Seeking Huntington Disease Biomarkers by Multimodal, Cross-Sectional Basal Ganglia Imaging

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Abstract: Neurodegeneration of the striatum in Huntington disease (HD) is characterized by loss of medium-spiny neurons, huntingtin nuclear inclusions, reactive gliosis, and iron accumulation. Neuroimaging allows in vivo detection of the macro- and micro-structural changes that occur from presymptomatic stages of the disease (preHD). The aim of our study was to evaluate the reliability of multimodal imaging as an in vivo biomarker of vulnerability and development of the disease and to characterize macro- and micro-structural changes in subcortical nuclei in HD. Macrostructure (T_1 -weighted images), microstructure (diffusion tensor imaging), and iron content (R_2^* relaxometry) of subcortical nuclei and medial temporal lobe structures were evaluated by a 3 T scanner in 17 preHD carriers, 12 early-stage patients and 29 matched controls. We observed a volume reduction and micro-structural changes in the basal ganglia (caudate, putamen, and globus pallidus) and iron accumulation in the globus pallidus in both preHD and symptomatic subjects; all these features were significantly more pronounced in patients, in whom degeneration extended to the other subcortical nuclei (i.e., thalamus and accumbens). Mean diffusivity (MD) was the most powerful predictor in models explaining more than 50% of the variability in HD development in the caudate, putamen, and thalamus. These findings suggest that the measurement of MD may further enhance the well-known predictive value of striatal volume to assess disease progression as it is highly sensitive to tissue micro-impairment. Multimodal imaging may detect brain changes even in preHD stages. *Hum Brain Mapp* 34:1625–1635, 2013. © 2012 Wiley Periodicals, Inc.

Key words: MRI; volume; mean diffusivity; anisotropy; iron; Huntington disease

Contract grant sponsor: Italian Association of HD Families “Associazione-Italiana-Corea-di-Huntington-Neuromed”; Contract grant number: 5x1000; Contract grant sponsor: IRCCS Neuromed; Contract grant number: 5x1000

Contract grant sponsors: Italian Ministry of Health (Ricerca Corrente); Italian Olympic Committee (CONI); European Huntington's Disease Network for the REGISTRY Study.

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Received for publication 26 May 2011; Revised 8 November 2011; Accepted 21 November 2011

DOI: 10.1002/hbm.22019

Published online 22 February 2012 in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

Huntington disease (HD) is an autosomal dominant disease characterized by a CAG repeat mutation that encodes for a polyglutamine stretch in the huntingtin (htt) protein. Mutated htt causes progressive neurodegeneration, which in turn results in a sequence of motor symptoms and behavioral and cognitive deficits leading to dementia [Walker, 2007].

There are as yet no effective therapies to stop this disease. However, because the disease is inherited, a genetic test allows the preclinical diagnosis to be made many years before the onset of clinical manifestations [Walker, 2007]. This preclinical phase offers a therapeutic window to investigate preventive treatments aimed at delaying its clinical onset. Finding markers of brain changes before HD begins, i.e., presymptomatic stages of disease (preHD) may help to assess preventive treatments and to monitor disease progression.

Neuropathologically, the striatum degenerates in the early stages of disease, exhibiting a progressive loss of neurons, nuclear htt inclusions, iron accumulation, reactive astrogliosis, microgliosis, and an increase in oligodendrocytes [Gomez-Tortosa et al., 2001; Vonsattel, 2008]. Thus, biomarkers that are sensitive to these various features of HD pathology are needed in clinical practice.

Neuroimaging reveals and quantifies in vivo the pattern of brain changes that occur in HD. Multimodal imaging is sensitive to a wide range of tissue variations that accompany the aging process [Cherubini et al., 2009] or neurodegeneration [Di Paola et al., 2010; Péran et al., 2010]. Various methods can be adopted to enhance imaging sensitivity: high-resolution T_1 -weighted images document volume decrease; diffusion tensor imaging (DTI) is sensitive to tissue microarchitecture and early alterations at the cellular and molecular levels [Basser and Pierpaoli, 1996]; MR-based relaxometry (R_2^*) is sensitive to iron/ferritin deposits in the basal ganglia [Cherubini et al., 2009; Péran et al., 2009; Langkammer et al., 2010]. In HD, neuroimaging studies have documented volumetric and microstructural changes in subcortical nuclei from the preHD stages [Aylward et al., 2000, 2011; Hobbs et al., 2009; Jurgens et al., 2008; Paulsen et al., 2006a; Rosas et al., 2006; Tabrizi et al., 2009, 2011] and iron deposition in the basal ganglia of patients [Barzokis et al., 1999, 2007; Vymazal et al., 2007]. However, subcortical neurodegeneration in HD has not previously been assessed by means of all these magnetic resonance imaging (MRI) parameters contemporaneously. High-resolution T_1 -weighted imaging, which quantifies the decrease in volume, has so far been considered to be the most suitable and sensitive MRI technique for the cross-sectional and longitudinal study of early HD. However, in view of the clinical heterogeneity of the disease and the variability of the patients' age at onset, a multimodal approach may offer a novel means of detecting simultaneous neurobiological changes that occur in the brain even in the preHD stages of the disease.

To identify MRI markers of the various neurobiological changes that occur in subcortical nuclei from the preHD stages, we assessed preHD subjects, early-stage patients, and healthy controls by means of T_1 -weighted images, DTI, and R_2^* relaxometry.

MATERIALS AND METHODS

Subjects

Thirty-four subjects with abnormal CAG repeats (≥ 40) were enrolled in the study. Because five of them were discarded for artifacts in the MRI, the final sample was composed of 29 subjects. All the subjects were clinically examined by the same neurologist (F.S.), who is an expert in HD, using the unified Huntington's disease rating scale (UHDRS) motor, cognitive, behavioral, and functional subscales [Huntington Study Group, 1996] and the minimal state examination (MMSE) for general cognitive assessment [Folstein et al., 1975]. The cohort was then grouped into 17 preHD and 12 symptomatic subjects. PreHD subjects included asymptomatic individuals (total motor score of ≤ 5 in the UHDRS and cognitive and behavioral assessment within the normality) and individuals with soft signs (suspicious clinical features that were insufficient to warrant a diagnosis of HD) [Paulsen et al., 2001]. The patients' age at onset was retrospectively established by interviews to family members regarding the first neurological manifestations [Squitieri et al., 2003], thus excluding subjects showing severe cognitive/psychiatric abnormalities that represented a permanent change from the normal state [Brinkman et al., 1997]. The predicted years to manifest disease were calculated on the basis of the survival analysis formula described by Langbehn et al. [2004]. Furthermore, in an attempt to estimate the progression of the pathological process from the preHD stage, we calculated an "HD development" index by combining the predicted years to onset for preHD subjects and disease duration (years from onset) for patients. The disease burden index was measured according to the previously described formula: age $\times$ (CAG-35.5) [Penney et al., 1997]. Twenty-nine, one-by-one age- and gender-matched healthy subjects were recruited as controls. Patients were excluded if they were in the advanced stages of disease (Stages III and IV) or had traumatic brain injury or MRI focal lesions. The demographic and clinical characteristics of the sample are shown in Table I. Our local ethics committee approved the study and written informed consent was obtained from all participants.

Brain Imaging

All the participants were examined using a 3 T Allegra scanner with a standard quadrature (Siemens Medical Solutions). The MRI protocol included whole-brain T_1 -weighted, T_2^* -weighted, DTI, conventional T_2 -weighted and fluid-attenuated inversion recovery scanning. All planar sequence acquisitions were acquired along the

TABLE I. Demographic and clinical characteristics of the sample

	preHD (<i>n</i> = 17)	preHD control (<i>n</i> = 17)	HD (<i>n</i> = 12)	HD control (<i>n</i> = 12)	X^2/T	<i>P</i> -value
Gender (M:F)	12:5	12:5	6:6	6:6	2.5	NS*
Age	39 (7.2)	39 (7.3)	50 (11.1)	50.4 (11.3)	3.2	0.012 ^{a,i} ; 0.01 ^{b,i}
CAG repeat length	44.1 (1.7)		45 (3.3)		0.8	NS [†]
Disease duration (years)	NA		4.5 (3.5)		NA	NA
MMSE	28.1 (1.7)		24.1 (3.7)		−3.7	<0.0001 ^{c,i}
UHDRS motor	13.71 (23)		38.5 (14.6)		3.2	0.003 ^{a,i}
UHDRS cognitive	254.06 (45.5)		132.8 (53)		−6.5	<0.0001 ^{c,i}
UHDRS behavioral	6.75 (6.3)		20.3 (7.8)		5.0	<0.0001 ^{a,i}
UHDRS functional	25 (0)		18.8 (3.3)		−7.7	<0.0001 ^{c,i}
TFC	13 (0)		8.5 (1.3)		−14.2	<0.0001 ^{c,i}
Independence scale	99.7 (1.2)		80 (7.1)		−11.3	<0.0001 ^{c,i}
Disease burden	331.8 (54)		444.5 (72.9)		4.7	<0.0001 ^{a,i}

Values expressed as mean (S.D.) with the exception of gender.

Abbreviations: TFC, total functional capacity; MMSE, mini-mental state examination corrected for age and education (normal value above 24); NA, not applicable; NS, not significant; preHD, presymptomatic Huntington's disease; HD, Huntington's disease.

^apreHD < HD, higher punctuations mean greater impairment.

^bcontrol preHD < control HD.

^cpreHD > HD.

*Pearson's Chi-square.

[†]*t*-student. Bonferroni correction.

AC/PC line. Six consecutive T_2^* -weighted gradient-echo whole-brain volumes were acquired using a segmented echo-planar imaging sequence at different TEs: 6, 12, 20, 30, 45, and 60 ms (TR = 5,000; bandwidth = 1,116 Hz/voxel; matrix size 128×128 ; 80 axial slices; flip angle 90° ; voxel size of $1.8 \times 1.8 \times 1.8 \text{ mm}^3$). Diffusion-weighted volumes were acquired using spin-echo echo-planar imaging (echo time (TE)/repetition time (TR) = 89/8,500 ms, bandwidth = 2,126 Hz/voxel; matrix size 128×128 ; 80 axial slices, voxel size $1.8 \times 1.8 \times 1.8 \text{ mm}^3$) with 30 isotropically distributed orientations for the diffusion sensitizing gradients at a *b*-value of 1,000 s·mm² and 6 *b* = 0 images. Scanning was repeated three times to increase the signal-to-noise ratio. Because DTI and T_2^* volumes both consisted of $128 \times 128 \times 80$ identical isotropic voxels, the slice positioning and orientation of the diffusion-weighted volumes were set to be identical to the T_2^* volumes to improve subsequent coregistration. Lastly, whole-brain T_1 -weighted images were obtained on the sagittal plane using a modified driven equilibrium Fourier transform sequence (TE/TR = 2.4/7.92 ms, flip angle 15° , voxel size $1 \times 1 \times 1 \text{ mm}^3$). The postprocessing steps were performed using FSL 4.1 the Oxford Center's Functional MRI of the Brain Software Library [FSL 4.1] (www.fmrib.ox.ac.uk/fsl/) and in-house developed software in Matlab (version 6.5, the MathWorks), according to previously published methods [Cherubini et al., 2009; Péran et al., 2009, 2010].

Anatomical regions of interest (ROI) on T_1 -weighted images were obtained automatically with the segmentation tool FIRST 1.1 integrated within the FSL software, following the methodology of previous studies [Cherubini et al., 2009; Péran et al., 2009, 2010]. Five subcortical nuclei (caudate, putamen, globus pallidus, thalamus, and accumbens) and

two medial temporal lobe (MTL) structures (hippocampus and amygdala) were segmented in each subject. The mean values for the volume, mean diffusivity (MD), fractional anisotropy (FA), and R_2^* were obtained for each nucleus. The ROI analysis was performed by an automated procedure, and an expert rater (C.S.C.), who was blinded to group allocation, evaluated the adequacy of the segmentation. Individual volume values were corrected with a multiplicative scaling factor derived from an affine transform calculated with the SIENAX tool within FSL. When computing the affine transform to the template, we used the skull images to constrain scaling and skew. This scaling factor was computed as the determinant of the affine transform connecting each individual to the montreal neurological institute (MNI) standard template [Buckner et al., 2004]. The scaling factor represents the whole-brain volume expansion (or contraction) required to register each individual to the template. Furthermore, we obtained two parameters from the DTI analysis: MD, which reflects the average displacement of water molecules and is affected by cellular size and integrity; and FA, which provides information on the structural integrity of tissue and fiber tracts [Basser and Pierpaoli, 1996]. As there were no right/left hemisphere differences in any of the measured indexes, we calculated an averaged-value for the statistical analyses.

Statistical Analysis

The statistical analyses were conducted using SPSS (11.5, SPSS) and Stata 11.2 (Stata Corp.). Differences between groups were assessed using one-way ANOVA with a post hoc *t*-student contrast and Bonferroni correction. The X^2 test was used for qualitative variables. The effect

of age was controlled by including it as a covariate when comparing the two disease groups. We also tested the relationship between changes observed in the different MRI parameters (volume, MD, FA, and R_2^*) within each subcortical nucleus, considering CAG repeats length as the covariate and evaluating the alpha rejection limit following Bonferroni's correction for multiple tests ($P < 0.05/6 = P < 0.008$). Lastly, multiple regression models were applied to establish the parameters that best predict HD development (time to/from onset) and disease burden. Variance explained by the model (Adjusted R^2) was used as the most important criterion in model building. Predictors were evaluated in z-score transformation to understand the relative strength of explanation of the dependent variable.

RESULTS

Differences in Normalized Volume

Overall, the HD subjects displayed decreased volume in all five subcortical nuclei (caudate, putamen, globus pallidus, thalamus, and accumbens; $P < 0.0001$) (Table II). When analyzed separately, basal ganglia volume in preHD and patient cohorts was lower than in their respective control groups (caudate, putamen, and globus pallidus, $P < 0.0001$; caudate, putamen, $P < 0.0001$; and globus pallidus, $P < 0.002$; caudate, putamen, and globus pallidus, $P < 0.0001$, respectively). Patients displayed significant volume decrease, which extended to the thalamus and accumbens, when compared with their age-matched controls (thalamus, $P < 0.0001$; accumbens, $P < 0.002$) as well as with preHD subjects (thalamus, $P < 0.0001$; accumbens, $P < 0.017$); (Table III, Fig. 1a). All the HD subjects displayed normal MTL structure volumes.

Differences in Mean Diffusivity

The higher diffusion of water within a nucleus reflects an impairment of its microstructure and integrity. All HD subjects presented increased MD in the caudate, putamen, thalamus, accumbens, and hippocampus ($P < 0.0001$; Table II). When we considered preHD subjects and patients separately, preHD subjects exhibited greater MD in the striatum than controls (caudate, $P = 0.034$; putamen, $P < 0.0001$), whereas patients exhibited greater MD in the striatum, which extended to the thalamus and hippocampus (vs. pre-HD and controls; $P < 0.0001$) as well as to the accumbens (vs. controls; $P = 0.014$; Table III, Fig. 1c).

Differences in Fractional Anisotropy

FA reflects microstructure and fiber tracts organization. When HD subjects were considered as a whole, FA was higher in caudate, putamen, and globus pallidus ($P < 0.0001$), but lower in the thalamus ($P = 0.001$), accumbens ($P = 0.011$), and MTL structures (hippocampus, $P < 0.0001$; amygdala, $P = 0.002$), in comparison with controls (Table II). When analyzed separately, preHD and

TABLE II. Significant differences in brain volume, R_2^* relaxometry, mean diffusivity, and fractional anisotropy between groups

	HD subjects (<i>n</i> = 29)	Control (<i>n</i> = 29)	<i>T</i>	<i>P</i> -value [†]
Volume				
Caudate	3,712 (830)	5,416 (761)	8.14	<0.0001
Putamen	5,435 (985)	7,294 (690)	8.31	<0.0001
Globus pallidus	2,293 (266)	2,665 (233)	6.35	<0.0001
Thalamus	11,276 (930)	12,187 (932)	3.72	<0.0001
Accumbens	622 (132)	751 (179)	4.21	<0.0001
Hippocampus	5,846 (679)	5,982 (790)	0.48	NS
Amygdala	1,934 (312)	1,937 (294)	0.03	NS
MD				
Caudate	1,154 (151)	988 (80)	-5.22	<0.0001
Putamen	828 (58)	470 (26)	-8.43	<0.0001
Globus pallidus	702 (44)	702 (27)	0.02	NS
Thalamus	1,007 (89)	914 (55)	-4.77	<0.0001
Accumbens	822 (87)	756 (42)	-3.64	0.001
Hippocampus	1,036 (47)	988 (41)	-5.15	<0.0001
Amygdala	1,016 (85)	979 (80)	-1.70	NS
FA				
Caudate	2,398 (235)	2,141 (147)	-4.97	<0.0001
Putamen	2,858 (241)	2,466 (100)	-8.07	<0.0001
Globus pallidus	5,074 (593)	4,190 (343)	-6.94	<0.0001
Thalamus	2,931 (225)	3,133 (195)	3.64	0.001
Accumbens	2,376 (372)	2,632 (368)	2.62	0.011
Hippocampus	1,514 (187)	1,680 (145)	3.77	<0.0001
Amygdala	1,505 (132)	1,618 (135)	3.21	0.002
R_2^*				
Caudate	4,435 (408)	4,184 (312)	-2.62	0.011
Putamen	5,385 (610)	4,959 (435)	-3.06	0.003
Globus pallidus	7,615 (525)	6,840 (415)	-6.22	<0.0001
Thalamus	4,262 (190)	4,205 (292)	-1.15	NS
Accumbens	4,195 (662)	4,117 (448)	-0.53	NS
Hippocampus	4,022 (305)	3,961 (353)	-0.69	NS
Amygdala	4,412 (658)	4,163 (558)	-1.55	NS

Values expressed as mean (SD).

Abbreviations: MD, mean diffusivity; FA, fractional anisotropy; NS, not significant.

[†]t-Student.

HD subjects displayed a selectively increased FA in basal ganglia compared with their respective age-matched controls (preHD: caudate, $P = 0.004$; putamen, $P < 0.0001$; globus pallidus, $P = 0.001$; HD: caudate, $P = 0.005$; putamen, $P < 0.0001$; globus pallidus, $P < 0.0001$). FA values were higher in patients than in pre-HD subjects in the putamen ($P = 0.001$), but lower in the thalamus ($P < 0.0001$) and hippocampus ($P = 0.001$). Moreover, FA in the thalamus and in both MTL structures was lower in patients than in controls (thalamus, hippocampus, $P < 0.0001$; amygdala, $P < 0.009$; Table III, Fig. 1d).

Differences in Mean Relaxation Time (R_2^*)

R_2^* is an indirect measure of ferritin/iron deposition in the brain. All HD subjects displayed a significant increase

TABLE III. Significant differences in brain volume, R_2^* relaxometry, mean diffusivity, and fractional anisotropy between groups (separating preHD and HD)

						P-value [†]		
	preHD (<i>n</i> = 17)	preHD control (<i>n</i> = 17)	HD (<i>n</i> = 12)	HD control (<i>n</i> = 12)	<i>F</i>	Pre versus CNT	HD versus CNT	Pre-HD versus HD Age as covariate
Volume								
Caudate	4,039 (774)	5,502 (854)	3,247 (695)	5,294 (622)	27.33	<0.0001	<0.0001	0.05
Putamen	5,865 (822)	7,354 (795)	4,826 (893)	7,209 (528)	31.7	<0.0001	<0.0001	0.010
Globus Pallidus	2,293 (266)	2,665 (233)	1,851 (311)	2,597 (315)	23.44	0.002	<0.0001	0.001
Thalamus	11,829 (589)	12,401 (870)	10,494 (747)	11,883 (968)	13.94	NS	<0.0001	<0.0001
Accumbens	622 (132)	751 (179)	463 (112)	679 (92)	10.7	NS	0.002	0.017
Hippocampus	5,971 (386)	6,008 (762)	5,668 (948)	5,946 (861)	0.57	NS	NS	NS
Amygdala	1,943 (263)	1,983 (248)	1,920 (384)	1,871 (350)	0.32	NS	NS	NS
MD								
Caudate	1,069 (111)	972 (71)	1,274 (115)	1,010 (89)	24.78	0.034	<0.0001	<0.0001
Putamen	828 (58)	470 (26)	917 (60)	747 (26)	42.42	<0.0001	<0.0001	<0.0001
Globus Pallidus	694 (44)	699 (27)	712 (43)	706 (29)	0.67	NS	NS	NS
Thalamus	949 (60)	906 (51)	1,089 (49)	926 (62)	28.19	NS	<0.0001	<0.0001
Accumbens	802 (75)	752 (43)	852 (99)	763 (40)	5.9	NS	0.014	NS
Hippocampus	1,036 (47)	988 (41)	1,141 (62)	1,011 (39)	25.78	0.003	<0.0001	<0.0001
Amygdala	981 (60)	987 (82)	1,062 (93)	969 (79)	4.02	NS	0.004	0.015
FA								
Caudate	2,353 (168)	2,114 (147)	2,460 (303)	2,180 (144)	9.34	0.004	0.005	NS
Putamen	2,749 (207)	2,472 (93)	3,011 (204)	2,458 (113)	34.13	<0.0001	<0.0001	0.001
Globus Pallidus	4,919 (633)	4,288 (344)	5,293 (472)	4,052 (301)	19.39	0.001	<0.0001	NS
Thalamus	3,065 (126)	3,123 (212)	2,741 (195)	3,146 (177)	13.74	NS	<0.0001	<0.0001
Accumbens	2,489 (319)	2,694 (333)	2,217 (397)	2,544 (412)	4.17	NS	NS	NS
Hippocampus	1,622 (141)	1,728 (107)	1,362 (130)	1,613 (168)	17.26	NS	<0.0001	0.001
Amygdala	1,553 (110)	1,620 (151)	1,436 (133)	1,614 (113)	5.60	NS	0.009	NS
<i>R</i> ₂ [*]								
Caudate	4,409 (339)	4,170 (274)	4,472 (504)	4,205 (371)	2.31	NS	NS	NS
Putamen	5,280 (487)	4,953 (419)	5,534 (749)	4,967 (475)	3.64	NS	NS	NS
Globus pallidus	7,631 (615)	6,815 (377)	7,591 (390)	6,876 (480)	12.52	<0.0001	0.004	NS
Thalamus	4,262 (190)	4,205 (292)	4,162 (210)	4,078 (131)	1.71	NS	NS	NS
Accumbens	3,949 (297)	4,141 (472)	4,545 (872)	4,082 (429)	3.06	NS	NS	0.042
Hippocampus	4,045 (295)	4,068 (392)	3,989 (328)	3,810 (224)	1.74	NS	NS	NS
Amygdala	4,282 (616)	4,321 (641)	4,598 (698)	3,939 (317)	2.47	NS	NS	NS

Values expressed as mean (SD).

Abbreviations: CNT, control subjects; preHD, presymptomatic Huntington's disease; HD, Huntington's disease; MD, mean diffusivity; FA, fractional anisotropy; NS, not significant.

[†]One-way ANOVA with post hoc *t*-Student contrast. Bonferroni correction.

in R_2^* in the basal ganglia (caudate; $P = 0.011$; putamen, $P = 0.003$; globus pallidus, $P < 0.0001$; Table II). When considered separately, preHD and HD subjects displayed a higher R_2^* in the globus pallidus than controls ($P < 0.0001$ and $P = 0.004$, respectively; Table III, Fig. 1b). Furthermore, patients exhibited a higher R_2^* in the accumbens than preHD subjects ($P = 0.042$).

Correlations Between MRI Parameters Within Subcortical Nuclei

To evaluate the relationship between the four MRI parameters within each subcortical nucleus, we performed linear regression analyses and corrected them for multiple

comparisons (Bonferroni corrected; Table IV). When we considered the effect of the CAG repeat length, we found the following pattern of correlation in the striatum: in both the caudate and putamen nuclei, decreased volume significantly correlated with microstructural impairment, i.e., increased MD ($P < 0.0001$ for both nuclei) and increased FA ($P = 0.004$ and $P < 0.0001$, respectively). A different pattern emerged in the globus pallidus: the decreased volume significantly correlated with microstructural changes in terms of tissue organization (FA; $P < 0.0001$), but not with MD changes. The correlations involving the R_2^* parameter do not survive Bonferroni's correction, but display a trend towards significance. Increased iron accumulation is weakly correlated with decreased MD ($P = 0.04$) in the

Figure 1.

Histogram showing volume (mm^3), R_2^* , MD, and FA mean values from the seven structures considered. *Significant differences between preHD and control preHD; as well as between HD and control HD. †Significant differences between preHD and HD subjects. (A) Volume mean values, (B) R_2^* mean values, (C) MD mean values, and (D) FA mean values.

globus pallidus and with increased FA in the striatum and globus pallidus (caudate, $P = 0.015$; putamen, $P = 0.009$; globus pallidus, $P = 0.03$). In the thalamus, we found a significant correlation between decreased volume and microstructural impairment [increased MD ($P < 0.0001$) and decreased FA ($P = 0.002$)].

Multiple Regression Analysis with HD Development as Predicted Variable

We also performed a multiple regression analysis to evaluate the relative contribution of each of the four MRI

parameters (volume, MD, FA, and R_2^*) in predicting brain changes related to disease development. HD development was entered as predicted value and the four MRI parameters were entered as predictors. More than 50% of HD development variance can be explained by the four predictors' model in the caudate, putamen, and thalamus nuclei, with only the MD coefficient emerging as statistically significant, as well as in the accumbens, with volume and FA coefficients showing statistical significance. Only about 30% of HD development variance is explained by the model in the globus pallidus, where only the volume shows statistical significance (Table V). All the predictors,

TABLE IV. Correlations between MRI parameters within subcortical nuclei, with CAG repeats as covariate (HD subjects, $n = 29$)

		Volume		MD		FA	
		r	P	r	P	r	P
Caudate	MD	−0.855	<0.0001				
	FA	−0.531	0.004	0.446	0.017		
	R_2^*	−0.158	NS	0.267	NS	0.457	0.015
Putamen	MD	−0.845	<0.0001				
	FA	−0.742	<0.0001	0.732	<0.0001		
	R_2^*	−0.144	NS	0.238	NS	0.486	0.009
Globus pallidus	MD	0.166	NS				
	FA	−0.703	<0.0001	−0.481	0.01		
	R_2^*	−0.256	NS	−0.389	0.041	0.408	0.031
Thalamus	MD	−0.697	<0.0001				
	FA	0.564	0.002	−0.811	<0.0001		
	R_2^*	−0.054	NS	0.042	NS	0.067	NS
Accumbens	MD	−0.268	NS				
	FA	0.247	NS	−0.833	<0.0001		
	R_2^*	−0.332	0.08	−0.327	0.09	0.197	NS

Bonferroni corrected alpha level for multiple testing = $P < 0.008$.

which resulted significant in a specific nucleus, were then entered into a stepwise backward multiple regression analysis, with the criteria of removing predictors not showing a significance $P < 0.2$. Caudate MD, thalamus MD, and accumbens FA were retained in a three variable model, where thalamus MD emerged as the strongest predictor (predicting an adjusted increase of 3.96 years from onset for 1 standard deviation variation in thalamus MD, Table VI).

Multiple Regression Analysis with Disease Burden as Predicted Variable

We also tested the relative contribution of each of the four MRI parameters (volume, MD, FA, and R_2^*) in quantifying the brain changes related to disease burden. Disease burden was entered as predicted value, and MRI parameters were entered as predictors. The amount of explained variance resulted marginal in all the nuclei, except in the thalamus, where the 4-predictors model explained 42% of

the variability, but without any variable emerging as statistically significant, perhaps due to the collinearity between MD and FA predictors (i.e., both resulted significant in the respective univariate regression analyses with a similar amount of variance explained, around 41% for both variables).

DISCUSSION

MRI Parameters in HD: Differences Between Groups

This is a preliminary cross-sectional multimodal MRI study designed to analyze the macro- and micro-structure abnormalities of subcortical nuclei by simultaneously combining four MRI parameters in preHD, HD subjects, and healthy controls. This is, to our knowledge, the first time these structures have been investigated using a multimodal MRI approach that is sensitive to the various properties of brain tissue.

TABLE V. Regression coefficients of the multiple linear model (forced entry)

		Nucleus			
	Caudate	Putamen	Globus pallidus	Thalamus	Accumbens
Volume	1.67 ($P = 0.510$)	0.64 ($P = 0.787$)	−4.26 ($P = 0.048$)	−1.74 ($P = 0.246$)	−2.92 ($P = 0.022$)
MD	6.96 ($P = 0.007$)	4.32 ($P = 0.05$)	1.67 ($P = 0.321$)	4.33 ($P = 0.038$)	−2.30 ($P = 0.262$)
FA	0.51 ($P = 0.732$)	2.58 ($P = 0.210$)	1.32 ($P = 0.568$)	−1.43 ($P = 0.431$)	−7.18 ($P = 0.001$)
R_2^*	1.59 ($P = 0.238$)	2.09 ($P = 0.124$)	0.99 ($P = 0.523$)	0.62 ($P = 0.570$)	2.03 ($P = 0.110$)
Adjusted R^2	0.49	0.57	0.29	0.57	0.61

Predicted variable: HD development. Results are presented as z-score coefficients (expected variations in “HD development” for 1 standard deviation variation in the predictor). The values in parenthesis are the P -values associated with each predictor in the model, and values in bold are the coefficients evaluated as significant.

TABLE VI. Regression coefficients of the multiple linear model (stepwise backward) including all the significant predictors on Table V

MRI parameter	Coefficient	Standard Error	<i>t</i>	<i>P</i>	95% CI	
MD caudate	2.086	1.28	1.63	0.117	−0.557	4.729
MD thalamus	3.965	1.27	3.13	0.004	1.355	6.575
FA accumbens	−2.736	1.03	−2.64	0.014	−4.869	−0.602
Adjusted R^2	0.70					

Predicted variable: HD development. Results are presented as z-score coefficients. Bold values are the coefficients evaluated as significant.

Our findings lend further support to the hypothesis that a selective basal ganglia deterioration starts in preHD subjects. Indeed, here, we describe two different MRI patterns: (1) the volume loss associated with microstructural impairment (i.e., increased MD and FA) that characterizes the striatum; and (2) the volume loss in the globus pallidus, associated with iron accumulation and microstructural impairment (increased R_2^* and FA). These macro- and micro-structural changes worsen in subjects with manifest HD features, extending to other subcortical nuclei (thalamus and accumbens).

The volumetric changes that we observed in the striatum and globus pallidus in our preHD subjects, and to a greater extent in HD subjects, have been consistently reported both in cross-sectional and in longitudinal studies, which are considered as the best markers of the disease [Aylward et al., 2000, 2011; Douaud et al., 2006; Kassubek et al., 2004; Mascalchi et al., 2004; Peinemann et al., 2005; Rosas et al., 2003; Ruocco et al., 2008; Tabrizi et al., 2009, 2011; Thieben et al., 2002].

From a microstructural perspective, increased MD in the striatum is present from the preHD stages, thereby reflecting an increase in tissue water. It is not surprising that this increased MD (i.e., reduced tissue integrity) correlates with striatal volume loss. In subjects with manifest disease, the increase in MD was greater in the striatum and even extended to the thalamus, accumbens and hippocampus. One unexpected result was, however, the absence of significant MD changes in the globus pallidus in either of the groups despite volume loss. This last finding may theoretically be ascribable to the remarkable degree of iron deposition within this nucleus, which would consequently reduce the extracellular space. Indeed, we found a correlation between increased iron and decreased MD in the globus pallidus.

These data highlight the sensitivity of MD to microstructural impairment in subcortical nuclei, even in the preHD stages of the disease, and point to the potential role of this parameter as a complementary biomarker of disease-related changes.

Another noteworthy observation concerns the FA, which was higher in preHD subjects, and even higher in patients,

and selectively affected the caudate, putamen, and globus pallidus. As FA is an index of neuronal fiber integrity, this finding suggests that the selective loss of some specific subcortical connections has transformed the striatum into a more organized structure [Douaud et al., 2009]. By contrast, HD patients displayed lower FA values in the thalamus, hippocampus, and amygdala than either preHD or control subjects. Neuropathological HD studies have demonstrated that the striatal loss of neurons is associated with increased astrocyte reactivity, which is in contrast to what happens in other subcortical nuclei [Myers et al., 1991; Vonsattel, 2008]. These data are in agreement with the white matter and neuron axon impairment documented HD [Gutkunst et al., 1999; Ciarmiello et al., 2006]. In keeping with these results, we found a correlation between decreased volume and increased FA in the caudate, putamen, and globus pallidus, even though decreased volume correlated with decrease FA values in the thalamus.

Lastly, we observed increased iron deposition (R_2^* parameter) in the basal ganglia (caudate, putamen, and globus pallidus) when we considered the HD subjects together, although significance was only reached in the globus pallidus when pre-HD and patients were considered separately. Physiologically, iron concentrations in the globus pallidus are higher than those in other parts of the brain, including other basal ganglia [Langkammer et al., 2010]. Both postmortem and MRI studies have described iron accumulation in the basal ganglia in manifest HD [Bartzokis et al., 1999, 2007; Dexter et al., 1992; Simmons et al., 2007; Vymazal et al., 2007]. Here, we report for the first time that iron is abnormally accumulated in the globus pallidus in HD well before the first symptoms appear. Iron deposition in the brain increases normally with age, especially in the caudate, putamen, and globus pallidus [Cherubini et al., 2009; Pfefferbaum et al., 2010], and has been suspected to be a causative factor in several neurodegenerative diseases, possibly because of its involvement in toxic free-radical reactions [Dexter et al., 1992; Gerlach et al., 1994; Hilditch-Maguire et al., 2000; Lumsden et al., 2007; Péran et al., 2009, 2010; Simmons et al., 2007]. This aging effect may explain the increased iron accumulation observed in the basal ganglia in two previous MRI studies that assessed HD patients [Bartzokis et al., 1999, 2007; Vymazal et al., 2007]. However, the relatively young mean age of our preHD sample (i.e., about 39 years), if compared with that of the samples in these two previous studies (i.e., about 50 years) and with a group of one-by-one age matched controls, appears to demonstrate that the increased iron observed in the basal ganglia in HD is independent of aging and starts before the disease becomes manifest. There are several possible explanations for our finding. First, increased iron accumulation in HD striatum may be related to the well-known neuronal loss in this area, as occurs in the *substantia nigra pars compacta* in Parkinson's disease patients [Péran et al., 2010]. The increased iron accumulation in the globus pallidus might thus be a consequence of the deafferentation of the striato-

pallidal pathway [Douaud et al., 2009; Wakai et al., 1993]. A second hypothesis might be that the premature myelin breakdown due to mutant htt affects vesicular axonal transport, thereby causing excessive oligodendrocyte production associated with iron accumulation, following a homeostatic attempt to repair/remyelinate these regions [Bartzokis et al., 2007]. Whatever the cause is, iron accumulation may be severely detrimental to many critical cellular functions as iron is involved in mitochondrial respiration, myelinogenesis, gene expression, and production of neurotransmitters (namely, dopamine or GABA) [Gerlach et al., 1994; Hilditch-Maguire et al., 2000].

Taken together, our findings demonstrate that preHD subjects and HD patients differ insofar as the latter suffer more extensive damage to the striatum and globus pallidus (i.e., decreased volume and microstructural impairment), which further extends to the thalamus and accumbens.

Multiple Linear Regression Analyses

Lastly, in this study, we hypothesized a relationship between subcortical impairment and HD progression as measured by a HD development index and the disease burden score. Therefore, we tested which MRI parameter best explained HD development and disease burden in our HD subjects for each subcortical nucleus. MD is the most powerful predictor in our multivariate models, which globally explained more than 50% of the variability in HD development in the caudate, putamen, and thalamus, and about 40% of the variability in disease burden in the thalamus. On the other hand, volume is the most powerful predictor of HD development in the globus pallidus multivariate model (that explains 34% of the variance), and together with FA, in the accumbens nuclei (in a model explaining 61% of the variance). The variance in these nuclei appears to be explained by the different MRI parameters in a more complex way, which is probably related to the role of iron accumulation. Our results confirm that MD is highly sensitive to tissue disintegration even long before the onset of symptoms in preHD subjects. Moreover, we found that microstructural changes in the caudate, thalamus, and accumbens explained 70% of the variance in HD development. These preliminary data suggest that since the striatum and globus pallidus were impaired in our preHD subjects, the study of other subcortical nuclei affected in HD patients (i.e., thalamus and accumbens) may provide complementary information and enhance the predictive power when disease onset and evolution are assessed. However, these results need to be confirmed in larger samples of subjects. There have been extensive descriptions of the important role played by striatum shrinkage in the evolution of this disease [Aylward et al., 2010], following which the pathological process extends in a dorso-ventral direction [Douaud et al., 2006; Kassubek et al., 2004]. In view of the cross-sectional approach adopted in our study, a longitudinal multimodal

study in a larger preHD population is warranted to confirm our findings.

CONCLUSIONS

In conclusion, the multimodal MRI approach provides a body of complementary quantitative information that may help to evaluate the subcortical nuclei as a whole. Our results provide in vivo evidence of early, selective and complex degeneration that affects the subcortical nuclei involved in HD. It also proves the sensitivity of the multimodal MRI assessment in detecting changes in volume, tissue microstructure, and iron content in the basal ganglia, even in preHD subjects. MRI changes observed in the striatum reflect the main focus of degeneration, whereas those observed in the globus pallidus, thalamus, and accumbens seem to be somewhat more complex in nature. Studies based on a multimodal approach in larger cohorts and applied to the whole brain are warranted to evaluate the role of the cortex and white matter in the pathological process underlying HD.

ACKNOWLEDGMENTS

We thank Lewis Baker for editing the English in the manuscript.

REFERENCES

- Aylward EH, Codori AM, Rosenblatt A, Sherr M, Brandt J, Stine OC, Barta PE, Pearlson GD, Ross CA (2000): Rate of caudate atrophy in presymptomatic and symptomatic stages of Huntington's disease. *Mov Disord* 15:552–560.
- Aylward EH, Liu D, Nopoulos PC, Ross CA, Pierson RK, Mills JA, Long JD, Paulsen JS; PREDICT-HD Investigators and Coordinators of the Huntington Study Group (2011): Striatal Volume Contributes to the Prediction of Onset of Huntington Disease in Incident Cases. *Biol Psychiatry* (in press)
- Aylward EH, Nopoulos PC, Ross CA, Langbehn DR, Pierson RK, Mills JA, Johnson HJ, Magnotta VA, Juhl AR, Paulsen JS; PREDICT-HD Investigators and Coordinators of Huntington Study Group (2011): Longitudinal change in regional brain volumes in prodromal Huntington disease. *J Neurol Neurosurg Psychiatry* 82:405–410.
- Bartzokis G, Cummings J, Perlman S, Hance DB, Mintz J (1999): Increased basal ganglia iron levels in Huntington disease. *Arch Neurol* 56:569–574.
- Bartzokis G, Lu PH, Tishler TA, Fong SM, Oluwadara B, Finn JP, Huang D, Bordelon Y, Mintz J, Perlman S (2007): Myelin breakdown and iron changes in Huntington's disease: Pathogenesis and treatment implications. *Neurochem Res* 32:1655–1664.
- Basser PJ, Pierpaoli C (1996): Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson* 111:209–219.
- Brinkman RR, Mezei MM, Theilmann J, Almquist E, Hayden MR (1997): The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. *Am J Hum Genet* 60:1202–1210.

- Buckner RL, Head D, Parker J, Fotenos AF, Marcus D, Morris JC, Snyder AZ (2004): A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: Reliability and validation against manual measurement of total intracranial volume. *Neuroimage* 23:724–738.
- Cherubini A, Péran P, Caltagirone C, Sabatini U, Spalletta G (2009): Aging of subcortical nuclei: Microstructural, mineralization and atrophy modifications measured in vivo using MRI. *Neuroimage* 48:29–36.
- Ciarmiello A, Cannella M, Lastoria S, Simonelli M, Frati L, Rubinsztein DC, Squitieri F (2006): Brain white-matter volume loss and glucose hypometabolism precede the clinical symptoms of Huntington's disease. *J Nucl Med* 47:215–222.
- Dexter DT, Jenner P, Schapira AH, Marsden CD (1992): Alterations in levels of iron, ferritin, and other trace metals in neurodegenerative diseases affecting the basal ganglia. The Royal Kings and Queens Parkinson's Disease Research Group. *Ann Neurol* 32 (Suppl): S94–S100.
- Di Paola M, Di Iulio F, Cherubini A, Blundo C, Casini AR, Sancesario G, Passafiume D, Caltagirone C, Spalletta G (2010): When, where, and how the corpus callosum changes in MCI and AD: A multimodal MRI study. *Neurology* 74:1136–1142.
- Douaud G, Behrens TE, Poupon C, Cointepas Y, Jbabdi S, Gaura V, Golestani N, Krystkowiak P, Verny C, Damier P, Bachoud-Lévi AC, Hantraye P, Remy P (2009): In vivo evidence for the selective subcortical degeneration in Huntington's disease. *Neuroimage* 46:958–966.
- Douaud G, Gaura V, Ribeiro MJ, Lethimonnier F, Maroy R, Verny C, Krystkowiak P, Damier P, Bachoud-Levi AC, Hantraye P, Remy P (2006): Distribution of grey matter atrophy in Huntington's disease patients: a combined ROI-based and voxel-based morphometric study. *Neuroimage* 32:1562–1575.
- Folstein MF, Folstein SE, McHugh PR (1975): "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12:189–198.
- Gerlach M, Ben-Shachar D, Riederer P, Youdim MB (1994): Altered brain metabolism of iron as a cause of neurodegenerative diseases? *J Neurochem* 63:793–807.
- Gomez-Tortosa E, MacDonald ME, Friend JC, Taylor SA, Weiler LJ, Cupples LA, Srinidhi J, Gusella JF, Bird ED, Vonsattel JP, Myers RH (2001): Quantitative neuropathological changes in presymptomatic Huntington's disease. *Ann Neurol* 49:29–34.
- Gutkunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, Rye D, Ferrante RJ, Hersch SM, Li XJ (1999): Nuclear and neuropil aggregates in Huntington's disease: Relationship to neuropathology. *J Neurosci* 19:2522–2534.
- Hilditch-Maguire P, Trettel F, Passani L, Auerbach A, Persichetti F, MacDonald ME (2000): Huntingtin: An iron-regulated protein essential for normal nuclear and perinuclear organelles. *Human Mol Genet* 9:2789–2797.
- Hobbs NZ, Henley SM, Wild EJ, Leung KK, Frost C, Barker RA, Scallan RI, Barnes J, Tabrizi SJ, Fox NC (2009): Automated quantification of caudate atrophy by local registration of serial MRI: Evaluation and application in Huntington's disease. *Neuroimage* 47:1659–1665.
- Huntington Study Group (1996): The unified Huntington's disease rating scale: Reliability and consistency. *Mov Dis* 11:136–142.
- Jurgens CK, van de Wiel L, van Es AC, Grimbergen YM, Witjes-Ané MN, van der Grond J, Middelkoop HA, Roos RA (2008): Basal ganglia volume and clinical correlates in "preclinical" Huntington's disease. *J Neurol* 255:1785–1791.
- Kassubek J, Juengling FD, Kioschies T, Henkel K, Karitzky J, Kramer B, Ecker D, Andrich J, Saft C, Kraus P, Aschoff AJ, Ludolph AC, Landwehrmeyer GB (2004): Topography of cerebral atrophy in early Huntington's disease: A voxel based morphometric MRI study. *J Neurol Neurosurg Psychiatry* 75:213–220.
- Langbehn DR, Brinkman RR, Falush D, Paulsen JS, Hayden MR (2004): A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. *Clin Genet* 65:267–277.
- Langkammer C, Krebs N, Goessler W, Scheurer E, Ebner F, Yen K, Fazekas F, Ropele S (2010): Quantitative MR imaging of brain iron: A postmortem validation study. *Radiology* 257:455–462.
- Lumsden AL, Henshall TL, Dayan S, Lardelli MT, Richards RI (2007): Huntingtin-deficient zebrafish exhibit defects in iron utilization and development. *Hum Mol Genet* 16:1905–1920.
- Mascalchi M, Lolli F, Della Nave R, Tessa C, Petralli R, Gavazzi C, Politi LS, Macucci M, Filippi M, Piacentini S (2004): Huntington disease: volumetric, diffusion-weighted, and magnetization transfer MR imaging of brain. *Radiology* 232:867–873.
- Myers RH, Vonsattel JP, Paskevich PA, Kiely DK, Stevens TJ, Cupples LA, Richardson EP Jr, Bird ED (1991): Decreased neuronal and increased oligodendroglial densities in Huntington's disease caudate nucleus. *J Neuropathol Exp Neurol* 50:729–742.
- Paulsen JS, Zhao H, Stout JC, Brinkman RR, Guttman M, Ross CA, Como P, Manning C, Hayden MR, Shoulson I; Huntington Study Group (2001): Clinical markers of early disease in persons near onset of Huntington's disease. *Neurology* 57:658–662.
- Paulsen JS, Magnotta VA, Mikos AE, Paulson HL, Penziner E, Andreasen NC, Nopoulos PC (2006a) Brain structure in pre-clinical Huntington's disease. *Biol Psychiatry* 59:57–63.
- Peinemann A, Schuller S, Pohl C, Jahn T, Weindl A, Kassubek J (2005): Executive dysfunction in early stages of Huntington's disease is associated with striatal and insular atrophy: A neuropsychological and voxel-based morphometric study. *J Neurol Sci* 239:11–19.
- Penney JB, Vonsattel JP, MacDonald ME, Gusella JF, Myers RH (1997): CAG repeat number governs the development rate of pathology in Huntington's disease. *Ann Neurol* 41:689–692.
- Péran P, Cherubini A, Luccichenti G, Hagberg G, Démonet JF, Rascol O, Celsis P, Caltagirone C, Spalletta G, Sabatini U (2009): Volume and iron content in basal ganglia and thalamus. *Hum Brain Mapp* 30:2667–2675.
- Péran P, Cherubini A, Assogna F, Piras F, Quattrocchi C, Peppe A, Celsis P, Rascol O, Démonet JF, Stefani A, Pierantozzi M, Pontieri FE, Caltagirone C, Spalletta G, Sabatini U (2010): Magnetic resonance imaging markers of Parkinson's disease nigrostriatal signature. *Brain* 133:3423–3433.
- Pfefferbaum A, Adalsteinsson E, Rohlfing T, Sullivan EV (2010): Diffusion tensor imaging of deep gray matter brain structures: Effects of age and iron concentration. *Neurobiol Aging* 31: 482–493.
- Rosas HD, Koroshetz WJ, Chen YI, Skeuse C, Vangel M, Cudkowicz ME, Caplan K, Marek K, Seidman LJ, Makris N, Jenkins BG, Goldstein JM (2003): Evidence for more widespread cerebral pathology in early HD: An MRI-based morphometric analysis. *Neurology* 60:1615–1620.
- Rosas HD, Tuch DS, Hevelone ND, Zaleta AK, Vangel M, Hersch SM, Salat DH (2006): Diffusion tensor imaging in presymptomatic and early Huntington's disease: Selective white matter pathology and its relationship to clinical measures. *Mov Disord* 21:1317–1325.

- Ruocco HH, Bonilha L, Li LM, Lopes-Cendes I, Cendes F (2008): Longitudinal analysis of regional grey matter loss in Huntington's disease: Effects of the length of the expanded CAG repeat. *J Neurol Neurosurg Psychiatry* 79:130–135.
- Simmons DA, Casale M, Alcon B, Pham N, Narayan N, Lynch G (2007): Ferritin accumulation in dystrophic microglia is an early event in the development of Huntington's disease. *Glia* 55:1074–1084.
- Squitieri F, Gellera C, Cannella M, Mariotti C, Cislighi G, Rubinsztein DC, Almqvist EW, Turner D, Bachoud-Lévi AC, Simpson SA, Delatycki M, Maglione V, Hayden MR, Donato SD (2003): Homozygosity for CAG mutation in Huntington disease is associated with a more severe clinical course. *Brain* 126:946–955.
- Tabrizi SJ, Langbehn DR, Leavitt BR, Roos RA, Durr A, Craufurd D, Kennard C, Hicks SL, Fox NC, Scahill RI, Borowsky B, Tobin AJ, Rosas HD, Johnson H, Reilmann R, Landwehrmeyer B, Stout JC; TRACK-HD Investigators (2009): Biological and clinical manifestations of Huntington's disease in the longitudinal TRACK-HD study: Cross-sectional analysis of baseline data. *Lancet Neurol* 8:791–801.
- Tabrizi SJ, Scahill RI, Durr A, Roos RA, Leavitt BR, Jones R, Landwehrmeyer GB, Fox NC, Johnson H, Hicks SL, Kennard C, Craufurd D, Frost C, Langbehn DR, Reilmann R, Stout JC; TRACK-HD Investigators (2011): Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: The 12-month longitudinal analysis. *Lancet Neurol* 10:31–42.
- Tai YF, Pavese N, Gerhard A, Tabrizi SJ, Barker RA, Brooks DJ, Piccini P (2007): Microglial activation in presymptomatic Huntington's disease gene carriers. *Brain* 130:1759–1766.
- Thieben MJ, Duggins AJ, Good CD, Gomes L, Mahant N, Richards F, McCusker E, Frackowiak RS (2002): The distribution of structural neuropathology in pre-clinical Huntington's disease. *Brain* 125:1815–1828.
- Vonsattel JP (2008). Huntington disease models and human neuropathology: Similarities and differences. *Acta Neuropathol* 115:55–69.
- Vymazal J, Klempíř J, Jech R, Zidovská J, Syka M, Růžicka E, Roth J (2007): MR relaxometry in Huntington's disease: Correlation between imaging, genetic and clinical parameters. *J Neurol Sci* 263:20–25.
- Wakai M, Takahashi A, Hashizume Y (1993): A histometrical study on the globus pallidus in Huntington's disease. *J Neurol Sci* 119:18–27.
- Walker FO (2007): Huntington's disease. *Lancet* 369:220–221.