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 ($r=-0.44; P=.03$) 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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tions and disability measures.\textsuperscript{9,13} Therefore, the aim of the present study will be to investigate the relationship of N-acetyl-aspartate levels between disability in PPMS (which has been shown in relapsing-remitting MS [RRMS]\textsuperscript{1,14,15}) 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.\textsuperscript{16}

**METHODS**

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\textsuperscript{1} and clinical progression for less than 5 years (which has been shown in relapsing-remitting MS [RRMS])\textsuperscript{1,14,15} 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.\textsuperscript{16}

**Figure 1.** Sagittal and axial views of slab and grid location and an example spectrum. A, T1-weighted image showing the approximate location of the magnetic resonance spectroscopic imaging (MRSI) slab. B, T1-weighted image with the MRSI grid overlaid on it. C, Spectrum extracted from the MRSI grid.
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 = .001) and glutamate-glutamine (−13.9%; P = .005). In NAWM, the tNAA concentration was reduced (−5.7%; P = .005) and the level of myo-inositol increased (+14.6%; P = .002) in patients compared with controls (Table 2).

Cortical GM tNAA concentration correlated with EDSS (r = −0.44; P = .03), Multiple Sclerosis Functional Composite scores (r = 0.49; P = .02) (Figure 2A), and with the 9-hole peg test (r = 0.48; P = .02). In NAWM, the level of myo-inositol correlated with the EDSS score (r = 0.41; P = .01) (Figure 2B), Multiple Sclerosis Functional Composite score (r = −0.48; P = .002), timed walk test (r = 0.37; P = .02) and 9-hole peg test (r = −0.481; P = .003). Glutamate-glutamine levels in NAWM correlated with age (r = −0.33; P = .048) and with the EDSS score (r = 0.41; P = .01). In NAWM, creatine-phosphocreatine concentrations correlated with disease duration (r = 0.34; P = .02). In CGM, tNAA concentrations correlated with proton density lesion load (r = −0.45; P = .03) and with WMF (r = 0.55; 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 lesion load (r = 0.519; P = .001).

**COMMENT**

The present study demonstrates metabolite 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.4–12 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, 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 RRMS13 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-

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**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>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>4.5 (3 to 7)</td>
<td></td>
</tr>
<tr>
<td>MSFC score</td>
<td>0.12</td>
<td>0.16 (−0.36 to 1.13)</td>
</tr>
<tr>
<td>BPF</td>
<td>0.83 (0.70 to 0.87)</td>
<td>0.79 (0.70 to 0.86)</td>
</tr>
<tr>
<td>WMF</td>
<td>0.28 (0.22 to 0.32)</td>
<td>0.26 (0.20 to 0.30)</td>
</tr>
<tr>
<td>GMF</td>
<td>0.55 (0.48 to 0.59)</td>
<td>0.53 (0.48 to 0.58)</td>
</tr>
<tr>
<td>Gd+ lesions, No.</td>
<td>0.11</td>
<td>1.1 (0 to 9)</td>
</tr>
<tr>
<td>Volume of Gd+ lesions, mL</td>
<td>0.14 (0 to 0.58)</td>
<td></td>
</tr>
<tr>
<td>PD lesion volume, mL</td>
<td>0.14</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 (0.05)</td>
<td>.29</td>
</tr>
<tr>
<td>Cr</td>
<td>6.00 (0.13)</td>
<td>5.59 (0.17)</td>
<td>.07</td>
</tr>
<tr>
<td>Ins</td>
<td>4.25 (0.13)</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>3.60 (0.12)</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.
correlation of NAWM myo-inositol with increased disability, consistent with most previous PPMS study findings (being found in only 11% of 3 studies10,11,13) but differing from studies in RRMS.14,15 The correlation of NAWM myo-inositol concentration with disability is remarkably concordant with studies of patients with RRMS16 and would suggest that myo-inositol is a marker of clinical progression in MS, although the mechanism is unclear. Measurements of myo-inositol concentration in NAWM may be of value in monitoring the effect of potential therapeutic agents. A positive correlation between NAWM glutamate-glutamine concentration and EDSS score has been found, suggesting that increasing concentrations of glutamate, glutamine, or both relate to worsening clinical status in patients with early PPMS. 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.

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