Metabolite Changes in Normal-Appearing Gray and White Matter Are Linked With Disability in Early Primary Progressive Multiple Sclerosis

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Background: Abnormalities in normal-appearing brain tissues may contribute to disability in primary progressive multiple sclerosis (PPMS), where few lesions are seen on conventional imaging.

Objectives: To evaluate the mechanisms underlying disease progression in the early phase of PPMS by measuring metabolite concentrations in normal-appearing white matter (NAWM) and cortical gray matter (CGM) and to assess their relationship with clinical outcomes.

Design: Case-control study.

Setting: Tertiary referral hospital.

Patients: Forty-three consecutive patients within 5 years of onset of PPMS and 44 healthy control subjects.

Main Outcome Measures: Concentrations of choline-containing compounds, phosphocreatine, myo-inositol, total N-acetyl-aspartate (tNAA), and glutamate-glutamine were estimated using proton magnetic resonance spectroscopic imaging. Brain parenchymal, white matter and gray matter fractions and proton density and gadolinium-enhancing lesion loads were calculated. The Expanded Disability Status Scale and Multiple Sclerosis Functional Composite scores were recorded.

Results: In CGM, concentrations of the tNAA (P < .001) and glutamate-glutamine (P = .005) were lower in patients with PPMS than in controls. In NAWM, myo-inositol levels were higher (P = .002) and tNAA levels were lower (P = .005) in patients with PPMS than in controls. The Expanded Disability Status Scale score correlated with the tNAA concentration in CGM (r = −.44; P = .03) and with myo-inositol (r = 0.41; P = .01) and glutamate-glutamine concentrations (r = 0.41; P = .01) in NAWM. Proton density lesion load correlated negatively with CGM tNAA concentration and positively with NAWM myo-inositol concentration.

Conclusion: Metabolite changes, which differ in CGM and NAWM, occur in early PPMS and are linked with disability.

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Changes in normal-appearing white matter (NAWM) in multiple sclerosis (MS) have been demonstrated early in the clinical course of the disease by a range of magnetic resonance (MR) techniques and are clinically relevant.¹ The most pathologically specific of these techniques, proton MR spectroscopy (MRS), which estimates the concentration of a number of metabolites in vivo, has shown reduced N-acetyl-aspartate, a putative marker of axonal loss or dysfunction, in the NAWM of patients with MS.² The use of short-echo MRS imaging (MRSI) together with more sophisticated quantification techniques³ makes it possible to determine the levels of other metabolites, such as myo-inositol and glutamate-glutamine, which could reflect other pathological processes, such as astrogliosis.

The study of normal-appearing brain tissue is particularly relevant in primary progressive MS (PPMS), where patients tend to have marked disability, despite having low lesion loads.⁴ Such studies need to include both gray and white matter (GM and WM, respectively) as GM is frequently involved in MS and may influence the clinical course.⁵ Segmentation of brain images into WM and GM facilitates the application of MRSI to both areas.⁶⁷

Finally, in studying patients with PPMS, it may be particularly productive to explore these relationships early in the disease course, at a time when there is a steady accumulation of disability.⁸ Previous studies have focused on later stages, which might contribute to the relative lack of correlations between metabolite concentra-
diagnostic criteria and clinical progression for less than 5 years (which has been shown in relapsing-remitting MS [RRMS]) and conventional radiological findings in a cohort of patients with early PPMS. Other potentially relevant metabolites will be investigated and measurements for NAWM and GM will be performed, as GM has been shown to be associated with disability in PPMS. 

METHODOLOGY

Forty-three consecutive patients with early PPMS and 44 healthy control subjects were recruited. Magnetic resonance spectroscopy imaging studies were performed on 41 patients (1 patient did not attend for MRSI and data from another patient could not be analyzed) and on all controls. Fulfillment of the PPMS diagnostic criteria and clinical progression for less than 5 years were the main inclusion criteria. Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Functional Composite were scored for all patients. The study had approval from the joint ethics committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology, London, United Kingdom. All subjects gave written informed consent.

A 1.5-T scanner (Signa; General Electric, Milwaukee, Wis) was used. Magnetic resonance spectroscopy imaging data were acquired from a volume located superior to the roof of the lateral ventricles (Figure 1) using a Point Resolved Spectroscopy localization sequence (echo time [TE], 30 milliseconds; repetition time [TR], 3000 milliseconds; number of excitations, 1; 24×24 phase encodes over a field of view of 30×30 cm; spectral width, 2500 Hz; number of points, 2048; section thickness [ST], 15 mm; and nominal voxel volume, 2.3 mL) with outer-volume suppression bands. Three other different sets of images were acquired: a 3-dimensional inversion-prepared fast spoiled gradient recall sequence (2279 voxels were still retained. Voxels with a Cramer-Rao minimum variance bound for any given metabolite 2 SDs above the mean of Cramer-Rao minimum variance bounds for that metabolite, with a content of less than 80% in GM plus WM or with a significant amount of lesional tissue (>1% of lesion fraction) were discarded. Of the 1620 voxels remaining, those containing more than 60% of WM (1115 voxels; 724 from controls and 391 from patients) were classified as NAWM and those containing more than 60% of cortical GM (CGM) (134 voxels: 113 from controls and 41 from patients) were classified as CGM. The differences in the final number of voxels available for analysis in patients and controls may relate to different factors: a larger number of voxels were available from the outset from controls (2634 vs 2371 voxels) and more severe atrophy in subjects with MS (13.41% vs 9.3% of mean cerebrospinal fluid content per voxel). A mean concentration for a given metabolite in CGM and NAWM voxels was obtained for each patient and control and entered into the statistical modeling.

Statistical analysis was performed using SPSS version 10.0 (SPSS Inc, Chicago, Ill). A general linear model analysis was used to determine the effect of MS on brain metabolite concentrations while allowing for age, sex, voxel tissue contents,
and potential partial volume effects associated with brain atrophy. Spearman correlation coefficients were used to assess the presence of linear associations among metabolite concentrations and clinical and radiological variables.

### RESULTS

Forty-one patients with early PPMS and 44 controls were studied (Table 1). Thirty-nine controls and 24 patients yielded usable CGM voxels, while 44 controls and 37 patients yielded usable NAWM voxels. No statistically significant differences were found in demographic or clinical variables between patients who provided voxels for CGM analyses and those who did not.

Significant differences were seen between patients and controls in metabolite concentrations in CGM for tNAA (−12.3% difference in marginal mean values favoring controls; P = .005) and glutamate-glutamine (−13.9%; P = .006), while glutamate-glutamine concentrations correlated with WMF (r = 0.47; P = .02). In NAWM, the level of myo-inositol correlated with the proton density load (r = 0.519; P = .001).

### Table 1. Demographic, Clinical, and Radiological Features of Healthy Control Subjects and Patients With Primary Progressive Multiple Sclerosis (PPMS)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Control Subjects (n = 44)</th>
<th>Patients With PPMS (n = 41)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF, %</td>
<td>50/50</td>
<td>56/44</td>
</tr>
<tr>
<td>Age, median (range), y</td>
<td>36 (23 to 67)</td>
<td>46 (22 to 65)</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>0 (2 to 5)</td>
<td>3.31 (2 to 5)</td>
</tr>
<tr>
<td>EDSS score, median (range)</td>
<td>0</td>
<td>4.5 (3 to 7)</td>
</tr>
<tr>
<td>MSFC score</td>
<td>0.26 (0.20 to 0.30)</td>
<td>0.35 (0.26 to 0.35)</td>
</tr>
<tr>
<td>BPF, mean (range)</td>
<td>0.83 (0.70 to 0.96)</td>
<td>0.70 (0.69 to 0.86)</td>
</tr>
<tr>
<td>WMF, mean (range)</td>
<td>0.55 (0.48 to 0.59)</td>
<td>0.38 (0.35 to 0.45)</td>
</tr>
<tr>
<td>Gd+ lesions, No.</td>
<td>0.14 (0.09 to 0.29)</td>
<td>1.1 (0 to 0.9)</td>
</tr>
<tr>
<td>Volume of Gd+ lesions, mL</td>
<td>0.14 (0.09 to 0.29)</td>
<td>0.14 (0.09 to 0.29)</td>
</tr>
<tr>
<td>PD lesion, mL</td>
<td>28.88 (1.43 to 119.46)</td>
<td>28.88 (1.43 to 119.46)</td>
</tr>
</tbody>
</table>

Abbreviations: BPF, brain parenchymal fraction; EDSS, Expanded Disability Status Scale; Gd+, gadolinium enhancing; GMF, gray matter fraction; MSFC, Multiple Sclerosis Functional Composite; PD, proton density; WMF, white matter fraction.

*Data are given as mean (range) unless otherwise indicated.
†N = 40 for brain fractions and Gd values.

### Table 2. Metabolite Concentrations in Cortical Gray Matter (CGM) and Normal-Appearing White Matter (NAWM) Voxels

<table>
<thead>
<tr>
<th>Metabolite*</th>
<th>Healthy Control Subjects (n = 39)</th>
<th>Patients With PPMS (n = 24)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cho</td>
<td>1.10 (0.04)</td>
<td>1.03 (0.06)</td>
<td>.29</td>
</tr>
<tr>
<td>Cr</td>
<td>6.00 (0.13)</td>
<td>5.69 (0.17)</td>
<td>.07</td>
</tr>
<tr>
<td>Ins</td>
<td>4.50 (0.18)</td>
<td>4.50 (0.18)</td>
<td>.27</td>
</tr>
<tr>
<td>tNAA</td>
<td>8.61 (0.14)</td>
<td>7.55 (0.19)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Glx</td>
<td>11.49 (0.31)</td>
<td>9.89 (0.42)</td>
<td>.005</td>
</tr>
<tr>
<td>NAWM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cho</td>
<td>1.26 (0.03)</td>
<td>1.30 (0.03)</td>
<td>.40</td>
</tr>
<tr>
<td>Cr</td>
<td>4.75 (0.07)</td>
<td>4.79 (0.08)</td>
<td>.69</td>
</tr>
<tr>
<td>Ins</td>
<td>4.21 (0.13)</td>
<td>4.21 (0.13)</td>
<td>.002</td>
</tr>
<tr>
<td>tNAA</td>
<td>8.55 (0.10)</td>
<td>8.06 (0.12)</td>
<td>.005</td>
</tr>
<tr>
<td>Glx</td>
<td>7.29 (0.12)</td>
<td>7.15 (0.13)</td>
<td>.48</td>
</tr>
</tbody>
</table>

Abbreviations: Cho, choline-containing compounds; Cr, creatine-phosphocreatine; Glx, glutamate-glutamine; Ins, myo-inositol; PPMS, primary progressive multiple sclerosis; tNAA, total N-acetyl-aspartate.

*Estimated marginal mean (SE) in millimoles per liter and P values obtained after linear modeling.

The present study demonstrates metabolic changes in both CGM and NAWM in the early stages of PPMS and suggests that tNAA concentrations in CGM and myo-inositol and glutamate-glutamine concentrations in NAWM are related to clinical disability in this subgroup of patients with MS.

### METABOLITE CHANGES

Studies of patients affected by PPMS with established disease have shown decreased tNAA/creatine-phosphocreatine or tNAA concentrations in NAWM.9,11,12 Our work extends these findings to the early clinical stages of PPMS, and, to our knowledge, is the first study to report on abnormalities of CGM metabolites in PPMS. Although the magnitude of the tNAA level decrease appeared greater in CGM than NAWM (mean decrease −12.3% vs −5.7%), the number of voxel and subjects available for CGM analysis was fewer and the potential for partial volume effects to influence CGM findings was greater. Nevertheless, since robust methods were used to address partial volume effects, the observed decrease in CGM tNAA concentration is likely to be a real biological finding, suggesting that neuronal dysfunction or loss occurs in the early stages of PPMS. Although high levels of NAWM myo-inositol have been recently found in patients with RRMS9 and clinically isolated syndromes,20 to our knowledge, our study is the first to demonstrate an increase in the levels of NAWM myo-inositol in patients with PPMS. Previous studies with MRS have identified myo-inositol as a po-
tential marker of astrogliosis,21 and histopathological studies in MS suggest that astrogliosis is a prominent abnormality in NAWM.22 Decreased glutamate-glutamine concentrations have not been reported previously in CGM of patients with PPMS. Although these 2 metabolites are closely associated in the brain,23 the concentration of glutamate is higher in neurons24,25 while that of glutamine is higher in glial cells.24 Therefore, although the present results appear consistent with CGM neuronal metabolic impairment or loss, further studies using techniques to resolve glutamate-glutamine signals are needed to clarify this issue.

CLINICORADIOLOGICAL CORRELATIONS

We found a moderate correlation between tNAA levels in CGM and disability, suggesting that CGM neuronal dysfunction or loss has an influence on clinical status in PPMS. A relationship was not found between tNAA levels in NAWM and disability, consistent with most previous PPMS study findings (being found in only 111 of 3 studies10,11,13) but differing from studies in RRMS.14,15 The present method cannot confirm whether this correlation relates to increased glial cellularity (increased glutamine concentration), increased excitotoxic reaction (increased extracellular glutamate concentration), or both. Magnetic resonance lesion load and atrophy in WM correlated with tNAA concentration in CGM, but not in NAWM, suggesting that WM disease influences the function or integrity of cortical neurons. The correlation between lesion load and myo-inositol concentration in NAWM has also been shown in RRMS.19

In summary, this study indicates that cortical neuronal dysfunction or loss (inferred from a decreased tNAA concentration) is an early feature in PPMS and is clinically relevant. Pathological change in NAWM (inferred from increased myo-inositol and glutamate-glutamine concentrations) also appears to be related to disability in this patient group. The value of these abnormalities in predicting disease progression needs to be investigated in follow-up studies.

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REFERENCES


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