In Vivo Voxel-Based Morphometry in Multiple System Atrophy of the Cerebellar Type

Karsten Specht, PhD; Martina Minnerop, MD; Michael Abele, MD; Jürgen Reul, MD; Ullrich Wüllner, MD; Thomas Klockgether, MD

Background: Multiple system atrophy (MSA) is a sporadic neurodegenerative disease. According to the clinical presentation a parkinsonian type and a cerebellar type (MSA-C) are distinguished.

Objective: To study the morphological alterations of MSA-C–affected brains in vivo using voxel-based morphometric analysis of magnetic resonance images.

Setting: University hospital.

Patients: Fourteen patients (5 men and 9 women) with MSA-C (mean age [SD], 59.4 [7.4] years; mean [SD] disease duration, 3.7 [1.4] years) and 13 healthy control subjects (5 men and 8 women) (mean [SD] age, 55.1 [6.9] years) were studied.

Methods: T1-weighted magnetic resonance images were normalized to a common stereotaxic space and segmented into gray and white matter. Data were analyzed using statistical parametric mapping (SPM99).

Results: Gray matter was reduced in the brainstem and the anterior lobe of the cerebellum. Reduction of white matter was observed in the middle cerebellar peduncles, cerebellar white matter, and brainstem. The inverted comparison revealed an increase of white matter density along the pyramidal tracts.

Conclusions: Voxel-based morphometry revealed a significant loss of cerebellar and brainstem tissue in MSA-C. It allowed a precise anatomical localization and a distinction between gray and white matter densities. In addition, our data point to a particular involvement of the pyramidal tract in MSA-C.

Arch Neurol. 2003;60:1431-1435

MULTIPLE SYSTEM atrophy (MSA) is a sporadic, adult-onset disease characterized by neurodegeneration in the basal ganglia, brainstem, cerebellum, and intermediolateral cell columns of the spinal cord.1,2 The neuropathological hallmark of MSA are α-synuclein–positive oligodendroglial cytoplasmic inclusions.3 Clinically, patients with MSA suffered from parkinsonism, cerebellar ataxia, and autonomic failure (most notably orthostatic hypotension and urinary incontinence).4,6 According to the clinical presentation, a parkinsonian type and a cerebellar type of MSA (MSA-C) are distinguished.5,6

Magnetic resonance imaging (MRI) has been extensively used to study the morphologic condition of the brain of patients with MSA. Magnetic resonance imaging abnormalities occurring in MSA include progressive atrophy of infratentorial and supratentorial brain structures as well as signal abnormalities.5,7-11 To quantify the atrophic changes in MSA, we previously used planimetric and volumetric region of interest–guided approaches. Compared with patients with idiopathic Parkinson disease and healthy control subjects, patients with MSA consistently had significant atrophy of the cerebellum, brainstem, putamen, and caudate nucleus.3,7

In morphometric MRI studies using region of interest–guided measurements, an inherent bias is introduced by selecting a limited number of brain regions for study. In addition, the segmentation procedure is often arbitrary and poorly reproducible. These problems are avoided by using voxel-based morphometric methods that allow an automated, unbiased, and comprehensive assessment of anatomical differences of gray and white matter throughout the brain.12,13 Voxel-based morphometry has been recently refined and successfully used to study structural brain...
correlates of aging and changes of gray and white matter volumes in neurodegenerative diseases. In this study, we used voxel-based morphometry to study the morphology of the MSA-affected brain in vivo. To increase the homogeneity of our patient group we restricted our analysis to those with MSA-C.

**METHODS**

**PATIENTS**

The study was performed in 14 consecutive patients (5 men and 9 women) with MSA-C whose mean (SD) age was 59.4 (7.4) years and mean (SD) disease duration was 3.7 (1.4) years compared with 13 healthy controls (5 men and 8 women) whose mean (SD) age was 55.1 (6.9) years. A diagnosis of MSA-C was made according to the criteria of Gilman et al. Alternative causes of cerebellar ataxia were excluded by history, conventional MRI, genetic testing for spinocerebellar ataxia mutations, cerebrospinal fluid studies, by laboratory tests including the levels of antineuronal antibodies, vitamin B12, vitamin E, and thyroid hormones, and by the results of lipid electrophoresis and VDRL. Of 14 patients, 9 met the clinical criteria for probable MSA-C and 5 for possible MSA-C (Table 1). The study was approved by the ethics committee of the medical faculty of the University of Bonn, Bonn, Germany. Informed and written consent was obtained from all participants.

**DATA ACQUISITION**

Magnetic resonance imaging measurements were performed using a 1.5-T scanner (Siemens Symphony; Siemens AG, Erlangen, Germany) with the standard head coil. The MRI protocol consisted of a T1-weighted, magnetization-prepared, rapid acquisition gradient-echo sequence. Repetition time was 11.08 milliseconds; echo time, 4.3 milliseconds; flip angle, 15°; excitation, 1 per phase encoding step; field of view, 230 mm; acquisition matrix, 256 × 256 pixels; and slice thickness, 180 mm; yielding 200 sagittal slices and a voxel size of 0.9 × 0.9 × 0.9 mm³. 

**MORPHOMETRIC ANALYSIS**

Data were preprocessed as described by Good et al. To prevent mismatch errors, each preprocessing step was controlled and verified for each subject and each sequence separately. After defining the anterior commissure in each image as the origin of the individual stereotaxic space, we reoriented all images to the axial view. For optimizing the stereotactic normalization procedure, the images were automatically seg-

---

**Table 1. Clinical Features**

<table>
<thead>
<tr>
<th>Variable</th>
<th>With MSA-C (n=14)</th>
<th>With Probable MSA-C (n=9)</th>
<th>With Possible MSA-C (n=5)</th>
<th>Control Subjects (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>5/9</td>
<td>3/6</td>
<td>2/3</td>
<td>5/8</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>59.4 (7.4)</td>
<td>59.2 (7.8)</td>
<td>60.0 (6.3)</td>
<td>55.1 (6.9)</td>
</tr>
<tr>
<td>Disease duration, mean (SD), y</td>
<td>3.7 (1.4)</td>
<td>4.1 (1.1)</td>
<td>3.2 (1.7)</td>
<td>NA</td>
</tr>
<tr>
<td>Patients with parkinsonism</td>
<td>3 (21)</td>
<td>3 (33)</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Patients with urinary incontinence</td>
<td>13 (93)</td>
<td>8 (89)</td>
<td>5 (100)</td>
<td>NA</td>
</tr>
<tr>
<td>Patients with orthostatic hypotension</td>
<td>13 (93)</td>
<td>8 (89)</td>
<td>5 (100)</td>
<td>NA</td>
</tr>
<tr>
<td>Patients with pyramidal signs</td>
<td>2 (14)</td>
<td>1 (11)</td>
<td>1 (20)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: MSA-C, multiple system atrophy-cerebellar type; NA, not applicable.

*The diagnosis of probable and possible MSA-C was made according to the clinical criteria of Gilman et al. Data are given as the number (percentage) of patients unless otherwise indicated.
mented into gray matter, white matter, and cerebrospinal fluid probability maps. Afterward, all nonbrain voxels were removed from the segmented images. The gray and white matter maps obtained by this procedure were separately normalized to a gray and white matter template representing the stereotactic standardized space, defined by a template from the Montreal Neurological Institute, Montreal, Quebec. After applying both transformations into the Montreal Neurological Institute template space to the original images, the spatially normalized images were resampled with a voxel size of $1.5 \times 1.5 \times 1.5$ mm$^3$. Scans were then segmented into gray and white matter. For further analysis we used the gray matter probability map derived from optimized gray matter normalization and the white matter probability map derived from optimized white matter normalization. The final probability maps were smoothed with a gaussian smoothing kernel size of 8 mm (full-width at half maximum of the gaussian kernel).

The normalized, segmented, and smoothed data were analyzed using statistical parametric mapping (SPM99). The statistical comparisons between the groups were calculated as 2-sample $t$ tests separately for the compartments. Each analysis was controlled for global differences in voxel intensity by including the overall mean of voxel intensities as a confounding covariate in the design matrix. The reported results are based on contrasts between the 2 groups at a threshold of $P<.05$ corrected and cluster level of at least 5 voxels, which reduces the false-positive rate by correcting the threshold for multiple testing. To investigate the areas of significant differences in more detail, we explored the statistical results on anatomical slices. For these overlays, the statistical parametric mapping (SPM99) results were thresholded at an uncorrected value of $P<.001$ for the voxel significance and a corrected value of $P<.05$ for the cluster level.

### RESULTS

#### GRAY MATTER FINDINGS

At a corrected threshold of $P<.05$ we found a reduction of gray matter in the upper part of the cerebellar vermis and in adjacent regions of the cerebellar hemispheres in MSA-C–affected patients. The gray matter loss was more pronounced in the right part of the cerebellum (Figure 1A). A more liberal analysis using an uncorrected threshold of $P<.01$ showed a widespread reduction of gray matter of the cerebellum sparing only parts of the hemispheres and the basal portion of the cerebellum. In addition, there were significant clusters in the tegmentum of the mesencephalon and in lateral and ventral parts of the pons (Figure 1B and Table 2). The inverted comparison did not reveal any increases of gray matter in the MSA-C–affected brains.

#### WHITE MATTER FINDINGS

Initial analysis using a corrected threshold of $P<.05$ revealed a bilateral reduction of white matter in the middle cerebellar peduncles of MSA-C–affected brains. In addition, smaller regions of white matter loss were found in the right posterior and left anterior parts of the cerebellum (Figure 2A). Subsequent analysis using an uncorrected threshold of $P<.001$ showed widespread white matter loss in MSA-C–affected brains affecting the middle cerebellar peduncles extending into the cerebellar white matter and the dorsal and lateral aspects of the pons. In addition, we found small clusters of white matter loss in several supratentorial regions including the right anterior cingulum, the left frontal lobe, the left temporal lobe, the left side of the thalamus, and the left caudate nucleus (Figure 2B and Table 2). The inverted comparison revealed an increase of white matter density in MSA-C along the right pyramidal tract (corrected threshold, $P<.05$) (Figure 3A). Reanalysis with an uncorrected threshold of $P<.001$ showed white matter increase in both pyramidal tracts in the right occipital lobe and the medulla oblongata (Figure 3B and Table 2).

---

<table>
<thead>
<tr>
<th>Table 2. Morphometric Analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Voxelwise Statistics</strong></td>
</tr>
<tr>
<td><strong>Extent, No. of Voxels</strong></td>
</tr>
<tr>
<td>Gray matter decrease</td>
</tr>
<tr>
<td>White matter decrease</td>
</tr>
<tr>
<td>184</td>
</tr>
<tr>
<td>186</td>
</tr>
<tr>
<td>360</td>
</tr>
<tr>
<td>169</td>
</tr>
<tr>
<td>White matter increase</td>
</tr>
<tr>
<td>227</td>
</tr>
<tr>
<td>149</td>
</tr>
<tr>
<td>185</td>
</tr>
</tbody>
</table>

* Morphometric analysis of magnetic resonance imaging density changes was made separately for gray and white matter in 14 patients with multiple system atrophy–cerebellar type compared with 13 healthy control subjects. Each reported anatomical location exceeded a voxelwise statistical threshold of $P<.001$ (uncorrected) and an extent threshold of $P<.05$ (corrected). The extent of each location is expressed as the number of voxels. The coordinates refer to the Montreal Neurological Institute space and denote the location of the most significant voxel within each area.
We used voxel-based morphometry to evaluate the MRIs of patients with MSA-C. The major findings were (1) a loss of gray matter in the brainstem and anterior lobe of the cerebellum; (2) a reduction of white matter in the middle cerebellar peduncles, cerebellar white matter, and brainstem; and (3) an increase of white matter density along the pyramidal tracts.

In contrast with region of interest–guided morphometric approaches, voxel-based morphometry is not limited to a number of preselected brain regions, but it does provide a comprehensive analysis of the entire brain allowing improved anatomical localization and differentiation between tissue loss in gray and white matter. To minimize the problems of normalization,21 we used an optimized protocol based on the gray and white matter compartments and a verification of each step. This refined method considerably improves the match between the individual brain images and the templates used. In an attempt to increase the sensitivity of the method, we performed a dual statistical analysis, with an initial conservative statistical approach using a corrected threshold of P<.001 and a subsequent more liberal approach with an uncorrected threshold of P<.001.

Our findings of prominent gray matter loss in the cerebellum and brainstem confirm earlier MRI studies5,7,10 and are in good agreement with the distribution of neuronal loss in MSA-C–affected brains at autopsy.23 Gray matter loss was pronounced in the upper cerebellar vermis and adjacent areas of the hemispheres, areas of the cerebellum that are anatomically referred to as the anterior lobe and receive mainly spinal afferents. It is generally assumed that cerebellar atrophy in MSA-C is diffuse whereas other types of cerebellar degeneration, in particular those caused by chronic alcoholism, are characterized by prominent involvement of the anterior lobe.24-26 Our findings seem to contradict this common view, suggesting that anterior lobe degeneration is also typical for MSA-C. Since our patients were in a medium disease stage, one may conclude that the cerebellar anterior lobe is the most vulnerable part of the cerebellum in MSA-C where degeneration starts whereas advanced cases that come to autopsy have a diffuse cerebellar degeneration.

Multiple system atrophy is unique among the neurodegenerative disorders as oligodendroglial cells forming central myelin are the primary cell type affected. α-Synuclein–positive inclusions are abundant in oligodendroglial cells and give above the view refer to the corresponding slices in the Montreal Neurological Institute reference space.
and distributed throughout the brain beyond areas of overt neuronal loss. A recent study using antibodies that specifically recognize an epitope of myelin basic protein that is exposed in areas of myelin degeneration detected widespread immunoreactivity in oligodendroglial processes suggesting significant myelin degeneration in MSA. Thus, structural abnormalities observed in myelinated fiber tracts in MSA may be a direct consequence of myelin pathology due to oligodendroglial cell dysfunction. In addition, fiber tracts may be also affected by axonal loss secondary to neuronal degeneration.

Our study shows loss of white matter, which was most prominent in the middle cerebellar peduncles. This confirms earlier MRI studies showing that the middle cerebellar peduncles undergo the most severe shrinkage of all brain structures analyzed. In addition, our data suggest that white matter loss in the cerebellum and brainstem significantly contributes to the infratentorial atrophy observed on conventional MRIs in MSA. Further, there was less pronounced white matter loss in the supratentorial brain regions.

To our surprise, the pyramidal tracts showed an increase of white matter density. This finding cannot be explained by degeneration of the pyramidal tracts since degeneration would result in decreased T1 signal. Correspondingly, most of our patients had no clinical signs of pyramidal tract dysfunction. Another observation arguing against pyramidal tract degeneration in our patients is a decreased T2 signal along the pyramidal tracts (our unpublished data, 2000). This finding, in conjunction with the increased T1 signal, points to decreased water content in the pyramidal tracts, possibly due to deposition of an unknown biochemical substrate. Whether these findings are related to altered myelin properties due to oligodendroglial pathology remains a matter of speculation.

Accepted for publication March 26, 2003.

Author contributions: Study concept and design (Drs Reul and Klockgether); acquisition of data (Drs Specht, Minnerop, Abele, and Wullner); analysis and interpretation of data (Drs Specht and Klockgether); drafting of the manuscript (Drs Specht, Minnerop, Abele, Reul, and Klockgether); critical revision of the manuscript for important intellectual content (Drs Specht, Abele, Wullner, and Klockgether); statistical expertise (Drs Specht and Klockgether); obtained funding (Drs Wullner and Klockgether); administrative, technical, and material support (Drs Specht, Minnerop, Abele, Wullner, and Klockgether); study supervision (Drs Reul and Klockgether).

Corresponding author: Thomas Klockgether, MD, Department of Neurology, University of Bonn, Sigmund-Freud-Straße 25, D-53105 Bonn, Germany (e-mail: klockgether@uni-bonn.de).

REFERENCES

2. Quinn N. Multiple system atrophy—the nature of the beast. J Neurol Neurosurg Psychiatry. 1989;suppl.78-89.