

## BRIEF COMMUNICATION

**Spinal cord atrophy in early Huntington's disease**

Mark Mühlau<sup>1,2,3</sup>, Christina Engl<sup>1,2</sup>, Christine C. Boucard<sup>1,2</sup>, Paul Schmidt<sup>1,2,4</sup>, Viola Biberacher<sup>1,2</sup>, Isabel Görsch<sup>1,5</sup>, Christian Sorg<sup>2,6,7</sup>, Afra Wohlschläger<sup>2,6</sup>, Claus Zimmer<sup>6</sup>, Bernhard Hemmer<sup>1,3</sup> & Adolph Weindl<sup>1</sup>

<sup>1</sup>Department of Neurology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

<sup>2</sup>TUM-Neuroimaging Center, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

<sup>3</sup>Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

<sup>4</sup>Department of Statistics, Ludwig-Maximilians-University München, Munich, Germany

<sup>5</sup>Department of Ophthalmology, University Hospital Hamburg, Hamburg, Germany

<sup>6</sup>Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

<sup>7</sup>Department of Psychiatry, Klinikum rechts der Isar, Technische Universität München, Munich, 81675, Germany

**Correspondence**

Mark Mühlau, Department of Neurology, Technische Universität München, Ismaningerstr. 22, D-81675 Munich, Germany. Tel: +49 89 4140 4606; Fax: +49 89 4140 4867; E-mail: muehlau@lrz.tum.de

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**Introduction**

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease resulting from an expanded CAG trinucleotide repeat size (>35) within the first exon of the IT15 gene on chromosome 4 leading to a polyglutamin stretch.<sup>1</sup> HD is characterized by the triad of motor deficits, dementia, and behavioral disturbances.<sup>2</sup> The most striking finding at gross pathology is atrophy of the striatum, where medium spiny neurons are dramatically reduced.<sup>2</sup> Accordingly, the vast majority of studies on HD have focused on changes in the brain. In contrast, data on an involvement of the spinal cord are rare. Yet several lines of evidence point in this direction. Some neuropathological reports described changes in the spinal cord, although in different parts of the cross section.<sup>3–5</sup> Clinical case reports described spinal involvement of the pyramidal tract, although interpreting this finding as exceptional.<sup>5,6</sup> Brain diffusion tensor imaging revealed involvement of the pyramidal tract,<sup>7</sup> which may extend to

**Abstract**

Despite evidence for spinal cord involvement, it remains unclear whether spinal cord atrophy exists in early Huntington's disease. We studied magnetic resonance images, covering both brain and upper cervical cord, in two cohorts of Huntington's patients and in one cohort of Alzheimer's patients. All cohorts included healthy controls comparable with regard to age and gender. We found significant spinal cord atrophy in both cohorts of Huntington's patients but not in the cohort of Alzheimer's patients. Furthermore, spinal cord atrophy correlated with motor symptoms indicating that spinal cord atrophy occurs in the clinical stages and does not result from abnormal development.

the spinal cord. Changes in spinal cord function have been reported, although usually attributed to changes in supraspinal modulation.<sup>8–10</sup> According to animal studies, the mutant product, Huntingtin, is also expressed in the spinal cord.<sup>11</sup> Moreover, Huntingtin seems to be indispensable for neural tube formation,<sup>12</sup> which could not only relate to slightly smaller total intracranial volumes (TIV)<sup>13</sup> but also to spinal cord changes. Finally, the rate of progression of motor symptoms is not completely explained by the rate of striatal atrophy,<sup>14,15</sup> so that spinal cord pathology could even contribute to the clinical picture.

Because of the lack of systematic studies on spinal cord involvement in HD, we searched for spinal cord atrophy in early stages of HD. We also analyzed whether spinal cord atrophy occurs in another neurodegenerative disorder with a similar degree of brain atrophy. Finally, we investigated whether spinal cord atrophy results from abnormal development or whether it occurs in the clinical stage of the disease.

## Methods

### Subjects

All three cohorts under investigation were taken from different magnetic resonance imaging (MRI) studies performed at our center. Each subject included here belongs to only one cohort. All studies were performed in accord with the Helsinki Declaration of 1975 and approved by the local ethics committee. Each cohort comprised two groups comparable with regard to age and gender, namely a group of patients and a group of healthy controls (HC). Cohorts 1 and 2 comprised HD patients; cohort 3 comprised patients with Alzheimer's disease (AD). Motor deficits of HD patients were quantified by the motor score of the Unified Huntington's Disease Rating Scale (UHDRS).<sup>16</sup> All AD patients met the National Institute of Neurological Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) Alzheimer's Criteria. Scores of the Clinical Dementia Rating were 1 ( $n = 23$ ) and 2 ( $n = 12$ ) corresponding to mild-to-moderate AD. Mini-mental state examination yielded a mean value of 21 with a standard deviation of 5.

In all three cohorts, some subjects had to be excluded from image analysis because of motion artifacts or because the field of view did not allow determination of spinal cord atrophy. Numbers of included and excluded subjects, numbers of CAG trinucleotide repeat size within the HD gene and demographics are given in Table 1.

### Magnetic resonance imaging

Images covered the brain and upper cervical cord. Images of cohort 1 were acquired on the same 1.5 Tesla (T) scanner (Magnetom Symphony, Siemens, Germany; sequence, T1-weighted magnetization prepared rapid gradient echo; repetition time [TR], 11.1 msec; echo time [TE], 4.3 msec; slices, 160; voxel size,  $1.0 \times 1.0 \times 1.0 \text{ mm}^3$ ). Images of cohort 2 and 3 were acquired on the same 3T scanner (Achieva, Philips, Netherlands; gradient echo T1-weighted sequence; TR, 9 msec; TE, 4 msec; slices, 170; voxel size,  $1.0 \times 1.0 \times 1.0 \text{ mm}^3$ ). To quantify spinal cord atrophy, we determined the cross-sectional area of the upper cervical cord (UCCA) at the level of the intervertebral disk C2/3 as previously described.<sup>17</sup> To quantify overall brain atrophy, we determined brain parenchymal fraction (BPF) as this parameter had proved a useful parameter in both AD<sup>18</sup> and HD<sup>19</sup> research. Brain gray matter (GM) and white matter (WM) volume was derived from the first segmentation process as implemented in version 5 of the software package Statistical Parametric Mapping (SPM5). TIV was also determined based on SPM5 as described elsewhere.<sup>20</sup> BPF was calculated by  $(\text{GM} + \text{WM})/\text{TIV}$ .

### Statistical analysis

Images of the cohorts cannot be compared directly as they were acquired at different scanners (cohort 1 vs. cohort 2 and 3) or as age ranges differed remarkably

**Table 1.** Demographics and baseline disease characteristics of all subjects.

	Huntington' disease						Alzheimer's disease		
	Cohort 1			Cohort 2			Cohort 3		
	HC	Pat.	2s P	HC	Pat.	2s P	HC	Pat.	2s P
<i>n</i> , Excluded	4	4	n/a	0	5	n/a	0	8	n/a
<i>n</i> , Included	175	31	n/a	22	20	n/a	30	35	n/a
Male/female	76/99	17/14	0.25	12/10	9/11	0.76	10/20	19/16	0.13
Age in years	48 ± 12	44 ± 11	0.08	48 ± 12	47 ± 11	0.9	67 ± 8.9	71 ± 8.8	0.10
	25–68	18–64		25–75	30–74		46–85	46–84	
Total intra-cranial volume in mL	1438 ± 135	1447 ± 125	0.75	1411 ± 128	1328 ± 138	0.051	1389 ± 162	1412 ± 166	0.57
Brain parenchymal fraction	0.78 ± 0.04	0.73 ± 0.05	<0.001	0.81 ± 0.05	0.74 ± 0.08	0.008	0.77 ± 0.05	0.66 ± 0.05	<0.001
CAG	n.d.	44 ± 2.4	n/a	n.d.	43 ± 3.2	n/a	n.d.	n.d.	n/a
		40–48			40–48				
Motor UHDRS	n.d.	21 ± 18	n/a	n.d.	20 ± 18	n/a	n.d.	n.d.	n/a
		0–62			1–54				
Upper cervical cord area in mm <sup>2</sup>	86 ± 8.9	81 ± 11	0.003	75 ± 7.9	68 ± 5.6	0.003	70 ± 9.4	70 ± 9.3	0.97

Values are given in mean ± standard deviation followed by range (age and CAG repeat number). HC, healthy controls; Pat., patients; 2s P, two-sided *P*-values derived from Fisher's exact test (male/female ratio) or unpaired *t* tests; motor UHDRS, motor score of the Unified Huntington's Disease Rating Scale; *n*, number of excluded and included subjects, note that the remaining data relate only the included subjects.

(cohort 1 and 2 vs. cohort 3). Therefore, we first analyzed cohort 1 (discovery cohort) and replicated our findings in cohort 2 (replication cohort). To test the hypothesis that any kind of brain atrophy inevitably goes along with spinal cord atrophy, we also searched for spinal cord atrophy in a cohort of patients with a similar degree of brain atrophy due to the most common neurodegenerative disorder, namely AD. For group comparisons, we used unpaired *t* tests. To estimate whether spinal cord atrophy results from abnormal development or whether it occurs later in the clinical stage of the disease, we performed correlation analyses (Pearson's correlation coefficient) of UCCA and motor deficits. To visualize this correlation and to relate spinal cord atrophy of HD patients in different stages to the normal range of HC, we pooled the data of cohort 1 and 2 and plotted the values of UCCA against motor scores of the UHDRS. Beforehand, we corrected the lower UCCA values of cohort 2 by adding the mean difference between both cohorts of HC.

For the discovery cohort 1, we applied two-sided *P*-values; for the replication cohort 2, we accepted one-sided *P*-values. We used standard software (IBM, SPSS statistics, version 21.0, Chicago, IL).

## Results

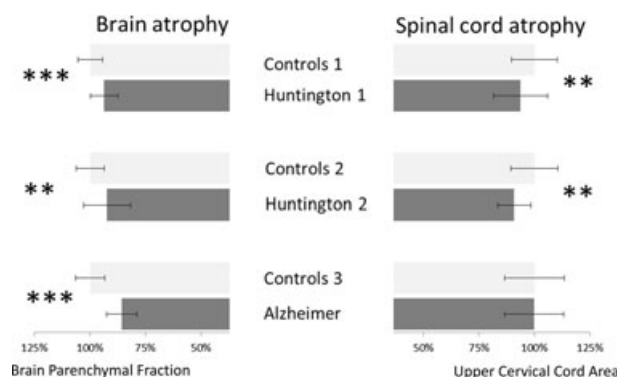
Besides numbers of excluded subjects and demographics, TIV, BPF, numbers of CAG repeats (HD patients), motor scores of the UHDRS (HD patients), values of UCCA, and two-sided *P*-values of group comparisons are given in Table 1. In our statistical analyses, we could include 175 HC and 31 patients of HD cohort 1, 22 HC and 20 patients of HD cohort 2, as well as 30 HC and 35 patients of AD cohort 3. As illustrated in Figure 1, all three cohorts showed significant and highly comparable brain atrophy (BPF of HC vs. patients, two-sided *P*-values: HD cohort 1,  $0.78 \pm 0.04$  vs.  $0.73 \pm 0.05$ ,  $<0.001$ ; HD cohort 2,  $0.81 \pm 0.05$  vs.  $0.74 \pm 0.08$ , 0.008; AD cohort 3,  $0.77 \pm 0.05$  vs.  $0.66 \pm 0.05$ ,  $<0.001$ ). Both cohorts including patients with HD showed significant spinal cord atrophy, while the cohort with AD patients did not show this effect (UCCA in  $\text{mm}^2$  of HC vs. patients, two-sided *P*-values: HD cohort 1,  $86 \pm 8.9$  vs.  $81 \pm 11$ , 0.003; HD cohort 2,  $75 \pm 7.9$  vs.  $68 \pm 5.6$ , 0.003; AD cohort 3,  $70 \pm 9.4$  vs.  $70 \pm 9.3$ , 0.97).

To estimate whether spinal cord atrophy develops in the clinical stage of the disease, we performed correlation analyses between the motor score of the UHDRS and UCCA in cohort 1 and 2. Both cohorts showed a significant decrease in UCCA with increasing motor disability (cohort 1: *R*-value,  $-0.432$ ; two-sided *P*-value, 0.02; cohort 2: *R*-value,  $-0.399$ ; one-sided *P*-value, 0.04). To

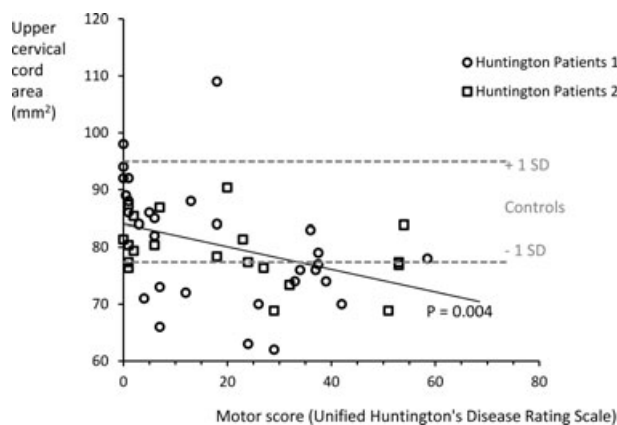
visualize this correlation and to relate UCCA values of HD patients in different disease stages to the normal range of HC, pooled data of UCCA (cohort 1 and 2) are plotted against the motor score of the UHDRS in Figure 2.

## Discussion

We demonstrated spinal cord atrophy in early HD. Since motor deficits correlated with spinal cord atrophy and since almost all patients without motor deficits displayed UCCA



**Figure 1.** Brain and spinal cord atrophy. For all three cohorts, bar charts of brain atrophy (left), as estimated by brain parenchymal fraction, and spinal cord atrophy (right), as estimated by the upper cervical cord area (UCCA), are shown. Values are given in percent and scaled according to the mean value of the respective control group. Error bars indicate  $\pm 1$  standard deviation. Significance is indicated by asterisks (two-sided *P*-value:  $<0.001$ , \*\*\*;  $<0.01$ , \*\*).



**Figure 2.** Spinal cord atrophy and motor deficits. Pooled data analysis of cohort 1 and 2 are shown. Values of the upper cervical cord area are plotted against motor scores of the Unified Huntington's Disease Rating Scale. The range ( $\pm 1$  standard deviation) of the controls is indicated by dotted horizontal lines. Pooled correlation analysis yielded an *R*-value of  $-0.399$  corresponding to a two-sided *P*-value of 0.004.

values within the normal range, most spinal cord atrophy seems to develop in the clinical stage of HD, which does not preclude subtle spinal cord atrophy preceding the development of motor or other symptoms. To address this issue, investigation of large numbers of pre-symptomatic individuals is necessary. Yet, our results raise the question of whether spinal cord involvement considerably contributes to the clinical picture of HD, which would make it even a rational target for therapy. Those conclusions must be regarded premature at this point given the limitations of our study and the issues to be clarified. We only measured the cross-sectional area at one level but spinal cord atrophy may occur across all spinal levels to different degrees. From the fact that we did not observe spinal cord atrophy in a group of AD patients with a similar degree of brain atrophy, we can only conclude that any kind of brain atrophy does not inevitably go along with spinal cord atrophy but we cannot exclude the possibility that spinal cord atrophy in HD results from brain atrophy rather than from direct damage by HD pathology. We assume that this question can be addressed adequately only by histopathological and animal studies. Finally, studies on the clinical importance of spinal cord changes may require more sophisticated approaches (e.g., diffusion tensor imaging of brain and spinal cord) and large numbers of subjects to include brain and spinal cord measures in common statistical models, which would most likely require multisite studies.

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## Conflicts of Interest

V. B. and M. M. received funding from Merck Serono for the project: "The Upper Cervical Cord Area Determined From Cranial MRI as a Marker of Disease Severity in Multiple Sclerosis". There are no further conflicts of interest.

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