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Smaller intracranial volume in prodromal Huntington's disease: evidence for abnormal neurodevelopment

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Huntington's disease is an autosomal dominant brain disease. Although conceptualized as a neurodegenerative disease of the striatum, a growing number of studies challenge this classic concept of Huntington's disease aetiology. Intracranial volume is the tissue and fluid within the calvarium and is a representation of the maximal brain growth obtained during development. The current study reports intracranial volume obtained from an magnetic resonance imaging brain scan in a sample of subjects ($n = 707$) who have undergone presymptomatic gene testing. Participants who are gene-expanded but not yet manifesting the disease (prodromal Huntington's disease) are compared with subjects who are non-gene expanded. The prodromal males had significantly smaller intracranial volume measures with a mean volume that was 4% lower compared with controls. Although the prodromal females had smaller intracranial volume measures compared with their controls, this was not significant. The current findings suggest that mutant huntingtin can cause abnormal development, which may contribute to the pathogenesis of Huntington's disease.

Introduction

Huntington's disease is an autosomal dominant disease manifesting motor, cognitive and psychiatric dysfunction. Although conceptualized as a neurodegenerative disease of the striatum, a growing number of studies in several lines of research have challenged this classic concept of Huntington's disease. Recent studies suggest that, although the primary neuropathology is that of neurodegeneration, there may also be a role of abnormal neurodevelopment (Mehler and Gokhan, 2000). Reports include: (i) studies on transgenic mice with pathogenic mutations that show subtle physiological and structural deficits that predate the occurrence of neurological symptoms and signs of cell injury/death (Hodgson *et al.*, 1999; Luthi-Carter *et al.*, 2000); (ii) a neuropathological study of brains from subjects with prodromal Huntington's disease (those who are gene-expanded but prior to diagnosis of disease) that showed evidence of developmental pathology with increased density of oligodendrocytes in the caudate (Gomez-Tortosa *et al.*, 2001). This finding confirmed a previous study also showing increased density of caudate oligodendrocytes in subjects with Huntington's disease (Myers *et al.*, 1991); (iii) neuroimaging studies of subjects with prodromal Huntington's disease showing brain changes decades prior to onset of disease (Paulsen *et al.*, 2006; Nopoulos *et al.*, 2007; Paulsen *et al.*, 2010); and (iv) a recent study demonstrating the impairment of developmental stem cell-mediated striatal neurogenesis and pluripotency genes in a knock-in mouse model of Huntington's disease (Molero *et al.*, 2009).

The Neurobiological predictors of Huntington's Disease (PREDICT-HD) is a multi-site, longitudinal, observational study of Huntington's disease, aimed at identifying biological and refined clinical markers of early disease in subjects who have tested as gene-expanded for the Huntington's disease mutation but are not yet clinically diagnosed as having the condition, and are thus referred to as having prodromal Huntington's disease. The current analysis is of a simple and basic biological measure from this study—measure of intracranial volume—that could test the hypothesis that abnormal development is a component of Huntington's disease pathology. The volume of the brain cavity [all tissues within the cranium including cerebrospinal fluid (CSF), or intracranial volume] is a proxy measure of maximum brain growth that peaks early in life (around adolescence) and then does not change after that. If intracranial volume is smaller in subjects who are gene-expanded compared with non-gene expanded subjects, this would lend support to the theory that abnormal brain growth may be a component of the aetiology of Huntington's disease.

Materials and methods

All subjects were from the PREDICT-HD study. Participants were subjects who are at risk for Huntington's disease (have a parent with Huntington's disease) and who had previously undergone elective presymptomatic genetic testing. Those that are found to be gene-expanded (CAG repeat length ≥36) but have not yet manifested the disease are referred to as subjects with prodromal Huntington's

disease. Those that are found to be non-gene expanded (CAG ≤30) are enrolled as comparison subjects.

Participants were recruited from 32 sites across the USA, Canada, Europe and Australia and underwent annual study visits consisting of a neurological motor examination, cognitive assessment, brain MRI (biennial), psychiatric and functional assessment, with blood samples for genetic and biochemical analyses. The data reported are based on images obtained from the beginning of the study to December 2008.

The current sample included 707 scans on 707 subjects (537 gene-expanded and 170 non-gene expanded; 464 female, 243 male) from 52 scanners at 32 sites. All aspects of the study were approved by the Institutional Review Board at each participating institution and are in compliance with the Declaration of Helsinki. Participants underwent informed consent procedures and signed consents for both participation and to allow de-identified research data to be sent to collaborative institutions for analysis.

Magnetic resonance imaging measures

All scans for this project were obtained using a standard multi-mode protocol that included an axial 3D volumetric spoiled gradient echo series ($\sim 1 \times 1 \times 1.5$ mm voxels) and a dual echo PDT2 ($\sim 1 \times 1 \times 3$ mm voxels) series. These two sequences were combined to obtain intracranial volume measures as described below. All sites used a General Electric 1.5 T scanner (with the exception of two sites using a 1.5 T Siemens scanner).

To measure intracranial volume, first an approximate rough brain tissue region was obtained using the 3D skull from the 'analysis of functional neuroimages' tool suite (Cox, 1996). Spatial intensity inhomogeneities correction fields were estimated over the approximated brain tissue region and applied using tools described in Styner *et al*. (2000) for each modality. An automated procedure rigidly aligned and re-sampled the three modes of each data set into a 1 mm^3 isotropic voxel lattice where a line passing through the anterior commissure and posterior commissure is parallel to the horizontal voxel lattice, the inter-hemispheric fissure is aligned with vertical voxel lattice and the anterior commissural point is located at the centre of the voxel lattice.

Tissue classification (Harris *et al.*, 1999) was performed using the 'brain research: analysis of images, networks and systems' tool (Magnotta *et al.*, 2002). Exemplars ($2 \times 2 \times 2$ mm plugs) for grey matter, white matter and CSF are selected by randomly sampling the images and keeping those plugs with low variance under the assumption that they represent a single tissue type. The selected plugs are then assigned to a compartment using *k*-means clustering. The labelled plugs are then used to define discriminant functions. The discriminant functions are then used to classify the multimodal data, producing an image where each voxel location is labelled with a code representing the grey, white and CSF composition. The final brain region is defined from the classified image using the automated neural network segmentation (Magnotta *et al.*, 2003) tool from the 'brain research: analysis of images, networks and systems' package. This brain region, defined as intracranial volume, is a measure of all tissue (grey and white matter) and CSF within the cranium and below the dura mater. The dura matter and blood vessels lining the inside of the skull are excluded from this measure.

The results of this procedure were visually inspected to verify that each stage was completed successfully. Greater than 90% of the scans analysed passed all stages successfully. The most common reasons for failure were poor co-registration of the multiple modes, erroneous inclusion of eye musculature near the temporal lobe when there was little CSF separation and erroneous inclusion of fat deposits near the cerebellum when there was little CSF separation. Scan failure was not

significantly predicted by any of the variables, including sex and Huntington's disease gene-expansion status that are the subject of this report.

Intracranial volume measure validation

A recent report has shown that some imaging measures of intracranial volume can be influenced by brain atrophy (Pengas et al., 2009). To validate our methods, we first completed a study in which 20 brain scans were manually traced and compared with the automated measures. These 20 brain scans were obtained from a database of subjects with schizophrenia and used a comparable, multimodal sequence on a GE 1.5 T Scanner. The raters manually traced intracranial volume using the dura mater as the defining limit of the measure. These same 20 scans were then processed using the automated method described above and the two measures compared. The average relative overlap (intersection/union) was 0.98 between the two methods.

Another way to investigate whether brain atrophy may potentially bias the intracranial volume measure is to correlate intracranial volume with estimated probability of onset. As age of onset is significantly correlated with CAG repeat length, it is possible to calculate an estimate of the probability of onset within the next 5 years (Langbehn et al., 2010). Those who are close to onset have significant brain tissue loss or brain atrophy compared with those that have a very low probability of onset within the next 5 years (i.e. those that are far from disease onset). If the measure of intracranial volume is confounded by degree of brain atrophy, then there should be an inverse correlation between intracranial volume and 5-year probability of onset. However, we found no significant relationship between intracranial volume and 5-year probability of onset ($r = -0.000$, $P = 0.98$). In addition, in our analysis on the reliability of the intracranial volume measure, we found the intra-subject correlation over time to be very high (0.977), again confirming that this measure does not change over time.

Statistical methods

Preliminary summary statistics were compared using t-tests with a check for variance equality. Given the known difference between males and females in intracranial volume (Nopoulos et al., 2000), the analyses included tests for different disease effects in the two sexes. Outlying observations were initially identified by scatterplots (e.g. height versus intracranial volume), and one subject whose very short height could not be verified was removed from analysis. Intracranial volume was treated as the outcome measure, with fixed predictor variables defined as CAG-expansion status (case versus control), sex, height and age. We expected sex and height to have a substantial effect on intracranial volume, which was verified. Age, which we would not expect to have an effect, was included as a precaution and check regarding the assumed approximate constant value of mature intracranial volume throughout the lifespan. Subject ID and MRI scanner ID were treated as random variables, with compound symmetric covariance assumed across repeated measures for both. Estimation and inference was via the residual (restricted) maximum likelihood method, and the Satterthwaite approximation was used in estimating degrees of freedom for all F-tests (Brown and Prescott, 1999).

The relationship between intracranial volume and CAG length was tested (using the CAG-expanded subjects only), employing a mixed linear model with intracranial volume as the outcome measure, and with fixed predictor variables defined as CAG length, sex, height and age. All calculations were performed in SAS, version 9.1.3 (SAS Institute, 2003).

Results

To determine reliability of the intracranial volume, we examined the stability of repeated intracranial volume measures in a subset of subjects who had more than one brain scan available (77 had two scans, 13 had three scans and one subject had four scans available). This was possible as this is a longitudinal study, although only the intake scan for each subject was used for the final analysis of intracranial volume (a total of 707 individuals with intake scans). After adjustment for height, the reliability (within-subject repeated measure correlation) of intracranial volume measurement was estimated to be 0.977. Of the 2.3% 'error' variance, we estimated that 1.8% was due to inter-scanner variability and 0.5% due to intra-scanner variability (including variation in post-scan analysis technique).

Table 1 displays the demographic information and summary statistics for both males and females in each group. The two male groups (prodromal Huntington's disease versus controls) did not differ in height or age. Preliminary comparison of the un-adjusted intracranial volume means using t-test shows the males with prodromal Huntington's disease to have significantly smaller intracranial volumes compared with the male control group ($t = 3.28$, degrees of freedom $= 241$, $P = 0.001$). This is a modest effect size with a Cohen's d of 0.492. For the females, the prodromal Huntington's disease group was significantly older (by ~2 years) than the female control group. Although female subjects with prodromal Huntington's disease had smaller intracranial volumes compared with the control females, this was not statistically significant (Cohen's d: 0.157).

Table 2 shows the results of the mixed linear model analysis in which intracranial volume was treated as an outcome measure. As expected, age had no significant effect on intracranial volume [$F(1,701) = 2$, $P = 0.1580$] while height and sex both had substantial effect [height: $F(1,698) = 27.71$, $P < 0.0001$; sex: $F(1,687) = 171.02$, $P < 0.0001$]. There was a significant group effect on the volume of intracranial volume [$F(1,687) = 13.14$, $P = 0.0003$]; so as a whole (males and females combined), the prodromal Huntington's disease group had smaller intracranial volume compared with controls. In addition, there was a significant sex by diagnostic interaction [$F(1,687) = 5.59$, $P = 0.0183$]. As indicated by the adjusted intracranial volume means displayed in Table 1, males with prodromal Huntington's disease have significantly smaller intracranial volumes compared with male controls ($t = 3.71$, $P = 0.0002$). The mean (unadjusted) volume difference between the males with prodromal Huntington's disease and the control males is 58 cc, which constitutes a difference of 4% in intracranial volume. There were no significant differences in intracranial volume between the female groups.

To ensure that the findings of low intracranial volume in prodromal Huntington's disease is not driven by outliers (potentially those with higher CAG repeats), we conducted two *post hoc* analyses. The first, conducted on the entire sample, looked at the relationship between CAG repeat and intracranial volume. This analysis revealed no main effect of CAG length on intracranial volume [$F(1,529) = 0.002$, $P = 0.963$] and no CAG length by sex interaction [$F(1,529) = 0.51$, $P = 0.475$]. In addition, we repeated

Table 1 Demographic information and summary statistics

Variable	Males CAG expanded prodromal Huntington's disease, $n = 185$ Mean (SD) range	CAG non-expanded $n = 58$ Mean (SD) range	t	P	Females CAG expanded prodromal Huntington's disease, $n = 352$ Mean (SD) range	CAG non-expanded $n = 112$ Mean (SD) range	t	P
CAG Repeat	42.1 (2.2) 38–52	20.1 (4.0) 16–34	n/a	n/a	42.4 (2.5) 38–61	20 (2.9) 15–29	n/a	n/a
Intracranial volume (cc) unadjusted	1444 (120) 1171–1754	1502 (111) 1253–1794	3.28	0.001	1262 (95) 1056–1610	1277 (98) 1052–1546	1.38	0.17
Intracranial volume (cc) adjusted[a]	1415	1472	3.71	0.0002	1276	1289	1.14	0.25
Age (years)	43.4 (10.5) 21.8–77.8	44.6 (13.4) 19.1–83.7	0.65	0.52	41.9 (10.0) 20.1–69.8	44.3 (10.9) 19.5–68.2	2.11	0.04
Height (cm)	178.8 (7.3) 145–201	179.0 (6.2) 169–193	0.24	0.81	164.3 (6.6) 147–183	164.5 (7.0) 139–179	0.23	0.82

a Adjusted for height using mixed linear model.
n/a: Not applicable.

Table 2 Results of mixed linear model evaluating intracranial volume as outcome measure

Effect	F	Degrees of freedom (numerator, denominator)	P-value
Age	2.00	1, 701	0.158
Height	27.71	1, 698	<0.0001
Sex	171.02	1, 687	<0.0001
Group	13.14	1, 695	0.0003
Group by sex	5.59	1, 687	0.0183

the main analysis on a subset of the gene-expanded subjects, restricted to those with CAG repeats ≤ 49. This removed a total of seven gene-expanded subjects (two males and five females). The results of the analysis remained the same in which there was a significant effect of group on intracranial volume ($F = 13.52$, $P < 0.0001$) and a significant sex by group interaction ($F = 5.32$, $P = 0.021$). Gene-expanded males had significantly lower intracranial volumes compared with non-gene expanded males.

Discussion

The current study found that intracranial volume, after controlling for body size (height), is significantly smaller in males with prodromal Huntington's disease compared with control males. Intracranial volume is a measure of the total tissue and CSF volume within the calvarium and is a representation of the maximal brain growth obtained during development and maturation. Peak intracranial volume is reached early in life, such that, by the age of 6 years, total brain volume is 90% of adult volume and reaches full adult volume during adolescence (Giedd, 2004). Although head circumference increases throughout childhood and adolescence, this is a gain in skull thickness rather than internal calvarium volume (Giedd, 2004). Once intracranial volume peaks and the skull sutures are completely fused, there is no

further change in this measure, regardless of changes that may occur in brain tissue (Morriss-Kay and Wilkie, 2005). For example, in a neurodegenerative process, intracranial volume would remain the same over time while brain tissue volume would decrease, replaced in compensation by increased volume of CSF (Bradley et al., 1986). This is an important concept to highlight, as abnormal brain structure has been documented in subjects with prodromal Huntington's disease decades prior to the onset of disease (Paulsen et al., 2010). In general, changes in tissue structure (volume) seen years prior to onset of disease have often been conceptualized as representing a very slow degenerative process. However, in the case of the current study, a difference in intracranial volume between two groups is unlikely to be caused by degenerative process as intracranial volume, once determined by maximal brain growth in childhood, does not change over time. Instead, an alternative explanation for this finding is that, in the subjects with prodromal Huntington's disease, the brain did not grow to its full capacity, raising the possibility of a global abnormality in the process of brain development.

The currently favoured hypothesis in the aetiology of Huntington's disease is that the elongated polyglutamine sequence of the huntingtin protein results in a toxic gain of function. However, there is also emerging evidence that in addition to this mechanism, loss of function of normal huntingtin may also be an important mechanism in the disease (Cattaneo et al., 2001, 2005). Huntingtin is expressed in the brain throughout development (Zeitlin et al., 1995; Bhide et al., 1996) and may play a vital role in neuronal survival and stability (Rigamonti et al., 2000). Studies of mouse models with inactivation of the Huntington's disease gene have shown that complete lack of huntingtin results in developmental arrest during gastrulation, while reduction of huntingtin levels results in abnormal brain development (White et al., 1997), manifest in cognitive and motor abnormalities (Nasir et al., 1995). Therefore, given huntingtin's potential key role in development, a partial loss of function of this protein may manifest in abnormal neural development.

Multiple studies have shown that subjects with prodromal Huntington's disease have subtle but significant abnormalities in

cognitive (Lemiere et al., 2004; Solomon et al., 2007), behavioural (Berrios et al., 2001; Duff et al., 2007) and motor function (Kirkwood et al., 2000; Hinton et al., 2007) long before a clinical diagnosis is given. Although some have interpreted these findings to indicate early degeneration, another interpretation is that they represent manifestations of subtle abnormal development, or a combination of both neurodevelopmental and neurodegenerative effects. Subtle forms of cognitive, psychiatric and even motor deficits presenting in childhood, then manifesting later during the prodrome and early course of a brain disease is a model very well studied in schizophrenia, another neuropsychiatric disorder with aetiology in abnormal brain development (Waddington and Buckley, 1996).

In addition to these studies in Huntington's disease, work in the field of molecular biology has supported the theory of 'neurodevelopmental mechanisms of degeneration' in other neurodegenerative diseases. For example, Alzheimer's disease may represent a novel class of developmental disorders in which subsets of neural populations are vulnerable due to abnormal development and exist in a mutant steady state before succumbing to environmental stressors that normally would not promote cell death (Mehler and Gokhan, 2000). A study looking at the transgenic mouse model of spinocerebellar ataxia Type 1 (like Huntington's disease, another genetic, polyglutamine neurodegenerative disorder) showed that abnormal neurodevelopment plays a crucial role in the evolution of the neural degeneration (Serra et al., 2006). More importantly, a recent study of a knock-in mouse model of Huntington's disease showed clear abnormalities in striatal cell development and maturation (Molero et al., 2009).

In the current study, intracranial volume was found to be substantially smaller in the prodromal Huntington's disease group (both males and females combined) compared with controls. However the significant sex by group interaction showed that this finding was mostly driven by the male prodromal Huntington's disease group (although the female prodromal Huntington's disease group had smaller intracranial volume, this did not reach statistical significance in comparison with controls). A common feature of neurodevelopmental disorders is a sex-specific pattern of prevalence and severity in which males are affected more frequently and more severely than females. Examples include autism, attention-deficit disorder, dyslexia and developmental reading disorders (American Psychiatric Association, 1994). This vulnerability to developmental aberration may be related to the differential role of oestrogens in brain development (McCarthy, 2008). Huntington's disease has equal sex prevalence. However, even in neurodevelopmental disorders with relatively equal sex prevalence, such as schizophrenia, the male brain shows more structural and functional abnormality compared with females having the same disease (Nopoulos et al., 1997). Thus, given the hypothesis that low intracranial volumes in Huntington's disease are due to abnormal brain development, the sex findings of the current study are not unexpected. Moreover, the sex effect is considered to be one of a difference in severity rather than a difference in pattern of brain abnormalities. That is, one would expect the female brain to be affected by Huntington's disease in the same manner as it affects male brains. Thus, given a particular abnormality of brain structure in the context of Huntington's disease (smaller-than-normal intracranial volume), one would expect males and females to both display the same abnormality, but for that abnormality to be more severe in males with Huntington's disease.

There is a paucity of studies documenting sex differences in the phenotypic expression of Huntington's disease. Two studies have found that females, compared with males, have a later age of onset (Roos et al., 1991) and a longer disease course (Foroud et al., 1999). However, in animal models of Huntington's disease, several recent studies have documented that males have a more severe phenotype of Huntington's disease compared with females (Dorner et al., 2007; Bode et al., 2008; Orr et al., 2008). Given the current study's findings and the recent reports in animal models, further research should focus on exploring possible sex-dependent features of Huntington's disease.

In conclusion, the current finding of lower intracranial volume in subjects with prodromal Huntington's disease compared with controls lends support to the theory that abnormal brain development may be a precursor to neurodegeneration in Huntington's disease.

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Supplementary material

Supplementary material is available at Brain online.

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