Spinal cord atrophy in early Huntington’s disease

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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.

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.1 HD is characterized by the triad of motor deficits, dementia, and behavioral disturbances.2 The most striking finding at gross pathology is atrophy of the striatum, where medium spiny neurons are dramatically reduced.2 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.3–5 Clinical case reports described spinal involvement of the pyramidal tract, although interpreting this finding as exceptional.5,6 Brain diffusion tensor imaging revealed involvement of the pyramidal tract,7 which may extend to the spinal cord. Changes in spinal cord function have been reported, although usually attributed to changes in supraspinal modulation.8–10 According to animal studies, the mutant product, Huntingtin, is also expressed in the spinal cord.11 Moreover, Huntingtin seems to be indispensible for neural tube formation,12 which could not only relate to slightly smaller total intracranial volumes (TIV)13 but also to spinal cord changes. Finally, the rate of progression of motor symptoms is not completely explained by the rate of striatal atrophy,14,15 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). 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 × 1.0 × 1.0 mm³). 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 × 1.0 × 1.0 mm³). 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. To quantify overall brain atrophy, we determined brain parenchymal fraction (BPF) as this parameter had proved a useful parameter in both AD and HD 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. BPF was calculated by (GM + WM)/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 |
| n, Excluded          | 4   | 4    | n/a  | 0   | 5    | n/a  | 0   | 8    | n/a  |
| n, 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 | n/a  | 25–75 | 30–74 | n/a  | 46–85 | 46–84 | n/a  |
| 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  | 43 ± 3.2 | n/a  | n.d. | n.d. | n/a  | n/a  |
|                      | 40–48 | 40–48 | n/a  | 40–48 | 40–48 | n/a  | 40–48 | 40–48 | n/a  |
| Motor UHDRS          | n.d. | 21 ± 18 | n/a  | 20 ± 18 | n/a  | n.d. | n.d. | n/a  | n/a  |
|                      | 0–62 | 1–54  | n/a  | 1–54  | n/a  | n.d. | n.d. | n/a  | n/a  |
| Upper cervical cord area in mm² | 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 ± 0.04 vs. 0.73 ± 0.05, <0.001; HD cohort 2, 0.81 ± 0.05 vs. 0.74 ± 0.08, 0.008; AD cohort 3, 0.77 ± 0.05 vs. 0.66 ± 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 mm² of HC vs. patients, two-sided P-values: HD cohort 1, 86 ± 8.9 vs. 81 ± 11, 0.003; HD cohort 2, 75 ± 7.9 vs. 68 ± 5.6, 0.003; AD cohort 3, 70 ± 9.4 vs. 70 ± 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 ±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 (±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.](image-url)

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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

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References

1. MacDonald ME. The Huntington’s Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. Cell 1993;72:971–983.
2. Walker FO. Huntington’s disease. Lancet 2007;369:218–228.
3. Ringelstein EB, Schroder JM. The human dorsal spinocerebellar tract: myelinated fiber spectrum and fiber density in controls, autosomal dominant spinocerebellar atrophy, Huntington’s chorea, radiation myelopathy, and diseases with peripheral sensory nerve involvement. Clin Neuropathol 1982;1:121–132.
4. Bruyn GW, Bots GTAM, Dom R. Huntington’s chorea: current neuropathological status. In: Chase TN, Wexder NS, Barbeau A, eds. Huntington’s disease (advances in neurology, volume 23). New York: Raven Press, 1979; 83–94.
5. Zweig RM, Koven SJ, Hedreen JC, et al. Linkage to the Huntington’s disease locus in a family with unusual clinical and pathological features. Ann Neurol 1989;26:78–84.
6. Akanuma J, Saito N, Aoki M, et al. [Chorea with prominent spasticity associated with an expansion of the CAG trinucleotide repeat in the IT15 gene: a case report]. Rinsho Shinkeigaku 1995;35:1253–1255.
7. Rosas HD, Tuch DS, Hevelone ND, et al. Diffusion tensor imaging in presymptomatic and early Huntington’s disease: selective white matter pathology and its relationship to clinical measures. Mov Disord 2006;21:1317–1325.
8. Noth J, Podell K, Friedemann HH. Long-loop reflexes in small hand muscles studied in normal subjects and in patients with Huntington’s disease. Brain 1985;108:65–80.
9. Ferrotta A, Serpino C, Cormio C, et al. Abnormal spinal cord pain processing in Huntington’s disease. The role of the diffuse noxious inhibitory control. Clin Neurophysiol 2012;123:1624–1630.
10. Priori A, Polidori L, Rona S, et al. Spinal and cortical inhibition in Huntington’s chorea. Mov Disord 2000;15:938–946.
11. Davies SW, Turmaine M, Cozens BA, et al. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 1997;90:537–548.
12. Lo Sardo V, Zuccato C, Gaudenzi G, et al. An evolutionary recent neuroepithelial cell adhesion function of huntingtin implicates ADAM10-N-cadherin. Nat Neurosci 2012;15:713–721.
13. Nopoulos PC, Aylward EH, Ross CA, et al. Smaller intracranial volume in prodromal Huntington’s disease: evidence for abnormal neurodevelopment. Brain 2011;134:137–142.
14. Mahant N, McCusker EA, Byth K, et al. Huntington’s disease: clinical correlates of disability and progression. Neurology 2003;61:1085–1092.
15. Tabrizi SJ, Scabill RI, Durr A, et al. Biological and clinical changes in premanifest and early stage Huntington’s disease in the TRACK-HD study: the 12-month longitudinal analysis. Lancet Neurol 2011;10:31–42.
16. Huntington Study Group. Unified Huntington’s Disease Rating Scale: reliability and consistency. Mov Disord 1996;11:136–142.
17. Engl C, Schmidt P, Arsic M, et al. Brain size and white matter content of cerebrospinal tracts determine the upper cervical cord area: evidence from structural brain MRI. Neuroradiology 2013;55:963–970.
18. Smith EE, Egorova S, Blacker D, et al. Magnetic resonance imaging white matter hyperintensities and brain volume in the prediction of mild cognitive impairment and dementia. Arch Neurol 2008;65:94–100.

19. Kassubek J, Juengling FD, Ecker D, et al. Thalamic atrophy in Huntington’s disease co-varies with cognitive performance: a morphometric MRI analysis. Cereb Cortex 2005;15:846–853.

20. Keihaninejad S, Heckemann RA, Fagiolo G, et al. A robust method to estimate the intracranial volume across MRI field strengths (1.5T and 3T). Neuroimage 2010;50:1427–1437.