

Rate and Acceleration of Whole-Brain Atrophy in Premanifest and Early Huntington's Disease

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Abstract: Huntington's disease (HD) produces progressive and ultimately widespread impairment of brain function. Neostriatal atrophy alone cannot account for whole-brain losses seen postmortem, and the mutant huntingtin protein and its neuropathologic sequelae are evident throughout the brain. Whole-brain atrophy quantification encompasses the totality of mutant huntingtin's effects on brain volume and may be useful in tracking progression in trials. We studied whole-brain atrophy in HD using a 2-year follow-up design, with three annual MRI scans. We recruited 20 control subjects, 21 premanifest mutation carriers, and 40 patients with early HD and used the brain boundary shift integral to study rate and acceleration of atrophy. Among subjects with an acceptable quality 2-year scan pair, age- and gender-standardized mean brain atrophy rate was greater ($P < 0.001$) in the patients with HD ($n = 21$; 0.88%/yr; 95% con-

fidence interval: 0.62–1.13%/yr) than that in controls ($n = 13$; 0.16%/yr; 0.00–0.32%/yr). In the 12 patients with early HD in whom acceleration could be directly assessed there was evidence ($P = 0.048$) of acceleration year-on-year (mean acceleration = 0.69% yr⁻²; 95% confidence interval: 0.01% yr⁻² to 1.37% yr⁻²), although this was not formally significantly different from that in controls ($n = 7$, $P = 0.055$). Statistically significantly increased atrophy rates and acceleration were not seen overall in the premanifest group, who were on average 18 years from predicted disease onset. We conclude that the study of whole-brain atrophy has the potential to inform our understanding of the neurobiology of HD and warrants further study as one means of assessing the outcomes of future clinical trials. © 2010 Movement Disorder Society

Key words: Huntington's disease; volumetric MRI; whole-brain atrophy

Huntington's disease (HD) is an incurable, autosomal dominantly inherited neurodegenerative disorder, caused by a CAG triplet repeat expansion in the gene

encoding huntingtin. HD usually begins in mid-adult life, causing widespread impairment of brain function resulting in clinical manifestations including movement disorders, cognitive impairment, and psychiatric disturbances.

Mutant huntingtin is expressed ubiquitously.^{1–3} The most striking macroscopic change in HD brains is striatal atrophy.^{4,5} However, while intracellular aggregates of mutant huntingtin are found widely throughout the brain, they are relatively sparse in the striatum.⁶ Moreover, the total loss of brain mass by end-stage HD is considerably greater than could be accounted for by striatal atrophy alone^{4,7,8} and numerous studies have confirmed that HD ultimately affects the whole brain.^{9,10} The rate and timing of the spread of HD

Additional Supporting Information may be found in the online version of this article.

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pathology through the brain is less well understood and difficult to study in vivo in humans.

Though there are no current treatments that slow the progression of HD in humans, several putative treatments have demonstrated disease-modifying effects in animal models,¹¹ and reversible neuronal dysfunction precedes cell death in HD.¹² A genetic test can reliably predict individuals destined to develop the disease, in whom the slowing of pathology would likely delay the onset of symptoms. Because HD progresses slowly and is heterogeneous, and clinical rating scales have limitations, trials of possible disease-modifying treatments in humans are likely to be facilitated by the availability of a panel of biomarkers to track progression and help distinguish between symptomatic and disease-modifying effects.^{13,14}

Numerous neuroimaging techniques for detecting changes in HD brains have been employed, with different abilities to detect the diverse effects of the mutant protein.¹⁴ Quantifying change in structures with well-defined anatomical boundaries may be more reproducible than measuring the volumes of less easily-defined regions. The brain boundary shift integral (BBSI), a semi-automated technique for quantifying whole-brain atrophy,¹⁵ has improved reliability over manual measures¹⁶ and has been employed as an outcome measure in Alzheimer's disease clinical trials.¹⁷ Whole-brain atrophy measurement captures the totality of mutant huntingtin's effects on brain volume, encompassing changes that occur in less prominently involved brain regions, that may nonetheless have importance for understanding the disease.

Whole-brain atrophy rates measured using the BBSI have been shown to be increased in early HD over 6 and 12 months.^{7,18} Larger cohorts and longer interscan intervals are expected to produce stronger signal-to-noise ratios, enabling more robust estimation of atrophy rates. The study of mutation carriers before manifest disease diagnosis may reveal important early changes in the HD brain. Furthermore, it is not known whether whole-brain atrophy proceeds at a constant rate, accelerates or decelerates, and this has been studied only to a limited extent for regional atrophy,^{8,19} but has important implications for both our understanding of the disease and the conduct of future clinical trials.

Over 2 years, we studied prospectively a cohort of patients with HD, premanifest mutation carriers and control subjects, with clinical assessment and volumetric MRI scans at baseline, 1 and 2 years. We measured whole-brain atrophy rates, and used within-subject changes in atrophy rates to assess acceleration of atrophy in HD.

SUBJECTS AND METHODS

Ethical Approval

All work was performed in accordance with the declaration of Helsinki and approved by University College London (UCL)/UCL Hospitals Joint Research Ethics Committee. All subjects gave informed written consent.

Subjects

Eighty-one subjects were recruited: 20 control subjects, 21 premanifest mutation carriers, and 40 patients with early HD. Controls were partners or spouses of mutation carriers, or individuals previously at risk of HD with a negative genetic test for the mutation. Premanifest HD was defined as a diagnostic confidence score of <4 on the Unified Huntington's Disease Rating Scale (UHDRS);²⁰ early HD as a diagnostic confidence score of 4 with a UHDRS total functional capacity score of 7 or more.²¹ No subjects were taking medications known or suspected to influence brain volume and subjects with concomitant neurological illnesses were excluded.

MRI

Scanning was consistent between subjects and timepoints. Subjects underwent 1.5T T1-weighted volumetric imaging using an IR prepared FAST spoiled GRASS sequence with $24 \times 18 \text{ cm}^2$ field of view and 256×256 matrix providing 124 contiguous 1.5 mm-thick coronal slices. In-plane voxel dimensions: $0.9375 \times 0.9375 \text{ mm}$. Acquisition parameters: TR = 13 ms; TE = 5.2 ms; flip angle = 13° ; inversion time = 650 ms; receiver bandwidth = 16 kHz, NEX = 1.

Scans were corrected for intensity inhomogeneities using the N3 algorithm.²² Whole brains were delineated using a semiautomated technique as previously described using MIDAS software.^{7,23} Scan pairs were aligned using affine registration with 12 degrees of freedom, from which the BBSI was calculated.^{15,24} Registrations were carried out across all three intervals from three timepoints: year 1 to baseline, year 2 to baseline and year 2 to year 1. Registrations were checked by one investigator, blinded to time point and subject identity. Scan pairs were rejected where there was a problem with scan quality on either scan (e.g., motion or other artifact) or a difference in scan quality in the pair (e.g., different contrasts on each scan) or where the registration was inadequate (e.g., evidence of stretch).

TABLE 1. Subject demographic characteristics in those with an acceptable quality baseline to 1-year and/or baseline to 2-year scan pair

	Control	Premanifest	Early HD
N	19	20	31
Gender, M:F	7:12	9:11	16:15
Age at baseline assessment, years	45.6 (10.7)	37.5 (8.1)	49.4 (9.1)
CAG repeat length		42.3 (1.8)	43.4 (1.7)
Years to predicted motor onset ²⁶	–	17.6 (7.3)	
Years since motor onset			4.9 (2.6)
Interval between baseline and 1-year scan, years	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)
Interval between baseline and 2-year scan, years	2.2 (0.1)	2.3 (0.1)	2.3 (0.1)

Values are given as mean (SD).

Statistical Analysis

Follow-up brain volumes were calculated from baseline brain volume and BBSI-derived absolute brain loss. Brain volumes were normalized to mean total intracranial volume (TIV) as previously described.²⁵ For the purposes of statistical analysis, changes were expressed as the logarithm of the ratio of the followup to the baseline value so that equivalent proportional increases and decreases (such as doublings and halvings) were treated as effects of equal magnitude. Annualized changes (atrophy rates) were calculated by dividing by follow-up time. Means and standard deviations of annualized percentage changes were obtained from back-transformation of results on the logarithmic scale.

Paired *t*-tests were used to compare first- and second-year atrophy rates in each subject group. Atrophy acceleration rates were calculated by subtracting the first- from the second-year annualized atrophy rate.

Linear regression models, adjusting for baseline age and gender, were used to compare 2-year atrophy rates and acceleration rates between groups, and to obtain atrophy and acceleration rates standardized to the mean age and gender mix in the subjects as a whole. Further regression models of this type were used to relate 2-year atrophy rates and atrophy acceleration rates to CAG repeat length, adjusting for baseline age and gender, in the premanifest and early HD groups.

RESULTS

Follow-Up and Demographics

Twenty control subjects, 21 premanifest mutation carriers and 40 patients with early HD attended for baseline assessment. Of these, 18, 19 and 39 subjects

respectively returned for 1 year assessments; 13, 17 and 28 subjects attended at 2 years. One control and one premanifest subject attended 2-year but not 1-year assessments. Over the longest interval from baseline to 2 years, 10, 17 and 21 subjects respectively produced acceptable quality scan pairs. In total 19 controls, 20 premanifest and 31 patients with early HD produced at least one acceptable quality one-year or 2-year scan pair. Retention and follow-up are detailed fully in Supporting Information Table 1.

Direct information concerning atrophy acceleration was obtained from those subjects with acceptable quality baseline to 1 year and 1 to 2 year scan pairs. 7 controls, 10 premanifest and 12 patients with early HD met these criteria. All of these subjects also had acceptable quality baseline to 2 year scan pairs.

Although scan pair rejection due to failed registrations was distributed roughly equally between groups, rejection of scans due to excessive movement was confined to the early HD group (5 of 107 scans). Rejection of registrations due to image artifact was most common in early HD (11% versus 2% in controls and 4% in premanifest HD). Reasons for rejection of scans and pairs are detailed in Supporting Information Table 2.

Table 1 shows demographic characteristics of subjects with at least one acceptable quality scan pair. There were no statistically significant intergroup differences in gender or interscan interval. The premanifest group had a significantly shorter mean CAG repeat length than the early HD group ($P = 0.02$) and was significantly younger than both controls ($P = 0.01$) and the early HD group ($P < 0.001$).

Whole-Brain Atrophy Over 2 Years

Whole-brain atrophy findings for the subjects in Table 1 are summarized in Figure 1 and examples of whole-brain atrophy over 1 and 2 years are given in Figure 2.

Whole-brain atrophy rates over 2 years were calculated for 48 subjects with an acceptable quality 2-year scan (Fig. 3A and Table 2).

The mean atrophy rate was significantly higher in the HD group than in both controls and the premanifest HD group ($P < 0.001$ in both cases; adjusted for age and gender). In the premanifest group the mean atrophy rate did not differ significantly from the control mean ($P = 0.55$).

After adjusting for age and gender, there was a statistically significant association between whole-brain atrophy rate and CAG repeat length in the group of 38 HD mutation carriers ($P = 0.009$). On average, a

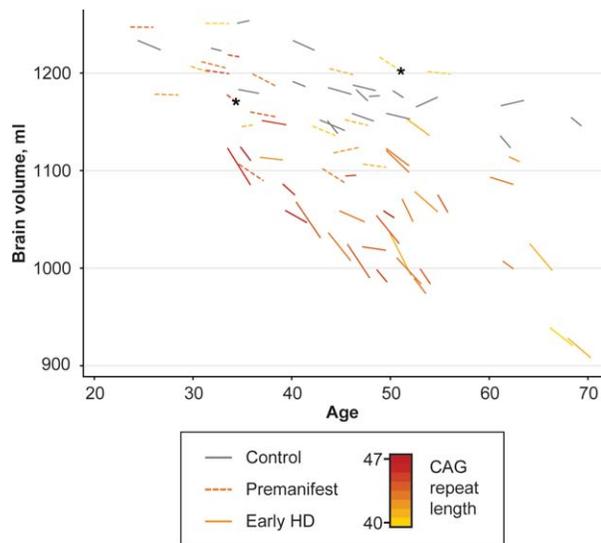


FIG. 1. Whole brain MRI findings in this cohort. Brain volumes are normalized to mean TIV; follow-up volumes are BBSI-derived. Asterisks highlight premanifest subjects who underwent motor onset during the study. Normal age-related atrophy is seen in controls, while some premanifest and many subjects with early HD have lower brain volumes and higher atrophy rates.

single triplet increase in repeat length was predicted to increase the whole-brain atrophy rate by 0.12%/year (95% confidence interval 0.03–0.22%/year). This model was designed to examine the effect of the length of the HD mutation per se, irrespective of the presence or absence of motor signs.

However, because early HD subjects had, on average, higher CAG repeat lengths than the premanifest group, a joint model was constructed, relating CAG repeat length and motor onset (early HD vs. premanifest) to atrophy, after adjusting for age and gender. Only motor onset was a statistically significant predictor of atrophy rate ($P = 0.03$). The CAG, age and gender adjusted difference in mean atrophy rates was 0.54%/year (95% confidence interval 0.06–1.02%/year).

Acceleration of Whole-Brain Atrophy

Using only data from those subjects with both acceptable quality baseline to 1-year and 1- to 2-year scan pairs (7 controls, 10 premanifest and 12 early HD) comparison of the first and second year atrophy rates within each group (Fig. 3B,C and Table 2) revealed statistically significant acceleration of atrophy in the early HD group ($P = 0.048$). In all groups, there was variability in rates of change of whole brain atrophy with some increases and some decreases. Mean acceleration in the premanifest group was around half

that seen in the early HD group, but neither this ($P = 0.29$) nor acceleration in controls ($P = 0.63$) was statistically significant. When acceleration was compared between groups after adjusting for age and gender, there was a trend for the HD group to have greater acceleration than controls, though the difference was not statistically significant ($P = 0.055$).

Across all 22 gene carriers there was a positive association between annual acceleration of atrophy and CAG repeat length, after correction for age and gender. On average, a single triplet increase was associated with a 0.39%/yr annual acceleration in atrophy (Fig. 3D; 95% confidence interval 0.18–0.59%/yr; $P = 0.001$). This difference remained statistically significant after adjustment for group differences.

A supplementary analysis, incorporating indirect information about atrophy acceleration from subjects with acceptable scan pairs over 2 years and only one intermediate interval, with the missing atrophy rate derived by subtraction, did not produce substantially different results.

DISCUSSION

Quantification of whole-brain atrophy over 2 years in this cohort confirms that atrophy rate is significantly

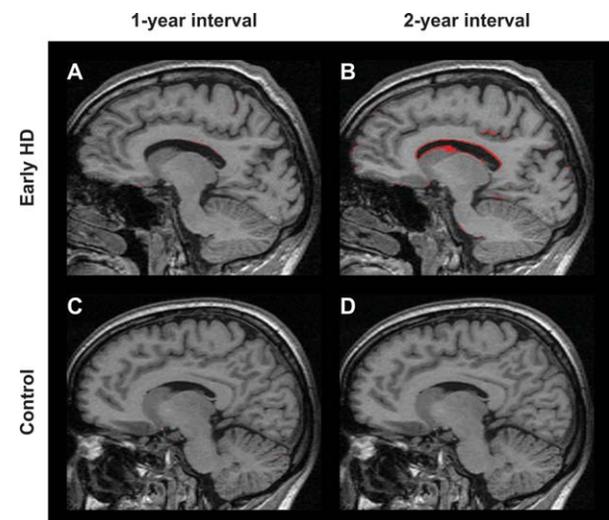


FIG. 2. Whole-brain atrophy in HD quantified by the BBSI technique. Parasagittal views of baseline MRI scans with overlays showing areas of volume loss (red) and gain (green) detected by BBSI. (A) Patient with early HD aged 33 with 47 CAG repeats, showing change over 1 year. (B) Change over 2 years in the same patient, with clearer evidence of atrophy. (C, D) Equivalent images from a 40-year-old control subject, showing little atrophy over either interval. Though apparent volume gain is not prominent in these examples, boundary shifts in either direction are incorporated in the calculation of overall brain volume change.

increased in early HD, to about five times the rate associated with normal aging. We did not observe a significant increase in mean atrophy rates in our group of premanifest subjects, likely because the premanifest

group was, on average, relatively far from predicted onset (mean 17.6 years).

The atrophy rate in early HD is consistent with our previous findings over shorter intervals.^{7,27} Studies of longer duration offer a more robust measure of atrophy rate: differences in volume measures, whether due to physiological reasons (e.g., hydration),²⁸ MRI acquisition variability or image analysis have less impact on atrophy measurement with a larger denominator (interval). However, the longer the study, the greater the chances of drop-out or changes in MR acquisition; optimal follow-up length is a balance between these competing considerations.

These atrophy rates are consistent with losses reported from other imaging studies and autopsy findings. The atrophy rate in our healthy control subjects is in keeping with findings of a gradual age-related reduction in brain weight of around 2% per decade and imaging studies that have reported brain volume losses of 0.1–0.3%/yr in this age group (for review, see Ref. 29). Postmortem studies reveal 10–20% loss in brain mass by end-stage HD,⁴ equating to ~180 mL (120–240 mL) for a typical brain (healthy controls have brain volumes of ~1,200 mL at this age; see Fig. 1). If the annual atrophy rate of 0.9% seen our subjects with early HD were sustained year-on-year, this amount of atrophy would be reached after about 15–20 years, corresponding to the typical period between onset and death.³⁰ However, our data suggest that such assumptions of linearity of whole-brain atrophy rate may not be valid. Within-subject comparisons of atrophy rates in this cohort suggest that atrophy may accelerate in early HD (albeit with wide 95% confidence intervals, from 0.01 to 1.4%/yr increase in atrophy rates).

The fact that the mean atrophy rate in the manifest group is higher than in the premanifest group or

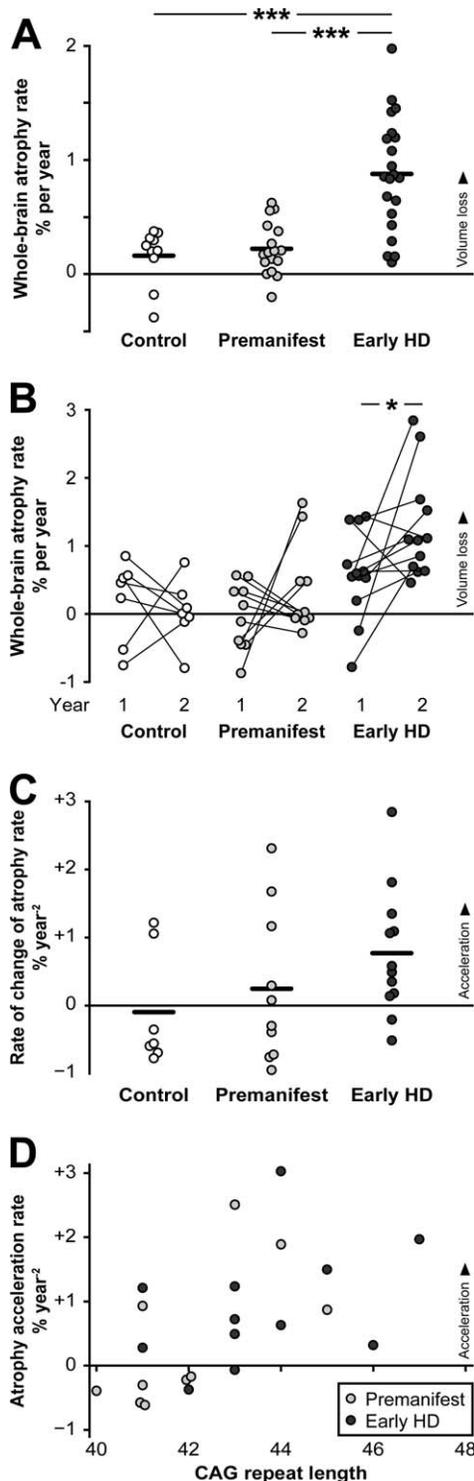


FIG. 3. Rate and acceleration of whole-brain atrophy in HD. (A) Whole-brain atrophy is increased in early HD. Whole-brain atrophy rates, adjusted for age and gender, were increased in patients with early HD compared with both controls and premanifest HD. $***P < 0.001$ by linear regression. Horizontal bars show group means. (B, C) Evidence for whole-brain atrophy acceleration in early HD. (B) Whole-brain atrophy rates by group, with each subject's first- and second-year atrophy rates joined, such that accelerating atrophy is indicated by a positive slope. (C) Atrophy acceleration rates, adjusted for age at baseline and gender, by group. Atrophy rates did not increase significantly in controls and premanifest HD but there was some evidence of acceleration in early disease ($*P = 0.048$, paired t -test; $P = 0.055$, age- and gender-adjusted comparison with control group acceleration). (D) Association between whole brain atrophy acceleration and CAG repeat length in early HD and premanifest subjects. Adjusted for age and gender. $P < 0.001$.

TABLE 2. Whole-brain atrophy rate and acceleration

	Control	Premanifest	HD
Whole-brain atrophy rate ^a			
N	10	17	21
Mean atrophy rate, %/yr (SD)	0.16 (0.25)	0.22 (0.23)	0.88 (0.50)
95% confidence interval	0.00–0.32	0.09–0.36	0.63–1.13
Whole-brain atrophy acceleration ^b			
N	7	10	12
0–1 year atrophy rate, %/yr (SD)	0.20 (0.60)	–0.03 (0.49)	0.58 (0.66)
1–2 year atrophy rate, %/yr (SD)	0.03 (0.47)	0.36 (0.67)	1.27 (0.79)
Mean annual atrophy acceleration rate from year 1 to year 2% yr ^{–2} (SD)	–0.17 (0.88)	+0.39 (1.10)	+0.69 (1.08)
95% confidence interval	–0.99 to +0.64	–0.40 to +1.18	+0.01 to +1.37

^aWhole-brain atrophy rates by group, adjusted to the mean age and gender case-mix in the 48 subjects with an acceptable quality two-year scan pair.

^bAssessment of atrophy acceleration by group in those subjects with acceptable quality baseline-1 year and 1 to 2 year scan pairs.

controls provides support for the hypothesis that there is acceleration in atrophy rate in HD (assuming that other differences between groups cannot explain this). The borderline statistically significant acceleration seen in the early HD group and the observed association between CAG repeat length and acceleration also support this hypothesis, even allowing for the uneven distribution of CAG repeat lengths between premanifest and manifest subjects with HD. What is unclear is when atrophy rates might start to accelerate. The mean acceleration in the premanifest subjects, far from predicted age at onset, was around half that in the early HD group, but this was not statistically significant. One possible explanation is that measurable acceleration occurs when individuals are closer to motor onset than the majority of our premanifest group; it is interesting that the two premanifest subjects who underwent motor conversion during follow-up appear to have increased rates of loss (Fig. 1). The relatively small sample size in the premanifest group ($n = 7$) including a high proportion of subjects many years from likely onset means that the study lacks statistical power to demonstrate acceleration in premanifest subjects. High variability in acceleration in the group is also a likely contributor. Similarly, it is important to note that our estimate of acceleration ($0.69\% \text{ yr}^{-2}$) in the early HD group has very wide confidence intervals ($+0.01$ to $+1.37\% \text{ yr}^{-2}$ yrs); an acceleration rate of over $0.5\% \text{ yr}^{-2}$ is unlikely to be plausibly sustained over a period of many years. These preliminary findings therefore merit further study in larger cohorts over multiple timepoints.

This study found an association between whole-brain atrophy rate and CAG repeat length when premanifest and manifest mutation carriers were considered

together. This association was present after correction for age, indicating an excess effect of CAG repeat length on atrophy, beyond the tendency of the disease to be more severe with aging for a given repeat length. However, our finding has to be interpreted cautiously: within the premanifest group we did not find an association between CAG length and atrophy rate; similarly we did not find an association within the manifest group on its own. The association may well have been driven by the fact that our manifest group had longer CAG lengths and the manifest individuals have greater atrophy rates—this association may therefore reflect disease stage rather than CAG length per se. Nonetheless it would be prudent to consider the possible effects of CAG length on outcome measures in future trials.

Further work in larger premanifest cohorts with subjects closer to predicted motor onset is required to investigate whether year-on-year acceleration of whole-brain atrophy is a predictor of clinical onset. Although acceleration of whole-brain atrophy rate in HD has not previously been studied, multi-interval longitudinal measurement of other brain structures has been carried out. Aylward and colleagues found that caudate volume was stable until 11 years before motor onset, then atrophy of the caudate proceeded linearly at $0.24 \text{ cm}^3/\text{yr}$ in premanifest subjects, while putaminal volume was stable until 9 years before motor onset then volume loss occurred at $0.23 \text{ cm}^3/\text{yr}$.⁸ In previous work, caudate atrophy rates in mild and moderate HD were determined to be 4.9 and 7.2% respectively, suggesting acceleration with progressing disease. However, because of the small volume of the caudate, these percentage changes corresponded to roughly equal absolute annual atrophy rates of 0.16 cm^3 and 0.18 cm^3 respectively.¹⁹ It is difficult to assess whether

this represents a true acceleration of pathology. Recent cross-sectional data from the large Predict-HD study appear to confirm the findings of Aylward and colleagues.³¹

One potential weakness of this study is the possible effect of the hyperkinetic movement disorder of HD on patients' ability to tolerate MRI scanning. Rejection of scans due to movement artifact was confined to the early HD group and affected 6% of scans. We acknowledge this group-specific suitability for scanning as a possible source of bias, though it is likely to have resulted in the exclusion of more severely-affected individuals and therefore an underestimation of atrophy rate in the early HD group. Such movement problems are likely to affect any study involving MR imaging of manifest HD subjects and we favor the application of rigorous quality control over the inclusion of poor quality scans.

In summary, quantification using the BBSI over 2 years confirms whole-brain atrophy rates to be increased in early HD with a suggestion of acceleration. Atrophy rates were not materially higher in far-from-onset premanifest subjects, but confidence intervals were wide. We conclude that study of whole-brain atrophy has the potential to inform our understanding of the neurobiology of HD. In assessing the outcomes of future clinical trials of putative disease-modifying treatments, several neuroimaging methodologies are likely to be required to accommodate the diverse regional and global brain changes at different stages of HD. We suggest that the BBSI technique warrants further study, alongside other regional and global imaging methods and particularly in early manifest HD, as one such possible measure.

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Author Roles: Edward J. Wild was involved in organization and execution of research project; design, execution, and review and critique of statistical analysis; writing of the first draft and review and critique of manuscript. Susie M. D. Henley was involved in conception, organization, and execution of research project; design and execution of statistical analysis; review and critique of manuscript. Nicola Z. Hobbs was involved in organization and execution of research project; design, execution, and review and critique of statistical analysis; review and critique of manuscript. Chris Frost was involved in design, execution, and review and critique of statistical analysis; review and critique of manuscript. David G. MacManus was involved in organization and execution of research project; review and critique of manuscript. Roger A. Barker was involved in organization of research project; review and critique of statistical analysis; review and critique of manuscript. Nick C. Fox was involved in conception and organization of research project; design and review and critique of statistical analysis; review and critique of manuscript. Sarah J. Tabrizi was involved in conception and organization of research project; design and review and critique of statistical analysis; review and critique of manuscript.

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