The Cerebellum
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SHORT REPORTS

# Cerebellar Degeneration Signature in Huntington's Disease

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## Abstract

Recent findings suggest a significant effect of the cerebellar circuit deterioration on the clinical manifestation of Huntington's disease, calling for a better understanding of the cerebellar degeneration in this disorder. Recent brain imaging analyses have provided conflicting results regarding the cerebellar changes during the progression of this disease. To help in resolving this controversy, we examined the cerebellar gray matter structural integrity from a cohort of HD patients. Whole brain voxel-based morphometry (VBM) and spatially unbiased atlas template of the human cerebellum (SUIT) analyses were done from T1-weighted brain images. Our results showed a significant cerebellar degeneration without any sign of volume increase. The highest cerebellar degeneration was identified in Crus I right lobule, Crus II bilaterally, and left VIIb, and left VIIIa lobules. The cerebellar degeneration signature, which controls for severity of degeneration, showed a degeneration pattern that included regions I–IV, Crus II, VIIb, VIIIa, VIIIb and X.

**Keywords** Gray matter decrease · VBM · cerebellum

## Introduction

Huntington's disease (HD) is an autosomal-dominant progressive neurodegenerative disease characterized mainly by significant neuronal loss in the striatum and cortex. The HD neurodegenerative process is manifested by progressive motor, cognitive, and psychiatric deterioration [1]. Although striatal degeneration is the hallmark of the neurodegenerative process in HD, other cortical and subcortical areas also show substantial neuronal loss [2]. Recent studies have started to uncover a significant impact of the cerebellar degeneration in the clinical manifestation of the disease that needs to be addressed [3], taking into account the intricate interactions between the basal ganglia and cerebellar circuitries [4]. In this regard, postmortem histopathological analyses have found a significant reduction on the number of cerebellar Purkinje cells in HD patients [5]. Significant reductions in cerebellar volume have also been found using in-vivo whole-brain [5, 6] and specialized cerebellar MRI analysis [7]. However, a recent study by de Azevedo and colleagues showed increased cerebellar gray matter density in a cohort of HD patients [8]. In their report, de Azevedo et al. used the spatially unbiased atlas template of the human cerebellum (SUIT) [9] that allows a better in-vivo characterization of this structure, and the possible disruptive consequences of cerebellar integrity in HD patients. Given the critical role of the cerebellum in a number of functions affected in HD, these controversial results prompted us to analyze the cerebellar integrity in a cohort of HD patients using both, whole-brain and specialized cerebellar approaches. Therefore, here we explored the gray matter integrity in these patients using the standard voxel-based morphometry as implemented in FSL, and the cerebellar SUIT toolbox. Additionally, we also produced the HD cerebellar degeneration signature to compare the unbiased degeneration pattern to other polyglutamine diseases [10, 11].

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## Materials and methods

### Participants

Nineteen patients with confirmed molecular diagnosis of HD (7/12 m/f, 47.57 years ± 12.05 SD), and nineteen healthy participants (9/10 m/f, 45.94 years ± 12.40 SD) with no neurological history, participated in this study. All participants were right-handed. The HD patients CAG mean repeat length was 44.11 ± 3.6 SD, their UHDRS mean motor score was 19.59 ± 18.79 SD. The patients Montreal Cognitive Assessment (MoCA) score mean was 24.66 ± 3.91 SD, while the controls was 27.4 ± 1.5 SD.

### Image acquisition

All images were acquired using a 3.0 Tesla Achieva MRI scanner (Phillips medical Systems, Eindhoven, The Netherlands) at the Instituto Nacional de Psiquiatria "Ramon de la Fuente Muñiz" in Mexico City. The high-resolution anatomical acquisition consisted of a 3-D_T1 Fast Field-Echo sequence with TR/TE of 8/3.7 ms, FOV of 256 × 256 mm, an acquisition and reconstruction matrix of 256 × 256, resulting in isometric resolution of 1 × 1 × 1 mm.

### Voxel-based morphometry

Whole-brain volume analysis were performed using voxel-based morphometry (VBM) as implemented on FSL following the standard procedure that can be find elsewhere [12]. We selected Gaussian isotropic kernel with a sigma of 3mm to smooth images and run a two-sample *t*-test between HD patients and healthy controls using FSL's randomise tool (running 10,000 permutations). The resulting maps showed areas in which the GM intensity is significantly different ($p < 0.05$ TFCE corrected) between groups controlling by age and CAG repeats.

### Spatially unbiased infratentorial (SUIT) analysis

SUIT toolbox performs an accurate normalization of the cerebellum compared to whole-brain methods [9] resulting in more reliable image statistics. The analysis was performed using MATLAB 2015b and SPM12. The procedure includes isolation of the cerebellum, segmentation of the gray and white matter components, normalization by diffeomorphic anatomical registration (DARTEL) algorithm of the gray matter segmentation to the SUIT template, and spatial smoothing using a 3-mm kernel. A two-sample *t*-test between the patient group and healthy controls was performed and the final map was corrected using false-discovery rate (FDR $p > 0.05$). The resulting parametric map was then projected into the flatmap representation of the cerebellar cortex in two-dimensional space.

### Degeneration signature

The degeneration signature normalizes the amount of degeneration of each patient controlled by their overall amount of cerebellar damage, thus penalizing patients with a large amount of degeneration. The procedure includes creating an image of the average GM intensity in the control group; then the GM image of each patient is subtracted from the mean GM image of the controls; for each patient the severity of degeneration is then computed as the mean intensity value of the resulting image. The severity value is used to control the intensity of the original GM segmentation of each patient [10]. Finally, the corrected GM images are averaged to create the degeneration signature. Such map reveals the underlying degeneration pattern avoiding skewed results led by patients with bigger amounts of degeneration [11].

## Results

Whole-brain VBM analysis confirmed significant GM decrease in caudate nucleus and putamen bilaterally, as well as in other cortical areas (Fig. 1a). SUIT flatmap shows the cerebellar data in two-dimensional space representation of significant GM decrement in HD patients compared to controls ($p < 0.05$, T = 2.5). No GM increases were found (Fig. 1b). The highest cerebellar degeneration was identified in Crus I right lobule, Crus II bilaterally, and left VIIb, and left VIIIa lobules (Fig. 1b). Similar to the VBM analysis, the SUIT analysis showed no regions with higher density in the HD group compared to the control group. Finally, the cerebellar degeneration signature showed a degeneration pattern that included regions I–IV, Crus II, VIIb, VIIIa, VIIIb and X (Fig. 1c).

## Discussion

Here we aimed to characterize the cerebellar volumetric changes in HD patients. Our cerebellar analysis, using two different methods, showed significant cerebellar degeneration in HD, without sign of cerebellar gray matter increase. Further analysis to delineate the cerebellar degeneration signature in HD showed a specific pattern of cerebellar areas that may be more susceptive to the disease mutation.

Our results confirm previous findings based on histopathological reports and brain imaging analyses that suggest a significant cerebellar degeneration in HD that has been overshadowed by the severe striatal and neocortical deterioration that are the hallmark of this disease [2, 4, 5]. It is

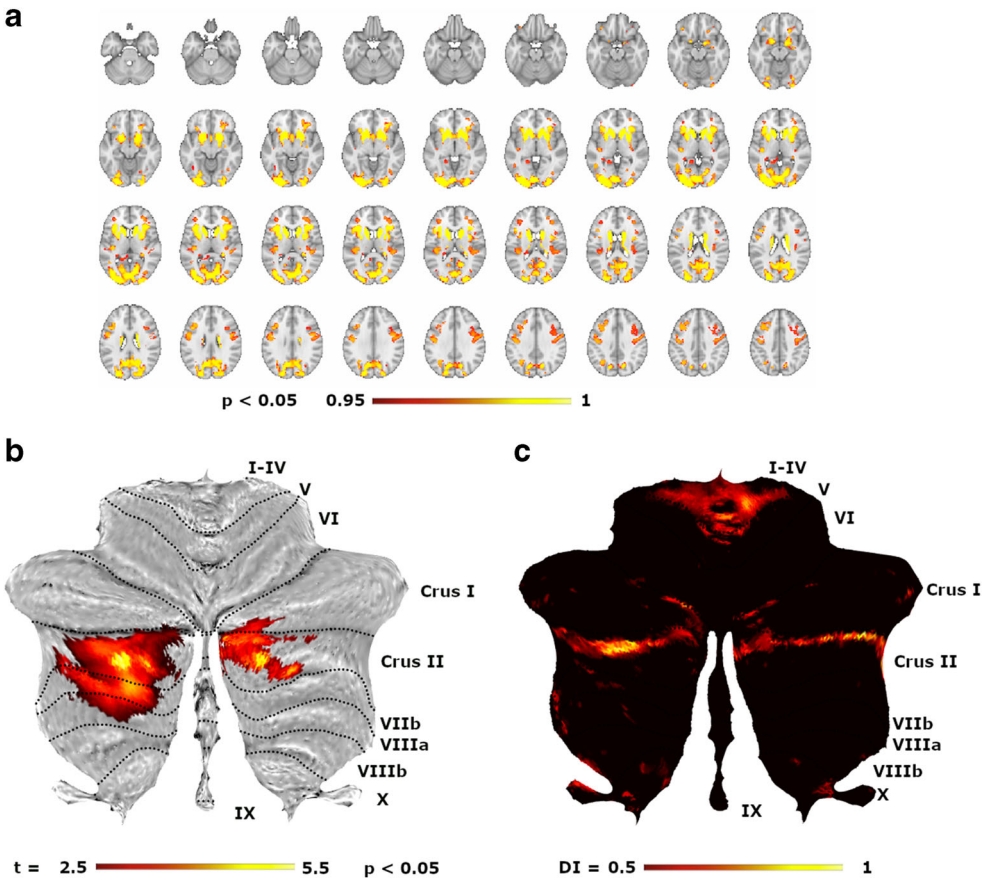


**Fig. 1** (*A*) Axial sections showing significant VBM results including HD striatal and neocortical degeneration when compared with the control group ($p < 0.05$). (***B***) Flat map representation of cerebellum GM showing significant GM decrement in right lobule of Crus I, and Crus II bilaterally, left VIIb, and left VIIIa ($p < 0.05$). (***C***) Degeneration signature of HD patients; color intensity indicates the pattern of degeneration (DI, degeneration Intensity).

becoming clear, however, that the cerebellar degeneration in HD has also an important impact in the clinical deterioration of these patients. For example, it has been suggested that cerebellar degeneration may contribute to HD symptoms such as deficit in rapid alternating movements, fine motor skills, ataxia and postural instability among other characteristic impairments observed in cerebellar patients [3]. The association between motor deficits and cerebellar degeneration in HD has been confirmed in two recent studies, an in-vivo study showing a significant correlation between cerebellar degeneration and motor impairment [13], and a postmortem study showing a correlation between Purkinje cell loss in the cerebellum and the presence of the motor symptom phenotype of the disease [14]. This last study is of special importance because in-vivo brain imaging studies usually are from patients in early stages, since it is difficult to obtain images once the patients are in more advanced stages. In contrast, post-mortem studies have shown cerebellar degeneration in more detail, suggesting that even the deep cerebellar nuclei are affected once the HD progresses to more advanced stages degeneration [3].

The degeneration signature revealed that besides Crus II, motor areas of the anterior cerebellum are susceptible to be the primary target of the disease. Controlling the severity of degeneration (see methods) is important, especially in small cohorts where a few subjects with a large amount of secondary degeneration can have a big impact on regular statistics, hence obscuring the original degeneration pattern [10]. In the absence of large longitudinal datasets, the degeneration signature provides an unbiased estimate of the primary target of the disease. In line with previous studies [4], the degeneration in the anterior lobule of the cerebellum can have a direct influence on the deficits in fine movement and postural stability in HD patients.

These results contrast with a previous finding suggesting increase in cerebellar GM consisting of GM excess found symmetrically in regions I–IV of the anterior cerebellar lobes, and GM density decrease mostly in areas VI of posterior cerebellar lobes bilaterally and V of anterior cerebellar lobe on the left [8]. Although those results are in line with studies that have shown increases in GM in other diseases, including some forms of dystonia [15], we cannot currently explain the differences with our study where the number of CAG repetitions, UHDRS and MoCA scores and the imaging processing steps were similar, as well as with previous in-vivo reports that did not find GM excess in HD [4, 6, 7]. One possibility, however, is that the HD cohorts could present yet unidentified differences. For example, recent studies found significant differences between spinocerebellar ataxia patients type 10 from Mexico and from Brazil. While the Mexican cohort showed

significant thalamic degeneration accompanied by the presence of seizures, the Brazilian cohort did not show these characteristics [11, 16].

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## Declarations

**Conflict of interest** The authors declare no competing interests.

**Ethics approval and consent to participate** This study was approved by the human research ethics committee at the Faculty of Medicine at UNAM.

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