

Striatal and extrastriatal atrophy in Huntington's disease and its relationship with length of the CAG repeat

H.H. Ruocco¹,
I. Lopes-Cendes²,
L.M. Li¹, M. Santos-Silva²
and F. Cendes¹

¹Departamento de Neurologia, ²Departamento de Genética Médica,
Faculdade de Ciências Médicas, Universidade Estadual de Campinas,
Campinas, SP, Brasil

Abstract

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that affects the striatum most severely. However, except for juvenile forms, relative preservation of the cerebellum has been reported. The objective of the present study was to perform MRI measurements of caudate, putamen, cerebral, and cerebellar volumes and correlate these findings with the length of the CAG repeat and clinical parameters. We evaluated 50 consecutive patients with HD using MRI volumetric measurements and compared them to normal controls. Age at onset of the disease ranged from 4 to 73 years (mean: 43.1 years). The length of the CAG repeat ranged from 40 to 69 (mean: 47.2 CAG). HD patients presented marked atrophy of the caudate and putamen, as well as reduced cerebellar and cerebral volumes. There was a significant correlation between age at onset of HD and length of the CAG repeat, as well as clinical disability and age at onset. The degree of basal ganglia atrophy correlated with the length of the CAG repeat. There was no correlation between cerebellar or cerebral volume and length of the CAG repeat. However, there was a tendency to a positive correlation between duration of disease and cerebellar atrophy. While there was a negative correlation of length of the CAG repeat with age at disease onset and with striatal degeneration, its influence on extrastriatal atrophy, including the cerebellum, was not clear. Extrastriatal atrophy occurs later in HD and may be related to disease duration.

Key words

- Neurodegeneration
- Dynamic mutation
- Genotype-phenotype correlation
- Basal ganglia
- Magnetic resonance imaging
- Huntington's disease

Correspondence

F. Cendes
Departamento de Neurologia
FCM, UNICAMP
13084-971 Campinas, SP
Brasil
Fax: +55-19-3289-1818
E-mail: fcendes@unicamp.br

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Introduction

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder that usually manifests in adulthood, resulting in familial adult onset chorea and subcortical dementia (1). In HD, the protein huntingtin is enlarged because of an ex-

tended CAG repeat chain present on the short arm of chromosome 4 (1,2). To date, the exact function of huntingtin is unknown and there is no information about its three-dimensional structure that suggests any putative function. The defect of this protein promotes neuronal apoptosis in various brain regions, especially the basal ganglia, and the

caudate nucleus in particular. Most descriptions were limited exclusively to damage to the striatum (3). However, neuronal degeneration also occurs in other areas of the brain (4-6). Generally the cerebellum is spared in the adult form, and in the great majority of patients the cerebellar changes have been reported to be discrete (5,6).

The length of the CAG repeat in the mutant gene has important implications for age at onset, penetrance, and stability of the gene between generations (7). While the relationship between CAG repeat and age at onset is relevant, the effect of trinucleotide length on the rate of HD progression is less clear. Although some investigators have suggested that larger CAG expansions result in more rapid progression (7), two prospective studies have failed to demonstrate a clear relationship between CAG repeat and illness progression (8-10). A plausible hypothesis to explain this discrepancy point is the instability in the length of the CAG repeat (10).

In the present study, we measured caudate, putamen, cerebral, and cerebellar volumes in a large series of HD patients and controls and we determined whether the HD genotype is associated with regional differences in brain structure, age at onset, duration of disease, and clinical disability.

Subjects and Methods

Subjects

All 50 consecutive patients studied had a clinical and molecular diagnosis of HD performed at the Neurogenetics clinic of UNICAMP. For each patient, age at onset of HD was determined by asking the patient and multiple unaffected family members to recollect the first occurrence of chorea, rigidity, irritability, sleep disturbance, frequent falls, sexual dysfunction, loss of energy, altered social behavior, or failing memory that was not an isolated incident but heralded a progressive decline. The control sub-

jects, 14 men and 19 women ranging in age from 18 to 55 years (mean age: 34 years; standard deviation, SD: 12.15), were healthy and had no family history of neurological or psychiatric illness. We excluded patients with significant co-morbidities, such as alcoholism, head injury, arterial hypertension, diabetes mellitus, stroke, drug abuse, and malignancy. Neurological exams were performed in all patients by the same investigator (HHR). All subjects gave written informed consent to participate in this study, which was approved by the Ethics Committee of our University Hospital. All patients had known trinucleotide repeats.

Clinical and molecular investigation

We used a structured clinical questionnaire for patients and family members. Each subject underwent formal motor examination. Subjects were scored according to the motor component of the United Huntington's Disease Rating Scale (UHDRS) (11). Clinical and genetic details of each patient were included in a database. Molecular testing was performed according to international standard protocols for laboratory testing of HD. Genomic DNA was isolated from peripheral lymphocyte by standard methods. PCR amplification of the CAG repeat in the IT15 gene was performed with primers HD1 and HD3 (1,12), which are adjacent to the CAG stretch. PCR reactions were performed in a total volume of 12.5 μ L containing 100 ng of genomic DNA, 1.6 mM of each dNTP (dATP, dGTP, dTTP), 4 pmol of each primer, 5% DMSO, 0.2 U perfect match (Stratagene[®], La Jolla, CA, USA), and 0.5 units Taq DNA polymerase. After an initial denaturation of 2 min at 95°C the amplification was accomplished in 30 cycles at the following temperatures: denaturing at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2 min, followed by a final extension at 72°C for 7 min. PCR products were separated by electrophoreses through a 6% dena-

turing polyacrylamide gel. Gels were transferred into Hybond N+ nylon membranes and hybridized with an α - 32 P 3'-end labeled (CAG) 15 probe. Allele lengths were determined by comparing migration relative to a sequencing ladder. Alleles were considered normal when they contained less than 35 CAGs and expanded when they contained more than 40 CAGs (13). We then assigned the patients to three subgroups: patients with early onset HD (N = 4, 2 males and 2 females, mean age at onset 8 years), patients with classical onset HD (N = 24, 8 males and 16 females, mean age at onset 30 years), and patients with late onset HD (N = 22, 19 males and 3 females, mean age at onset 44 years).

Magnetic resonance imaging acquisition

Magnetic resonance imaging was obtained with a 2 Tesla system (Elscent Prestige®, Haifa, Israel). Images were acquired on the coronal, sagittal, and axial planes. Our HD protocol consists of: a) sagittal T1 spin-echo, 6 mm thick (repetition time (TR), 430; echo time (TE), 12) for optimal orientation of the subsequent images; b) coronal T1 inversion recovery, 3 mm thick (flip angle = 200°; TR = 2700

ms; TE = 14 ms; T1 = 840 ms; matrix = 130 x 256; field of view (FOV) = 16 x 18 cm); c) coronal T2-weighted fast spin-echo, 3 mm thick (flip angle = 120°; TR = 4800 ms; TE = 129 ms; matrix = 252 x 320; FOV = 18 x 18 cm); d) axial images parallel to the long axis of the hippocampus; T1 gradient echo, 3 mm thick (flip angle = 70°; TR = 200 ms; TE = 5 ms; matrix = 180 x 232; FOV = 22 x 22 cm); e) axial T2 fast spin-echo, 4 mm thick (flip angle = 120°; TR = 6800 ms; TE = 129 ms; matrix = 252 x 328; FOV = 21 x 23 cm).

Volumetric studies

Basal ganglia and cerebral volumes were measured using coronal inversion recovery images and cerebellar volume was measured using sagittal T1 spin-echo acquisition. Caudate and putamen structures and total intracranial and cerebellar volumes (Figure 1) were manually delineated using the NIH Image program (<http://rsb.info.nih.gov/nih-image>). The volume of each region was compared to that of the control group of 33 healthy volunteers. Values below two SD from the mean for the control group were considered abnormal.

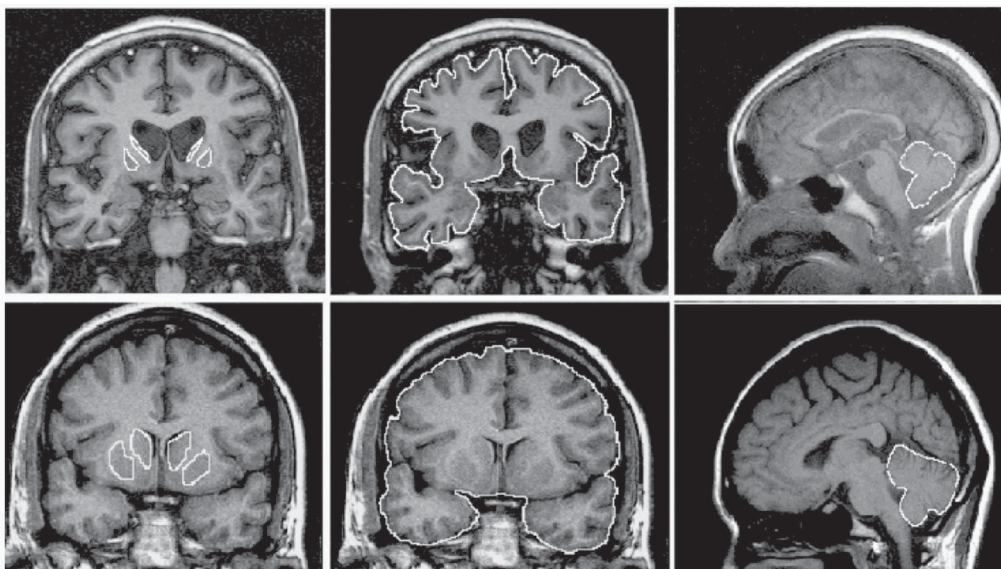


Figure 1. Samples of coronal and sagittal magnetic resonance imaging from a patient with Huntington's disease (top row) and a normal control (bottom row) showing the outlines of caudate and putamen (left), cerebral (center) and cerebellar volumes (right).

Statistical analysis

We report the minimum, maximum and mean values and SD for the volumes of the structures analyzed, and P values less than 0.05 were accepted as statistically significant. We used analysis of variance (ANOVA) to compare continuous variables between groups. Left/right asymmetry (asymmetric index, AI) was calculated according to the following formula: $AI = (left - right)/0.5 (left + right)$. To assess the relationship between volumes and length of the CAG repeat, and age at onset of the disease we used Spearman correlation analysis. We used the Mann-Whitney test to compare AI between right and left basal ganglia structures and cerebellar and cerebral volumes. We used linear regression to assess the relationship between age at onset of disease and length of the CAG repeat. For the clinical score analyses we used the Spearman correlation test.

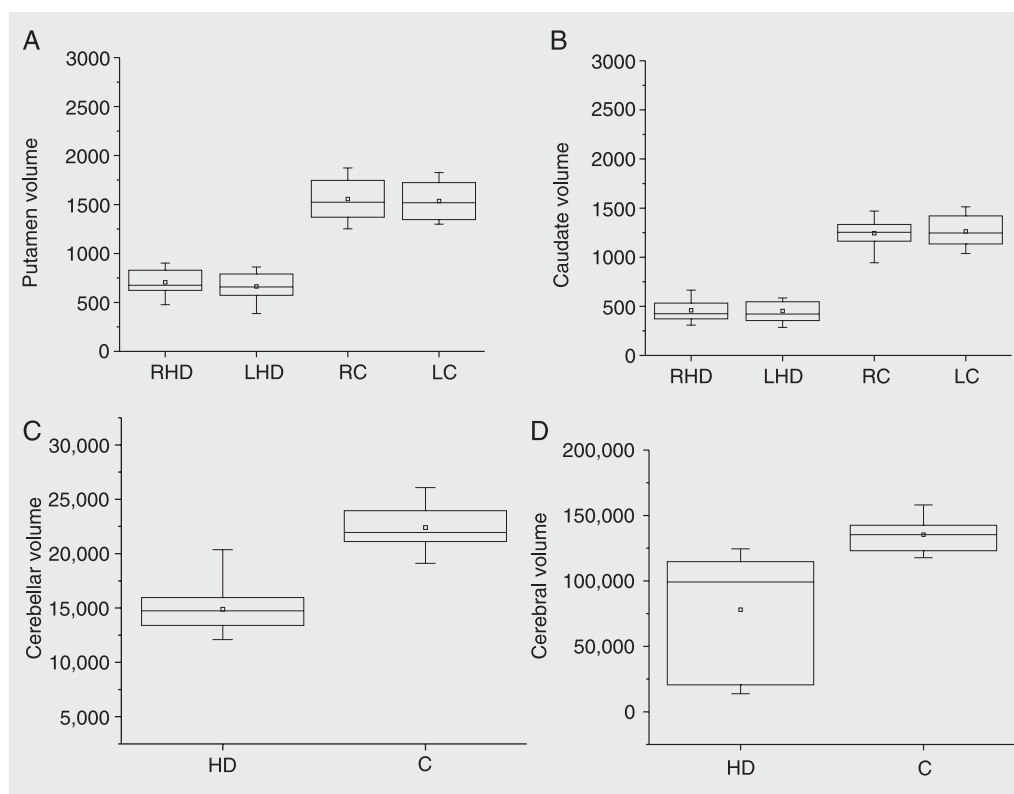
We also performed statistical analyses

comparing the three subgroups of HD subjects according to age at disease onset. We used ANOVA to compare continuous variables between groups such as putamen and caudate nuclei, and clinical analysis. We used the exact Mann-Whitney test to compare cerebellar and cerebral volumes between HD and control groups and the Kruskal-Wallis test when these structures were compared with the three subgroups of HD.

Results

We studied 50 consecutive patients with a clinical and molecular diagnosis of HD (21 women and 29 men; mean age: 43.1 years; SD: 13.25; range: 4 to 73 years) and compared them to normal controls (14 men and 19 women) ranging in age from 18 to 55 years (mean: 34 years; SD: 12.15). The age at onset of HD ranged from 2 to 66 years (mean: 35.1 years; SD: 12.19). Among patients followed at our HD clinic we identi-

Figure 2. Box plot with putamen (A), caudate (B), cerebellar (C), and cerebral (D) volumes in 50 patients with Huntington's disease (HD) and 33 controls (C). The box extends from the 25th percentile to the 75th percentile, with a horizontal line at the median (50th percentile). Whiskers extend down to the smallest value and up to the largest. RHD = right-sided volumes in patients with HD; LHD = left-sided volumes in patients with HD; RC = right-sided volumes in controls; LC = left-sided volumes in controls. There was a significant difference between patients and controls for all comparisons ($P < 0.0001$; ANOVA for basal ganglia and Mann-Whitney test for cerebellar and cerebral volumes).



fied 4 patients with the juvenile form of the disease (2 women) with a mean age of 14.5 years (SD: 7.85; range: 4 to 21 years) that were analyzed separately. The CAG repeat in the HD gene ranged from 40 to 69 (mean: 47.2 CAG; SD: 5.87) and normal alleles ranged from 18 to 26 CAG units (mean: 22 CAG; SD: 2.65). There was no significant difference in the length of the expanded alleles between affected men (mean 45.7; SD: 3.30) and affected women (mean 49.1; SD: 7.86; $P = 0.08$). There was a significant inverse correlation between age at onset of the disease and length of the CAG repeat ($P = 0.0001$, $r = -0.60$, Spearman test), but not between duration of disease and length of the CAG repeat ($P = 0.54$, $r = -0.08$). There was a marked reduction of caudate and putamen nuclei and of cerebral and cerebellar volume in HD patients, with a significant difference from controls ($P < 0.0001$; Figure 2). Left-sided putamen volumes were slightly smaller than right-sided putamen volumes ($P = 0.05$), but there was no asymmetry for the caudate. There was a significant correlation between the length of the CAG repeat and the volumes of right caudate ($P = 0.024$, $r = -0.31$), left caudate ($P = 0.0047$, $r = -0.39$), right putamen ($P = 0.023$, $r = -0.32$), and left putamen nuclei ($P = 0.05$, $r = -0.27$). However, there was no correlation between putamen and caudate volumes and duration of disease ($P = 0.53$, $r = 0.09$ for right putamen; $P = 0.60$, $r = 0.07$ for left putamen; $P = 0.43$, $r = -0.11$ for right caudate; $P = 0.49$, $r = -0.10$ for left caudate). In addition, we did not find a correlation between the length of the CAG repeat and cerebellar ($P = 0.72$, $r = -0.05$) or cerebral ($P = 0.91$, $r = -0.01$) volumes. We found a tendency to a correlation between duration of disease and cerebellar volumes ($P = 0.08$, $r = -0.25$), but not cerebral volumes ($P = 0.48$, $r = -0.10$).

When the juvenile group was compared to the group of classic and late onset HD, there was no statistical difference in basal ganglia, cerebellar or cerebral volume ($P =$

0.1, $P = 0.4$, and $P = 0.3$, respectively). In addition, we found a highly significant difference in the mean size of the expanded allele between early- and late-onset HD and between classic-onset and late-onset HD ($P < 0.05$).

The UHDRS scores ranged from 15 to 128 (mean: 65.5; SD: 33.74; Table 1). There was a correlation between UHDRS scores and age at onset ($P = 0.03$, $r = 0.3$), and a tendency for length of the CAG repeat ($P = 0.08$, $r = 0.24$), but there was no correlation between duration of disease, cerebral volume, right putamen and caudate volumes, and clinical scores ($P > 0.1$). Interestingly, there was a correlation between UHDRS scores and left putamen volume and a tendency to a correlation between UHDRS score and left caudate volume ($P = 0.05$, $r = 0.27$ and $P = 0.07$, $r = 0.25$, respectively).

Discussion

Since the original discovery that the HD phenotype results from an expanded CAG trinucleotide repeat within the IT15 gene located on chromosome 4 (1,2), several hypotheses have been raised in an attempt to explain the mechanisms of expanded CAG repeats in the pathogenesis of HD. Certain

Table 1. Summary of clinical and molecular data in patients with Huntington's disease and controls.

	Huntington's disease (N = 50)	Normal controls (N = 33)
Male:female	29:21	14:19
Age (years; range)	4-73	18-55
Age (years; mean \pm SD)	43.1 \pm 13.25	34 \pm 12.15
Age at onset (years; range)	2-66	
Age at onset (years; mean \pm SD)	35.1 \pm 12.19	
CAG repeat (range)	40-69	18-26
CAG repeat (mean \pm SD)	47.2 \pm 5.87	22 \pm 2.65
UHDRS (range)	15-128	
UHDRS (mean \pm SD):	65.5 \pm 33.74	
CAG repeat (men; mean \pm SD)	45.7 \pm 3.30	
CAG repeat (women; mean \pm SD)	49.1 \pm 7.86	

UHDRS = United Huntington's Disease Rating Scale.

brain areas and also specific neuronal subtypes differ significantly in their susceptibility to injury in the course of acute and chronic disorders, suggesting that specific protective factors are expressed differently among neurons or even among different cell types in the same tissue. This can be clearly seen in the striatum, where GABAergic projection cells (12,14-16), but not cholinergic interneurons, are precociously damaged in the course of HD (12,15-18).

There is evidence of distributed gray matter pathology and progressive white matter pathology before the clinical onset of HD (5,19), and loss of basal ganglia volume has also been reported in asymptomatic individuals with HD mutation (20-24), indicating that striatal neuronal loss occurs before or early during the clinical course.

Even though striatal atrophy has been detected and reported by many investigators (3,25-29), neuronal degeneration also occurs in other areas of the brain such as the cerebral and cerebellar cortex (4,5), amygdala, hippocampus, and brainstem (6), supporting the concept that HD is a multisystem disorder. In agreement with this concept, subcortical gray matter loss in the thalamus, substantia nigra, and anterior basomedial diencephalic region has been described, but in later stages of the disease (5).

It is well known that the most affected brain structure in HD patients is the striatum (3). However, the mechanisms underlying the regional preferences for striatum atrophy are still controversial. One possible explanation is the instability in the length of the CAG stretch (10). The resulting polyglutamine load can vary as a result of the regional instability of the CAG mutation related to the tissue biochemical features (30), therefore causing different rates of cell death in different tissues. On the other hand, there is some evidence that polyglutamine differs between tissues due to differential instability of the CAG mutation (30). Perhaps then the striatum is more vulnerable to

the effects of accumulating mutant huntingtin than other tissues. Curiously, in our study left-sided putamen volumes were slightly smaller than right-sided putamen volumes, but we did not find asymmetry in caudate nuclei. Although HD has been generally considered to be a symmetric disease (31), findings of higher lactate levels in the left than in the right striatum have been attributed to increased motor activation in the dominant hemisphere, especially increased glutamatergic synaptic input to striatal cells (32). In agreement with this finding, based on the UHDRS motor component, we detected a slight correlation between clinical disability and left-sided putamen atrophy and a tendency to a correlation between clinical disability and left-sided caudate atrophy. On the other hand, studies have shown that the putamen is more affected than the caudate (27,28), whereas others have shown the opposite (20,29). Longitudinal data from neuropathology and neuroimaging studies suggest that the earliest changes in HD occur in the caudate nucleus, while the putamen nucleus is more affected with disease progression (3,33,34). In agreement with previous studies (29,31,35,36), we found that the caudate and putamen were disproportionately atrophic in HD patients, with this atrophy correlating with the length of the CAG repeat, but not with duration of the disease (23,37). On the other hand, atrophy also occurred in cerebral and cerebellar volumes, but without correlation with the length of the CAG repeat (29,36). In addition, there was a non-significant trend towards an inverse correlation between cerebellar atrophy and duration of the disease. This may support the hypothesis that neocortical and cerebellar degenerations are explained, at least in part, by retrograde changes due to massive neuronal loss in the striatum or due to progressive white matter loss in the course of the disease, suggesting that cerebellar neuronal loss occurs later in the clinical course.

The present results suggest a more wide-

spread and global disease process in patients with HD, and that the extent of the degeneration process is not completely explained by the length of the CAG repeat. Recent evidence showed that environmental factors modify the onset and progression of neurodegeneration (38) and variations in the glutamate receptor 6 genotype may explain up to 13% of the variability in the age at onset of

HD not accounted for by the length of the CAG repeat (39,40).

Longitudinal studies with larger series will be necessary to characterize the exact rate of atrophy of different brain structures and to determine the kinetics of neuronal degeneration during the various stages of HD and this knowledge could be helpful for the targeting of new therapeutic measures.

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