4 mm slices, 2.0 × 2.0 mm² in-plane resolution) was performed, including 30 volumes with noncollinear diffusion gradients (b-value 900 s/mm²) and 5 volumes without diffusion weighting. Prior to further analysis, the 3D images were corrected for geometrical distortions due to gradient nonlinearity.

**Lesion segmentation and whole-brain MRI measures**

A detailed description on the WM lesion segmentation and the whole-brain measures in the present cohort was published previously (24). In short, WM lesions were automatically segmented using the kNN-TTP algorithm (31). Subsequently, in each patient, the lesion
volume was quantified and normalized for head size resulting in normalized lesion volume (NLV). Normalized whole brain (NBV), WM (NWMV) and GM (NGMV) were computed using the T1-weighted image and SIENAX (part of FSL 5.0.4, http://www.fmrib.ox.ac.uk/fsl) after lesion filling (32) to minimize the impact of WM lesions on atrophy measurements.

Deep GM structures

Deep GM structures were segmented using the T1-weighted images and FSL-FIRST (33) (also part of FSL). This provided segmentations of 7 deep GM structures per hemisphere, including the nucleus accumbens, amygdala, caudate, hippocampus, globus pallidus, putamen and thalamus. The volumes of all deep GM regions were measured and normalized to account for differences in head size.

Cortical thickness

Cortical thickness was measured using the FreeSurfer 5.3 processing stream (34,35). In short, FreeSurfer uses the T1-weighted images to locate the pial and WM surface of the cortex. The distance between these surfaces gives the vertex-wise cortical thickness (i.e., the perpendicular thickness at each location). The cortical thickness was subsequently averaged in different ‘lobes’ (i.e., frontal, precentral, postcentral, parietal, temporal, occipital, cingulate and insula) for global description, and in 34 cortical regions per hemisphere for regional analysis (36). Vertex wise analysis (see ‘Statistical analysis’ section below) was used to compare the long-standing MS population to previous cortical thickness studies. All cortical segmentations were manually checked and re-run after manual editing if errors occurred.

Construction of the connectivity atlas and quantification of WM pathology in tracts

The DTI images were corrected for head movement and eddy current distortions using FMRIB’s Diffusion Toolbox (FDT; also part of FSL). Subsequently the diffusion tensor was fitted, from which the fractional anisotropy (FA), mean (MD), axial (AD) and radial diffusivity (RD) were calculated.

DTI was then used to obtain the tract distributions typically associated with the previously segmented deep and cortical GM regions. Because tractography in the presence of MS pathology might lead to unreliable results, this was done by means of a probabilistic atlas based on the healthy controls. The pipeline was as follows (see Figure 1):

1. For each healthy control, the construction of the atlas first involved running bedpostx (also part of FSL) to estimate the voxelwise diffusion parameter distributions of a two-
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Figure 1. Construction of the connectivity atlas and quantification of pathology in white matter tracts. 1) For each healthy control, probabilistic tractography is run from 82 gray matter regions to identify the connected tracts; 2) the healthy controls are non-linearly registered to standard space and averaged to obtain an atlas with 82 probabilistic tracts; 3) the atlas is non-linearly registered to subject space to obtain an subject-specific tract segmentation; 4) the average weighted lesion volume and weighted average FA, are computed for each subject and tract separately.

fiber model. Then, for each healthy control, 82 GM region–tract pairs were obtained by running whole-brain probabilistic tractography (protrackx2, also part of FSL, 5000 streamlines per voxel) for each GM region separately, using the previously obtained deep and cortical GM masks as seed regions. Except the brain mask, no stopping criteria were applied.

2. The probabilistic maps of the tracts were non-linearly registered to MNI152 space and averaged to construct a single probabilistic map for each region in standard space. The 82 probabilistic maps (left/right, 7 deep and 34 cortical GM) formed the atlas of the tracts associated to the GM regions.

3. To obtain a subject-specific segmentation, the probabilistic atlas was propagated to the
individual-space of each subject using nonlinear registration (FNIRT, also part of FSL) of
the FA image while masking out the lesions from the registration cost-function. Linear
registration was used in the patients to obtain the lesion mask in FA space.

4. For each subject and tract distribution separately, the weighted lesion volume, as well
as the weighted average FA values inside the NAWM of the tract were computed using
the atlas probability values as a weighting value. Weighting was performed to emphasise
lesions and FA values in the centers of the tracts associated to each GM region. In order
to only include WM, the weighting values of voxels belonging to GM and CSF were set to
zero using the previously derived SIENAX segmentation. Note that the weighted lesion
volume is a relative instead of an absolute value, and cannot be compared with normal
(unweighted) whole-brain lesion volume.

Statistical analysis

Statistical analyses of the demographic, clinical and descriptive MRI measures were performed
in SPSS 20 (Chicago, IL, USA). Kolmogorov–Smirnov tests and visual inspection of the
histogram were used to assess normality of the variables. When the variables were normally
distributed, a multivariate general linear model (GLM) was used to assess group differences.
When variables were not normally distributed, the Mann–Whitney or the Kruskal–Wallis
test was used, as appropriate. Where applicable, the analyses were Bonferroni-corrected.
P-values < 0.05 were considered as statistically significant.

To analyze local cortical thickness differences in MS patients and disease types, vertex-wise
GLM analysis was performed using the FreeSurfer software. Prior to vertex-wise analysis, the
surface maps of cortical thickness were smoothed using a Gaussian kernel with a FWHM
of 10 mm. Cluster wise correction for multiple comparisons was applied using Monte Carlo
Z simulation while thresholding the statistical maps at \( p < 0.001 \), using 5000 iterations and
setting the cluster-level threshold at \( p < 0.05 \).

The relationship between atrophy in GM regions and pathology in the connected WM tracts
was assessed using a two step procedure. First, linear regression was used for every region
separately to remove the effects of normal aging and sex on deep GM volume and cortical
thickness. In this step, the regression coefficients were estimated based on the healthy controls
and the resulting model was used to eliminate the normal aging and sex effect from the GM
atrophy measures. A second linear regression analysis was then used to assess the relationship
between regional GM atrophy and pathology in the connected WM tract. Here the residuals
of the previous analysis were used as dependents, and the weighted lesion volume as well as
weighted average \( FA_{\text{NAWM}} \) of the tract connected to the respective GM region as independent
# Table 1. Demographic, clinical and MRI measures.

<table>
<thead>
<tr>
<th>Measure</th>
<th>HC (n = 60)</th>
<th>MS (n = 208)</th>
<th>RRMS (n = 130)</th>
<th>SPMS (n = 53)</th>
<th>PPMS (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50.33 ± 7.08</td>
<td>53.70 ± 9.62***</td>
<td>50.68 ± 9.53</td>
<td>57.00 ± 6.76***</td>
<td>62.40 ± 7.66***</td>
</tr>
<tr>
<td>F/M</td>
<td>37/23</td>
<td>141/67</td>
<td>97/33</td>
<td>34/19</td>
<td>10/15</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>-</td>
<td>20.20 ± 7.08</td>
<td>19.03 ± 6.23</td>
<td>23.05 ± 8.50†</td>
<td>20.22 ± 6.44</td>
</tr>
<tr>
<td>EDSSb</td>
<td>-</td>
<td>4.0 (3.0 - 6.0)</td>
<td>3.0 (2.5 - 4.0)</td>
<td>6.0 (4.0 - 7.0)†</td>
<td>6.0 (4.5 - 6.5)‡</td>
</tr>
<tr>
<td>NBV, L</td>
<td>1.49 ± 0.07</td>
<td>1.41 ± 0.09***</td>
<td>1.43 ± 0.10***</td>
<td>1.39 ± 0.08***</td>
<td>1.41 ± 0.10***</td>
</tr>
<tr>
<td>NGMV, L</td>
<td>0.80 ± 0.05</td>
<td>0.75 ± 0.06***</td>
<td>0.76 ± 0.06*</td>
<td>0.73 ± 0.05***</td>
<td>0.74 ± 0.07***</td>
</tr>
<tr>
<td>NWMV, L</td>
<td>0.70 ± 0.03</td>
<td>0.66 ± 0.05***</td>
<td>0.66 ± 0.05***</td>
<td>0.66 ± 0.05***</td>
<td>0.67 ± 0.04</td>
</tr>
<tr>
<td>LV, mL</td>
<td>-</td>
<td>11.24</td>
<td>9.70</td>
<td>13.93</td>
<td>12.49</td>
</tr>
<tr>
<td>NLV, mLb</td>
<td>-</td>
<td>18.09</td>
<td>16.80</td>
<td>24.65</td>
<td>15.24</td>
</tr>
<tr>
<td>(9.93 - 29.67)</td>
<td>(8.58 - 26.81)</td>
<td>(15.92 - 41.50)</td>
<td>(9.35 - 28.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDGMV, mLb†</td>
<td>63.45 ± 4.71</td>
<td>57.27 ± 6.62***</td>
<td>58.00 ± 6.73***</td>
<td>55.21 ± 6.28***</td>
<td>57.60 ± 6.26***</td>
</tr>
<tr>
<td>Accumbens</td>
<td>1.26 ± 0.20</td>
<td>1.10 ± 0.29***</td>
<td>1.16 ± 0.26*</td>
<td>1.00 ± 0.28***</td>
<td>1.02 ± 0.36***</td>
</tr>
<tr>
<td>Amygdala</td>
<td>3.92 ± 0.55</td>
<td>3.64 ± 0.53***</td>
<td>3.66 ± 0.52*</td>
<td>3.58 ± 0.55**</td>
<td>3.65 ± 0.55</td>
</tr>
<tr>
<td>Caudate</td>
<td>9.30 ± 1.01</td>
<td>8.30 ± 1.12***</td>
<td>8.42 ± 1.16***</td>
<td>8.06 ± 0.93***</td>
<td>8.18 ± 1.21***</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>10.33 ± 1.09</td>
<td>9.39 ± 1.32***</td>
<td>9.63 ± 1.20**</td>
<td>9.00 ± 1.31***</td>
<td>8.94 ± 1.66***</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>4.76 ± 0.51</td>
<td>4.36 ± 0.64***</td>
<td>4.42 ± 0.61**</td>
<td>4.18 ± 0.67**</td>
<td>4.43 ± 0.70</td>
</tr>
<tr>
<td>Putamen</td>
<td>13.01 ± 1.15</td>
<td>12.02 ± 1.60***</td>
<td>12.08 ± 1.61***</td>
<td>11.73 ± 1.63***</td>
<td>12.29 ± 1.47</td>
</tr>
<tr>
<td>Thalamus</td>
<td>20.83 ± 1.59</td>
<td>18.43 ± 2.40***</td>
<td>18.61 ± 2.44**</td>
<td>17.66 ± 2.36***</td>
<td>19.09 ± 1.90***</td>
</tr>
<tr>
<td>Cortical thicknessd</td>
<td>2.48 ± 0.09</td>
<td>2.39 ± 0.10***</td>
<td>2.40 ± 0.10***</td>
<td>2.35 ± 0.09***</td>
<td>2.38 ± 0.11***</td>
</tr>
<tr>
<td>Frontal</td>
<td>2.51 ± 0.09</td>
<td>2.43 ± 0.12***</td>
<td>2.45 ± 0.10**</td>
<td>2.40 ± 0.10***</td>
<td>2.39 ± 0.19***</td>
</tr>
<tr>
<td>Precentral</td>
<td>2.53 ± 0.11</td>
<td>2.42 ± 0.14***</td>
<td>2.45 ± 0.13**</td>
<td>2.37 ± 0.13**</td>
<td>2.37 ± 0.17***</td>
</tr>
<tr>
<td>Postcentral</td>
<td>2.07 ± 0.11</td>
<td>1.99 ± 0.12***</td>
<td>2.01 ± 0.11*</td>
<td>1.96 ± 0.11***</td>
<td>1.97 ± 0.15*</td>
</tr>
<tr>
<td>Parietal</td>
<td>2.35 ± 0.09</td>
<td>2.27 ± 0.12***</td>
<td>2.28 ± 0.11***</td>
<td>2.24 ± 0.11***</td>
<td>2.25 ± 0.17**</td>
</tr>
<tr>
<td>Temporal</td>
<td>2.82 ± 0.10</td>
<td>2.70 ± 0.15***</td>
<td>2.72 ± 0.13**</td>
<td>2.65 ± 0.13**</td>
<td>2.67 ± 0.25***</td>
</tr>
<tr>
<td>Occipital</td>
<td>2.04 ± 0.09</td>
<td>1.96 ± 0.11***</td>
<td>1.97 ± 0.10***</td>
<td>1.95 ± 0.09***</td>
<td>1.95 ± 0.17**</td>
</tr>
<tr>
<td>Cingulate</td>
<td>2.57 ± 0.13</td>
<td>2.50 ± 0.14**</td>
<td>2.53 ± 0.12</td>
<td>2.48 ± 0.13**</td>
<td>2.44 ± 0.19***</td>
</tr>
<tr>
<td>Insular</td>
<td>3.06 ± 0.11</td>
<td>2.98 ± 0.15**</td>
<td>3.00 ± 0.14**</td>
<td>2.97 ± 0.09**</td>
<td>2.93 ± 0.25</td>
</tr>
<tr>
<td>FA&lt;sub&gt;NAWM&lt;/sub&gt;</td>
<td>0.39 ± 0.02</td>
<td>0.37 ± 0.03***</td>
<td>0.37 ± 0.03***</td>
<td>0.36 ± 0.02***</td>
<td>0.37 ± 0.02**</td>
</tr>
<tr>
<td>MD&lt;sub&gt;NAWM&lt;/sub&gt;</td>
<td>0.83 ± 0.03</td>
<td>0.85 ± 0.03***</td>
<td>0.85 ± 0.03**</td>
<td>0.86 ± 0.03***</td>
<td>0.85 ± 0.03**</td>
</tr>
<tr>
<td>AD&lt;sub&gt;NAWM&lt;/sub&gt;</td>
<td>1.19 ± 0.03</td>
<td>1.20 ± 0.03</td>
<td>1.20 ± 0.03</td>
<td>1.20 ± 0.03</td>
<td>1.20 ± 0.03</td>
</tr>
<tr>
<td>RD&lt;sub&gt;NAWM&lt;/sub&gt;</td>
<td>0.65 ± 0.03</td>
<td>0.68 ± 0.04***</td>
<td>0.68 ± 0.04***</td>
<td>0.70 ± 0.04***</td>
<td>0.67 ± 0.04*</td>
</tr>
</tbody>
</table>

Abbreviations: HC = healthy controls; MS = multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis; PPMS = primary-progressive multiple sclerosis; EDSS = Expanded Disability Status Scale; NBV = normalized brain volume; NGMV = normalized gray matter volume; NWMV = normalized white matter volume; NLV = normalized lesion volume; NAWM = normal-appearing white matter; FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity.

*Values listed are mean ± standard deviation for normally distributed variables, p-values were Bonferroni- corrected.*
variables. Cortical regression variables and statistics were displayed on a 3D surface to enable visual interpretation of the results. Possible differences between disease types were investigated by repeating the analysis for all disease types as well as the relapse-onset (RO) MS group separately. Regional regression analyses were performed in Matlab R2011a (Natick, MA, USA).

**Results**

**Demographic, clinical and MRI characteristics**

A detailed discussion on the demographic, clinical and whole-brain MRI findings in the present cohort was published previously (24). In short, a total of 208 (67% female) patients with MS and 60 (62% female) healthy controls were included (see Table 1). The MS patients had an average age of 53.70 (± 9.62) years, had an average disease duration of 20.20 (± 7.08) years and consisted of 130 relapsing-remitting (RR), 53 secondary-progressive (SP) and 25 primary-progressive (PP) patients. Of the MS patients, 10 were using glatiramer, 40 beta-interferon and 9 natalizumab. Age was different between the total patient group and controls, but not between RRMS patients and controls. PPMS and SPMS patients had higher EDSS scores than RRMS patients.

The total MS group displayed atrophy in all deep GM structures. Compared to the RRMS patients, SPMS patients had more severe atrophy in the accumbens, hippocampus and thalamus, whereas in the PPMS patients only the hippocampus showed more pronounced atrophy (see Table 1). The MS patients displayed reduced cortical thickness in all lobes. Compared to RRMS patients, SPMS patients showed more cortical atrophy in the frontal, precentral, postcentral and temporal region, while in the PPMS patients more cortical atrophy was only found in the precentral and cingulate gyrus (see Table 1).
Supplementary Figure 1. Thickness difference maps highlighting significantly thinner regions in the MS patients as a group compared to the healthy controls. Figure displays vertex-wise $p$-values in significant clusters.

**Local cortical thickness**

Supplementary Figure 1 shows the areas with significant cortical thinning in the MS patients as a group compared to the controls. Bilateral thinning was found in the insula, precuneus, lingual, precentral, superior frontal, superior parietal and inferior parietal cortex. Thinning was also present in other regions, such as the left supramarginal, paracentral and rostral middle frontal cortex, as well as in the right entorhinal, lateral occipital, post central, fusiform and superior temporal cortex. The analyses were repeated with age and sex as covariates. Adding age as a covariate showed a slight reduction of the size, but not position of the clusters of significant cortical thinning in patients. No additional differences were seen with sex as a cofactor (data not shown).

Comparing the individual subtypes with healthy controls revealed a more widespread pattern of cortical thinning in the SPMS patients than the RRMS patients, while the pattern of PPMS was comparable to RRMS patients (see Figure 2). A direct comparison of the SPMS to the RRMS patients revealed small regions with more thinning in the bilateral temporal lobes and the superior part of the left precentral cortex (data not shown). No significant differences were found in the direct comparison of PPMS with RRMS patients.

**Association between regional GM atrophy and pathology in connected WM tracts**

Figure 3 and Table 2 display the results of the regional linear regression analyses. In the
Figure 2. Thickness difference maps highlighting significantly thinner regions in
(continued)
regression analyses, variables measured in the left and right hemispheres were averaged as the patterns only differed marginally. The results (i.e., the linear regression models in which regional age and sex corrected cortical thickness or deep GM volume were the dependent variables measured in the left and right hemispheres were averaged as the patterns only differed marginally. The results (i.e., the linear regression models in which regional age and sex corrected cortical thickness or deep GM volume were the dependent variables measured in the left and right hemispheres were averaged as the patterns only differed marginally. The results (i.e., the linear regression models in which regional age and sex corrected cortical thickness or deep GM volume were the dependent
Regional GM atrophy and pathology in connected WM tracts in MS

Table 2. Average regional linear regression results.

<table>
<thead>
<tr>
<th>Result</th>
<th>MS</th>
<th>ROMS</th>
<th>RRMS</th>
<th>SPMS</th>
<th>PPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>#sign</td>
<td>35 (85%)</td>
<td>33 (80%)</td>
<td>34 (83%)</td>
<td>15 (37%)</td>
</tr>
<tr>
<td></td>
<td>(n = 41)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>adjusted R²</td>
<td>0.120</td>
<td>0.122</td>
<td>0.154</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>stdβ_LV</td>
<td>-0.153</td>
<td>-0.144</td>
<td>-0.214</td>
<td>-0.063</td>
</tr>
<tr>
<td></td>
<td>stdβ_FA</td>
<td>0.196</td>
<td>0.206</td>
<td>0.193</td>
<td>0.137</td>
</tr>
<tr>
<td>Subcortical</td>
<td>#sign</td>
<td>7 (100%)</td>
<td>7 (100%)</td>
<td>7 (100%)</td>
<td>7 (100%)</td>
</tr>
<tr>
<td></td>
<td>adjusted R²</td>
<td>0.200</td>
<td>0.204</td>
<td>0.194</td>
<td>0.207</td>
</tr>
<tr>
<td></td>
<td>stdβ_LV</td>
<td>-0.332</td>
<td>-0.318</td>
<td>-0.349</td>
<td>-0.264</td>
</tr>
<tr>
<td></td>
<td>stdβ_FA</td>
<td>0.172</td>
<td>0.191</td>
<td>0.128</td>
<td>0.290</td>
</tr>
<tr>
<td>Cortical</td>
<td>#sign</td>
<td>28 (82%)</td>
<td>26 (76%)</td>
<td>27 (79%)</td>
<td>8 (24%)</td>
</tr>
<tr>
<td></td>
<td>adjusted R²</td>
<td>0.104</td>
<td>0.105</td>
<td>0.145</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>stdβ_LV</td>
<td>-0.116</td>
<td>-0.108</td>
<td>-0.187</td>
<td>-0.021</td>
</tr>
<tr>
<td></td>
<td>stdβ_FA</td>
<td>0.201</td>
<td>0.209</td>
<td>0.207</td>
<td>0.105</td>
</tr>
</tbody>
</table>

Abbreviations: MS = multiple sclerosis; ROMS = relapse-onset MS; RRMS = relapsing-remitting MS; SPMS = secondary-progressive MS; PPMS = primary-progressive MS; #sign = number of regions with significant models.

Figure 4. Graphical visualization of the deep GM regional regression model results. Gray areas indicate regions without significant thinning (Z-scores), non-significant models (adjusted R²) or variables non-significantly contributing to the model (standardized β), whereas the colors correspond to the results of the respective regional model.

Variables and weighted lesion volume and average FA_NAWM were the independent variables) are displayed in an alternative fashion in Figures 4 and 5. In general, twice as much of the variance was explained in the models for deep GM volume compared to the models for cortical thickness (in the total MS group on average 20.0% and 10.4%, respectively). The volumes of all deep GM structures could be explained significantly, whereas only in 28 of the 34 (82%
cortical regions, atrophy could be explained on the basis of pathology in connected WM tracts. Deep GM atrophy was particularly explained by lesion volume in the connected tract (in the total MS group, on average $\text{std}^\beta_{\text{LV}} = -0.332$ versus $\text{std}^\beta_{\text{FA}} = 0.172$), whereas cortical GM atrophy was particularly associated with normal-appearing tract FA (in the total MS group, on average $\text{std}^\beta_{\text{FA}} = 0.201$ versus $\text{std}^\beta_{\text{LV}} = -0.116$).

Looking into the clinical subtypes (see Table 2), all models for deep GM volume were significant, except the models for the amygdala, putamen and thalamus in PPMS patients. Large differences were however observed in the models for cortical thickness: in the RRMS patients, 26 of the 34 (76%) models for regional cortical thickness were significant, whereas only 8 (24%) models were significant in the SPMS patients and 7 (21%) in the PPMS patients. To exclude the possibility that difference in group size has led to the differences between the RRMS and SPMS patients, we combined the RRMS and SPMS patients in a ROMS group. The deep GM model parameters in this ROMS group were similar to those of the separate RRMS and SPMS groups. However, in the cortical models, on average more variance was explained in the RRMS patients compared to the ROMS patients (14.5% versus 10.5%, respectively), emphasizing the difference between RRMS and SPMS.

Typically, the models for cortical thickness in the RRMS patients explained most of the variance in the temporal, parietal and posterior part of the frontal lobes (see Figure 5).
pattern showed a widespread reduction in the SPMS patients; only a few of the models in the temporal lobe remained (marginally) significant. In contrast to the RR and SPMS patients, the PPMS patients displayed a much more scattered pattern of explained variance, with two regions displaying very high numbers of explained variance. These regions were regions with very little atrophy, stressing that the results of the PPMS models should be interpreted with care as the PPMS group was small.

Discussion

To our knowledge, this is the first study in a large sample of long-term MS patients directly investigating the association between regional GM atrophy and pathology in connected WM tracts at the whole-brain level. Our results demonstrate that normal-appearing white matter damage plays an important role in explaining deep and cortical GM atrophy, whereas lesion volume is particularly associated with deep GM atrophy. Large differences were observed in the association patterns of regional GM atrophy and connected WM pathology of RRMS patients and SPMS patients, indicating that GM atrophy and WM damage become (at least partly) independent after conversion to progressive disease.

Our results confirm findings from previous studies, in which MS patients displayed extensive deep and cortical GM atrophy in the bilateral temporal lobes, frontal areas, insula, precentral gyrus and medial occipital lobes (7,13,14,37–40). Compared to RRMS patients, SPMS patients showed a decreased cortical thickness, while PPMS patients did not show a significant difference. More pronounced GM atrophy in patients with SPMS has been reported before, both at a whole-brain level and specifically in the cortical and deep GM regions (10,15,41,42). The absence of statistically more cortical atrophy in PPMS patients compared to RRMS patients is contrary to our expectations (11), but might be explained by a different atrophy pattern in the two groups, or by the relatively low number of PPMS patients in our study cohort.

While previous studies, including our own work on this same patient cohort, have focused on whole-brain measures of atrophy and other pathology, the current work takes the investigation of these associations to another level. By identifying specific WM tracts to anatomically defined GM regions, and quantifying the focal and diffuse damage in those WM tracts, we were able to directly assess the relationship between regional GM atrophy and pathology in connected WM tracts.
An atlas of WM connectivity based on healthy controls was used to quantify the WM pathology in the tracts connected to individual deep and cortical GM regions. To minimize the effects of atrophy and lesions on registration accuracy in the patients, nonlinear registration and masking of lesions were used. Regional linear regression models in the MS patients revealed that WM explained deep GM atrophy better than cortical GM atrophy. Whereas NAWM damage showed comparable associations with both deep and cortical GM atrophy, lesion volume showed stronger associations with deep than cortical GM atrophy. Associations were found between regional GM atrophy and pathology in connected WM tracts in the RRMS patients, but in the SPMS patients, only deep GM atrophy, and not cortical GM atrophy, could be explained by pathology in the connected WM tracts. The findings in RRMS patients are in line with previous studies reporting a spatial relationship between deep GM atrophy and WM pathology (18,26–28). Studies investigating regional cortical GM and whole-brain lesion load in RRMS indicated that GM atrophy in frontotemporoparietal regions is partially dependent upon increasing lesion load (18,20,29,43,44). Our results confirm these findings, with the difference that the strongest cortical associations were found in the temporal lobe.

An interesting finding is the absence of a relationship between GM atrophy and pathology in the connected WM tracts in the cortical GM of the SPMS patients. To exclude the possibility that the smaller SPMS sample size caused the absence of this relationship, we performed a

### Supplementary Table 1

Average regional linear regression results for the mildly disabled relapse-onset MS patients (MilROMS) and severely disabled relapse-onset MS patients (SevROMS).

<table>
<thead>
<tr>
<th></th>
<th>Result</th>
<th>MS</th>
<th>ROMS</th>
<th>MilROMS</th>
<th>SevROMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td>#sign</td>
<td>35 (85%)</td>
<td>33 (80%)</td>
<td>32 (78%)</td>
<td>25 (61%)</td>
</tr>
<tr>
<td>$(n = 41)$</td>
<td>adjusted $R^2$</td>
<td>0.120</td>
<td>0.122</td>
<td>0.136</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>$\text{std}_L$</td>
<td>$-0.153$</td>
<td>$-0.144$</td>
<td>$-0.203$</td>
<td>$-0.081$</td>
</tr>
<tr>
<td></td>
<td>$\text{std}_F$</td>
<td>0.196</td>
<td>0.206</td>
<td>0.182</td>
<td>0.193</td>
</tr>
<tr>
<td><strong>Subcortical</strong></td>
<td>#sign</td>
<td>7 (100%)</td>
<td>7 (100%)</td>
<td>6 (86%)</td>
<td>7 (100%)</td>
</tr>
<tr>
<td>$(n = 7)$</td>
<td>adjusted $R^2$</td>
<td>0.200</td>
<td>0.204</td>
<td>0.199</td>
<td>0.178</td>
</tr>
<tr>
<td></td>
<td>$\text{std}_L$</td>
<td>$-0.332$</td>
<td>$-0.318$</td>
<td>$-0.387$</td>
<td>$-0.224$</td>
</tr>
<tr>
<td></td>
<td>$\text{std}_F$</td>
<td>0.172</td>
<td>0.191</td>
<td>0.093</td>
<td>0.275</td>
</tr>
<tr>
<td><strong>Cortical</strong></td>
<td>#sign</td>
<td>28 (82%)</td>
<td>26 (76%)</td>
<td>26 (76%)</td>
<td>18 (52%)</td>
</tr>
<tr>
<td>$(n = 34)$</td>
<td>adjusted $R^2$</td>
<td>0.104</td>
<td>0.105</td>
<td>0.123</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>$\text{std}_L$</td>
<td>$-0.116$</td>
<td>$-0.108$</td>
<td>$-0.165$</td>
<td>$-0.005$</td>
</tr>
<tr>
<td></td>
<td>$\text{std}_F$</td>
<td>0.201</td>
<td>0.209</td>
<td>0.200</td>
<td>0.176</td>
</tr>
</tbody>
</table>

Abbreviations: MS = multiple sclerosis; ROMS = relapse-onset MS; MilROMS = mildly disabled ROMS; SevROMS = severely disabled ROMS; #sign = number of regions with significant models.
Regional GM atrophy and pathology in connected WM tracts in MS

A post-hoc analysis in the ROMS patients with a similar approach, but now using a median split based on EDSS (EDSS < 4 and EDSS ≥ 4, respectively) to construct two equally sized groups. This procedure resulted in a mildly impaired group of 91 patients (83 RR and 8 SPMS) and a severely impaired group of 92 patients (45 RR and 47 SPMS). The results of this post-hoc analysis showed a similar behavior as the separate RR and SPMS analyses; indicating a stronger association between cortical GM atrophy and pathology in connected WM tracts in mildly impaired patients compared to severely impaired patients (see Supplementary Table 1).

Studies describing the relation between regional GM atrophy and WM pathology in SPMS patients are limited, but Fisher et al. described accelerated GM atrophy in these patients (42) and Bendfeldt et al. found a clearly different GM atrophy pattern in patients with low versus high lesion load (45).

It should be noted that the current study was performed in a cohort of patients with long-standing disease. This might be beneficial for addressing questions related to the neurodegenerative aspects of the disease, as measures of pathological and physical decline will be more pronounced in these patients compared to patients with a short disease duration. However, due to the same reason, some of the RRMS patients in our cohort have a rather benign (and thereby somewhat atypical) disease course.

Taken together, our data suggests that in RRMS patients GM atrophy can (at least partially) be explained by retrograde neurodegeneration, whereas GM atrophy may be much more independent of WM pathology after conversion to progressive disease. Possible explanations for this difference in SPMS may include a more widespread inflammatory disease (46,47), or gradually increasing (indirect) remote effects of local WM and GM damage in the cortex of these patients, but future studies are required to investigate these hypotheses.

Some limitations apply to this work. Although the RRMS patients were well matched in age, the whole MS group was on average older than the healthy controls. To prevent an unwanted influence of age on the study outcomes, we removed the effects of age (and sex) based prior to performing the regional regression analyses. Although it might be that after removal of healthy aging effects some additional age dependencies can be found in the MS data, we believe the current approach is justified for various reasons: first, we selected this approach to limit the number of candidate predictors in the regional analysis and assure robust regression results; second, given that older MS patients are more likely to have more pathology, it may be that entering age (and sex) as a covariate in the regional models erroneously corrects for some of the disease-related effects; and thirdly, although we do currently extrapolate the healthy control data to older MS patients, brain changes due to healthy aging are thought to be fairly smooth processes and the age difference between the healthy controls and the SPMS group is fairly small (~7 years, approximately the standard deviation of both groups).
second limitation is the relatively small size of the PPMS and SPMS groups compared to the RRMS group. Therefore, especially the results concerning PPMS should be interpreted with great care. In the SPMS patients however, the results from the combined RRMS and SPMS (ROMS) and post-hoc analysis (i.e., mildly impaired versus severely impaired patients) can be used to confirm the lower amount of GM atrophy explained in SPMS patients. Indeed, the ROMS analysis displayed a lower amount of explained variance compared to the RRMS group only whereas the post-hoc analysis displayed less explained variance in the severely impaired patients compared to the mildly impaired patients, supporting the interpretation of a different mechanism underlying cortical atrophy in the SPMS patients. Third, although the effect of (juxta)cortical lesions on cortical atrophy is thought to be limited (48), it cannot be ruled out that (juxta)cortical lesions have had an influence on the results as they might result in anterograde axonal degeneration (49). Future studies elaborating on this topic are required to overcome this limitation. Finally, the cross-sectional design of the present study makes it impossible to make statements about causality. Longitudinal studies are required to overcome this limitation.

In conclusion, in RRMS patients, both deep and cortical GM atrophy were associated with pathology in connected WM tracts. In SPMS patients, only regional deep GM atrophy could be explained by pathology in connected WM tracts. This suggests that in SPMS patients cortical GM atrophy and WM damage are (at least partly) independent disease processes.
References


Chapter 3.2


