Response to acetylcholinesterase inhibitor (AchE-I) treatment varies substantially between individual patients with Alzheimer disease (AD).1 At present, no tools predicting treatment outcome are available. In a recent proton magnetic resonance spectroscopy (1H-MRS) study an increase of the neuronal marker N-acetylaspartate (NAA) after 3 months of treatment with donepezil compared to placebo has been reported.2 Here we assessed whether changes of NAA in the medial temporal lobe (MTL) and the parietal lobe correlated with individual treatment response and whether baseline NAA levels predicted treatment outcome.

Methods. Seventeen patients with mild to moderate probable AD (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria) were consecutively recruited from the outpatient memory clinic of the Department of Psychiatry, University of Bonn (6 men, 11 women; mean age 72.8 years, SD 7.3; mean Mini-Mental State Examination 21.9, SD 3.2). None of the patients had ever received treatment with AchE-I or any other dementia drugs before the study. All gave written informed consent before participation. The ethical committee of the University of Bonn approved of the study. The inclusion of a placebo group was considered unethical.

Subjects initially received 5 mg donepezil with a subsequent increase to 10 mg after 4 weeks. Three subjects did not tolerate the dose increase due to nausea and remained on the 5 mg dose. The 1H-MRS protocol and the Alzheimer’s Disease Assessment Scale—Cognitive Subscale (ADAS-Cog) as neuropsychological outcome parameter were performed at baseline (T1) and after 12 weeks of treatment (T2). The ADAS-Cog was used in two parallel versions. Treatment response was measured by subtracting the scores ADAS-Cog (T1) from ADAS-Cog (T2). The neuropsychological rater was blinded to the 1H-MRS data and the neuroradiologist was blinded to the neuropsychological data.

1H-MRS protocol. The study was performed on a Gyroscan Intera 1.5 T MR-scanner (Philips Medical Systems, Best, The Netherlands). The MTL voxel (35 × 25 × 20 mm; ap/h/pl/mm) was positioned around the left hippocampus (figure 1). The second volume of interest (25 × 25 × 30 mm) was positioned in the left parietal lobe (figure 1).

Water-suppressed 1H-MRS spectra were collected with PRESS (repetition time [TR]/echo time [TE] = 2,000 msec/272 msec; 128 averages) (figure 1). Metabolite signal ratios for N-acetyl groups (NAA), choline containing compounds (Cho), and total creatine (Cr) were obtained from the corresponding metabolite peak areas using JAVA-MRUI. Absolute concentration of NAA (and of Cr and Cho by applying the measured metabolite ratios extrapolated to TE 0 and TR = ∞) were obtained by acquiring unsuppressed 1H-MRS spectra (TR/TE = 3,000 msec/272 msec, 32 averages) with brain water signal as internal reference.3 To determine NAA/H2O ratios by extrapolation to TE 0, and to correct for partial CSF volume within the voxels, T2 relaxation times and relative fractions of tissue water and CSF were obtained by a bi-exponential fit to a series of seven unsuppressed spin-echo spectra (TR = 6,000 msec, TE = 30/70/136/272/400/700/1,000 msec, 4 averages each). Metabolite concentrations were expressed as mmol/L brain tissue, assuming a water content of 72% in mixed gray/white matter corresponding to 40 mol/L.4 The reproducibility of the metabolic measures over time with this protocol has been demonstrated in a healthy subject.4

Statistics. Neuropsychological and metabolic data at both time points were compared using paired t tests. The relationship of changes between both time points in the ADAS-Cog with the changes of NAA and NAA/Cr ratio in the MTL and the parietal voxel and the relationship of these metabolic measures with cognition at T1 and T2 were assessed with the Pearson’s correlation coefficient. Treatment prediction was assessed using a stepwise linear regression model with change in ADAS-Cog as dependent variable and ADAS-Cog, NAA, and NAA/Cr of the MTL and the parietal lobe at baseline and age and sex as independent variables. Cho, Cr, and Cho/Cr were not included in the analysis due to the lack of a clear hypothesis regarding these measures in AD and to limit the number of statistical tests. However, means of those measures are given in the table and exploratory analyses with the identical statistical methods are given below.

Results. Due to technical or subject-related reasons, the following 1H-MRS data were missing: MTL at T1 (one person), parietal lobe at T2 (one person), parietal lobe at T1 and T2 (one person).

The initial ADAS-Cog scores (mean 19.1, SD 5.4) were not different from the ADAS-Cog scores at T2 (mean 17.1, SD 6.7) (T = 1.5, df = 16, p = 0.16).

There were no significant group differences of NAA or NAA/Cr in either region between both time points. These
metabolic measures also did not correlate significantly between T1 and T2.

For the MTL voxel, the change of the ADAS-Cog was neither correlated with change of NAA/Cr ratio nor with change of absolute concentration of NAA.

For the parietal voxel, there was a correlation of change in the ADAS-Cog with change of NAA/Cr ($r = -0.69$, $n = 16$, $p = 0.003$) (figure 2A) and with change of the absolute concentration of NAA ($r = -0.63$, $n = 15$, $p = 0.012$) (figure 2B).

At T1, none of the metabolic measures correlated with the ADAS-Cog. At T2, only parietal NAA correlated with the ADAS-Cog ($r = -0.64$, $n = 16$, $p = 0.011$).

Of age, sex, baseline ADAS-Cog, baseline NAA, and baseline NAA/Cr of the MTL and the parietal lobe only NAA/Cr ratio of the parietal lobe predicted treatment response (model: $F = 12.68$, $p = 0.003$; parietal NAA/Cr: $R^2 = 0.494$; beta $= -0.703$; $T = -3.56$, $p = 0.003$). An initially low NAA/Cr was associated with a cognitive improvement (figure 2C).

Exploratory analyses of Cho, Cr, and Cho/Cr revealed no differences of these markers between T1 and T2, no correlation of change with change in cognition between T1 and T2, and no correlation with cognition at either T1 or T2. None predicted treatment outcome.

**Discussion.** We observed a high correlation of change in ADAS-Cog scores and changes of both the NAA/Cr ratio and the absolute concentration of NAA in the left parietal lobe after 3 months of donepezil treatment in mild to moderate AD. In addition, parietal NAA/Cr ratio predicted treatment response. Patients with a low initial NAA/Cr ratio improved, while those with high initial NAA/Cr did not improve.

Experimental evidence suggests a link between NAA and the cholinergic system. NAA synthesis is located in mitochondria of neurons. The main donor of the acetyl-group of NAA is acetyl-CoA and conditions that lower the level of acetyl-CoA in mitochondria, lower NAA.$^5$ Lesions to the cholinergic innervation of cortical neurons are followed by a displacement of acetyl-CoA from the mitochondria to the cytoplasm.$^6$ Decreased mitochondrial acetyl-Co level may lead to a reduction of NAA synthesis. According to this model, patients with AD with a substantial cholinergic deficit should have low NAA levels. In these patients, cholinergic treatment should improve cognition and in addition restore NAA levels. Patients with AD with only a minor cholinergic deficit should have higher initial NAA levels and should not benefit from treatment. Both those effects are consistent with our results.

We were not able to observe this relationship of NAA changes and cognition in the MTL voxel. Possible explanations for this include that the substantial cholinergic deficit in the MTL of patients with AD$^7$ might be too pronounced to induce measurable NAA changes, or that the ADAS-Cog score might reflect changes of cortical function better than changes of hippocampal function, or that the methodologic challenges associated with $^1$H-MRS measurement of the MTL increase data variance.

For ethical reasons we did not include a placebo group. Therefore, our results might be unrelated to cholinergic treatment altogether. This possibility would weaken the model of interaction of cholinergic

![Figure 1. $^1$H-MRS voxel positioned around the left MTL (left) and in the left parietal lobe (right). $^1$H-MRS spectral data from T1 and T2 of one individual are depicted below the respective voxel.](image)

**Table Data of metabolic measures at T1 and T2**

<table>
<thead>
<tr>
<th></th>
<th>NAA, mmol/L brain tissue</th>
<th>NAA/Cr</th>
<th>Cr, mmol/L brain tissue</th>
<th>Cho, mmol/L brain tissue</th>
<th>Cho/Cr</th>
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<tr>
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<td>T1</td>
<td>T2</td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
</tr>
<tr>
<td>MTL</td>
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<td>10.2 (2.2)</td>
<td>2.0 (0.4)</td>
<td>1.9 (0.3)</td>
<td>7.8 (1.7)</td>
</tr>
<tr>
<td>Parietal</td>
<td>12.5 (2.0)</td>
<td>13.0 (1.7)</td>
<td>2.4 (0.3)</td>
<td>2.4 (0.3)</td>
<td>7.5 (1.0)</td>
</tr>
</tbody>
</table>

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neurons and NAA, but it does not affect the demonstrated high correlation between NAA changes and changes in cognition or the finding that low NAA/Cr in the parietal lobe predicts positive treatment outcome.

References


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