Functional and structural neuroimaging in Huntington's disease

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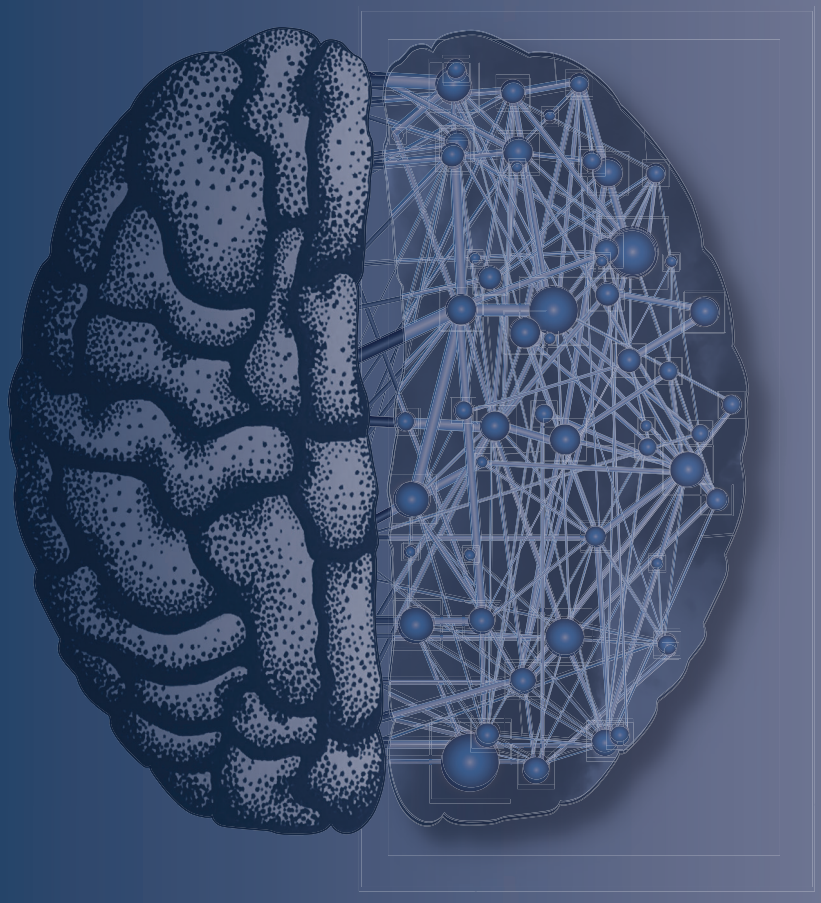

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Microstructural brain abnormalities in Huntington's disease: a two-year follow-up

CHAPTER 3

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Abstract

Background

Diffusion Tensor Imaging (DTI) provides indirect information about the quality of the microstructural organization of tissues. In this 2-year follow-up study, we assess both cross-sectional and time-related changes of striatal and whole-brain microstructural properties in different stages of Huntington's disease (HD) using DTI.

Methods

From the TRACK-HD study, 22 premanifest gene carriers (preHD), 10 early manifest HD and 24 controls were scanned at baseline and 2-year follow-up. Stratification of the preHD group into a far (preHD-A) and near (preHD-B) to predicted disease onset was performed. Age-corrected histograms of whole-brain white matter (WM), grey matter (GM) and striatal diffusion measures were computed and normalised by the number of voxels in each subject's data set.

Results

Higher cross-sectional mean, axial and radial diffusivities were found in both WM ($p \leq 0.001$) and GM ($p \leq 0.001$) of the manifest HD compared to the preHD and control groups. In preHD, only WM axial diffusivity (AD) was higher than in controls ($p \leq 0.01$). This finding remained valid only in preHD-B ($p \leq 0.001$). AD was also higher in the striatum of preHD-B compared to controls and preHD-A ($p \leq 0.01$). Fractional anisotropy (FA) lacked sensitivity in differentiating between the groups. Histogram peak heights were generally lower in manifest HD compared to the preHD and control groups. No longitudinal differences were found in the degree of diffusivity change between the groups in the two year follow-up. There was a significant relationship between diffusivity and neurocognitive measures.

Conclusions

Alterations in cross-sectional diffusion profiles between manifest HD subjects and controls were evident, both in whole-brain and striatum. In the preHD stage, only AD alterations were found, a finding suggesting that this metric is a sensitive marker for early change in HD prior to disease manifestation. The individual diffusivities were superior to FA in revealing pathologic microstructural brain alterations. Diffusion measures were well related to clinical functioning and disease stage.

Introduction

Huntington's disease (HD) is a neurodegenerative autosomal dominant disorder. It is caused by an increased CAG (Cytosine-Adenine-Guanine) repeat within the huntingtin gene on the short arm of chromosome 4.[1] The mutant huntingtin protein triggers a pathogenic cascade responsible for neuropathology in the brain.[2,3] This results in cognitive, motor, and psychiatric symptoms. The brain as a whole is impacted, though preferential striatal volume loss has been extensively documented by post-mortem histopathological as well as *in vivo* magnetic resonance imaging (MRI) studies.[4-9]

Even though no medication is currently available to cure or slow-down the disease, it remains crucial to have a clear understanding of the typical evolution of brain changes in the disease to determine when microstructural changes start and how fast degeneration occurs. This is necessary to define optimal intervention starting points as well as possibly providing an objective tool to determine the impact of candidate therapies, especially in the premanifest (preHD) phase where clinical measures are lacking.

Diffusion tensor imaging (DTI) is an MRI technique that can quantify water diffusion within tissue.[10-13] The diffusion tensor in every voxel can be described by its three eigenvectors and eigenvalues ($\lambda1, \lambda2, \lambda3$). These eigenvalues quantify the diffusion in three orthogonal orientations and are typically synthesized to axial ($= \lambda1$) and radial ($= (\lambda2 + \lambda3)/2$) diffusivities.

Another popular diffusion measure is fractional anisotropy (FA), which is a function of the eigenvalues, and ranges from 0 (completely isotropic diffusion) to 1 (completely anisotropic diffusion), with higher values generally corresponding to a higher directional coherence of tissue organization. High FA occurs for example in healthy white matter (WM) which typically has a parallel-oriented micro-architecture. Another commonly reported diffusivity measure is the mean diffusivity (MD), which is the average of the three eigenvalues. In this study we evaluate and report these measures as well as the separate underlying eigenvalues, as these may provide complementary information about the nature of microstructural change.[14,15] It is possible that certain metrics are more selectively affected and, therefore, might be more sensitive to longitudinal change. For example, when changes in axial diffusivity (AD) are proportional to radial diffusivity (RD), the FA value may not be very informative.[16]

In a previous study, we evaluated cross-sectional group differences in FA and MD between controls, preHD and manifest HD subjects using a region-of-interest and fiber tractography analysis approach.[17] In that study, MD proved to be more sensitive in differentiating between the groups compared to FA. Findings from previous longitudinal reports remain inconsistent.[18-20] With inherent limitations such as inter-user variability to nonautomated methods such as hand drawn regions-of-interest, we chose an automated histogram analysis method in this work to assess cross-sectional as well as time-related changes of diffusivity measures occurring within 2 years. We hypothesized that lower FA and higher MD, AD and RD values would be found in

subjects with manifest HD when compared to preHD subjects and controls, reflective of higher microstructural disorganization in the manifest group. In addition, we hypothesized that MD would be elevated in preHD subjects when compared to controls based on results from our previous work.[17] Grey matter (GM) diffusivity was assessed separately to assess potential higher sensitivity towards alteration compared to WM, fully bearing in mind the limitations of the tensor model in GM. Associations between neurocognitive measures and diffusivity findings were assessed for potential usage as surrogate markers or predictors for these findings. Also, associations between diffusivity and the expected time to disease onset were assessed to test the hypothesis that sensitivity of diffusivity measures in detecting disturbances in preHD subjects increases with shorter proximity to expected disease onset.

As a subanalysis, diffusion in the left and right hemispheres was assessed individually. This was done to explore the hypothesis of preferential degeneration of the dominant versus the non-dominant hemisphere. Plausibly increased lifetime excitotoxic exposure due to higher activation could lead to such a finding in HD. We hypothesized that diffusion parameters indicative of greater neuronal damage were represented more readily in the dominant hemisphere, as findings from previous studies have suggested.[21-24] To the best of our knowledge, this is the first study exploring this hypothesis and the first to apply histogram analysis to (longitudinal) DTI data in HD as well as to separately assess microstructural properties of both whole-brain GM and WM.

Materials and methods

Participants

As part of the TRACK-HD study, 90 participants were included at baseline at the Leiden University Medical Center (LUMC) study site (for details see Tabrizi et al.).[7] DTI was added to the standard MRI protocol. At baseline, DTI was not performed in ten participants because of claustrophobia, and another nine were excluded from analysis due to excessive movement artefacts. Of the remaining 71 subjects, 62 subjects completed DTI scans at both visits. Of these 62, a further six subjects were excluded from analysis due to excessive movement artefacts at the second visit. The longitudinal cohort included in this work was thus comprised of 56 subjects: 24 healthy controls, 22 preHD and ten early manifest HD (Table I).

Inclusion criteria for the preHD group were a CAG repeat ≥ 40 with a total motor score on the Unified Huntington's Disease Rating Scale (UHDRS-TMS) ≤ five. Inclusion criteria for the early manifest HD group were a CAG repeat ≥ 40, with a UHDRS-TMS ≥ five and a Total Functional Capacity score (TFC) ≥ seven. A further inclusion criterion for both the preHD and early manifest HD group consisted of a burden of pathology score greater than 250 ((CAG repeat length - 35.5) x age).[7,25] Healthy gene negative family members or partners were recruited as control subjects. None of the participants suffered from a concomitant neurological disorder, a major psychiatric diagnosis or had a history of severe head injury.

Table I. *Group characteristics and clinical scores*

		Healthy controls	preHD (A and B)	preHD-A	preHD-B	Manifest HD
N		24	22‡	11	11	10
Gender M/F		11/13	9/13	4/7	5/6	4/6
Age in years (at V1), mean (SD)		49.0 (8.2)	43.6 (8.7)	44.2 (5.7)	43.0 (11.2)	50.2 (9.3)
Handedness R/L		20/4	18/4	9/2	9/2	9/1
Level of education (ISCED), median (range)		4 (3)	4 (3)	4 (3)	4 (3)	4 (3)
DART-IQ, mean (SD)		105.0 (9.4)	100.5 (11.2)	101.3 (9.7)	99.6 (13.0)	101.8 (13.5)
CAG repeat length, mean (SD)		n/a	42.6 (2.7)	41.3 (1.4)	43.9 (3.1)^	42.5 (1.2)
Estimated years to onset, mean (SD)		n/a	11.8 (4.7)	14.9 (4.7)	8.6 (1.8)^	n/a
Total functional capacity, mean (SD)						
	V1	13.0 (0.2)	12.8 (0.5)	12.7 (0.7)	12.8 (0.4)	11.0 (1.5)Φ
	V2	12.9 (0.5)	12.6 (0.9)	12.7 (0.6)	12.5 (1.0)	10.3 (2.2)Φ
UHDRS-TMS, mean (SD)						
	V1	2.6 (2.5)	2.6 (1.5)	2.0 (1.5)	3.1 (1.2)	14.6 (7.7)Φ
	V2	2.1 (1.6)	5.7 (5.1) ¥	3.5 (2.2)	8.3 (6.1)*^	23.0 (12.1)Φ
SDMT, mean (SD)						
	V1	49.4 (8.9)	50.1 (11.0)	53.5 (9.3)	46.7 (11.9)	41.2 (9.2)Φ
	V2	50.9 (9.3)	50.6 (10.0)	54.7 (10.0)	46.6 (8.5)^	39.2 (10.6)Φ
SWR, mean (SD)						
	V1	100.1 (13.2)	91.9 (14.2)*	95.6 (9.6)	88.3 (17.3)*	87.7 (14.7)*
	V2	102.0 (15.6)	87.9 (15.7)*	91.4 (9.4)	84.4 (20.0)*	86.4 (18.6)*
BDI-II, mean (SD)						
	V1	4.1 (4.4)	6.4 (6.4)	4.9 (6.0)	7.9 (6.8)	10.2 (8.2)*
	V2	3.9 (4.1)	5.1 (5.6)	3.2 (4.9)	6.9 (5.9)	8.2 (8.4)
Between-scan interval in months, mean (SD)		23.0 (0.8)	23.0 (0.7)	23.2 (0.6)	22.7 (0.7)	23.5 (0.7)

*N = number of participants, SD = Standard deviation, n/a = not applicable, ISCED = International Standard Classification of Education, DART-IQ = Dutch Adult Reading Test Intelligence Quotient, CAG = Cytosine-Adenine-Guanine, UHDRS-TMS = Unified Huntington's Disease Rating Scale-Total Motor Score, SDMT = Symbol Digit Modalities Test, SWR = Stroop Word Reading task, BDI-II = Beck Depression Inventory-II, V1 = visit 1, V2 = visit 2. Significance at p ≤ 0.05 level: * significantly different from controls, Φ significantly different from controls and preHD, ¥ significantly different from controls and HD, ^ significantly different from preHD-A.*
‡ Including five subjects progressing to the early manifest stage during the two year follow-up period.

Hemispheric dominance was defined using a standardised neuropsychological questionnaire.[26] For preHD subjects, the predicted years to disease onset was calculated using the CAG repeat length and age-based survival analysis of Langbehn et al.[27]

As previously applied by Tabrizi et al.,[7] to assess the effect of expected proximity to disease onset on diffusion parameters, the preHD group was divided at baseline according to the median (10.9 years) for the predicted years to disease onset into preHD-A (≥ 10.9 years) and preHD-B (< 10.9). This resulted in two groups each consisting of eleven subjects (Table I).

The study was approved by the Medical Ethics Committee of the LUMC and written informed consent was obtained from all participants. For full details of study parameters, see Tabrizi et al.[7]

Clinical measures

To monitor disease state, the following clinical measures were evaluated longitudinally for all groups: UHDRS-TMS, TFC, Symbol Digit Modalities Test (SDMT), Stroop Word Reading (SWR) and Beck Depression Inventory-II (BDI-II) scores.

The UHDRS-TMS is the traditional measure which defines manifest disease state in HD. The SDMT and SWR in particular have been shown to be sensitive longitudinal neurocognitive measures in HD, independent of disease related motor effects.[28]

Magnetic resonance imaging acquisition

MRI acquisition was performed with a 3-Tesla whole-body scanner (Philips Achieva, Healthcare, Best, The Netherlands) with an eight channel SENSE head coil. T1-weighted image volumes were acquired using a 3D MPRAGE acquisition sequence with the following imaging parameters: TR = 7.7 ms, TE = 3.5 ms, FOV = 24 x 24 cm^2, matrix size 224 x 224, number of slices = 164, slice thickness = 1.00 mm, and no slice gap. A single-shot echo-planar diffusion tensor imaging sequence was applied with 32 measurement directions and the following scan parameters:[11] TR = 10,004 ms, TE = 56 ms, FOV = 220 x 220 mm^2 with an acquisition matrix of 112 x 110, 2.00 mm slice thickness, transversal slice orientation, no slice gap, flip angle = 90°, reconstruction voxel dimensions of 1.96 x 1.96 x 2.00 mm^3, number of slices = 64, *b*-value = 1,000 s/mm^2, halfscan factor = 0.61. Parallel imaging (SENSE) was used with a reduction factor of two, NSA = one, and fat suppression was applied. DTI acquisition time was 6.55 min.

Image processing

The DTI data was processed as described in Deprez et al.[29] In summary, this consisted of the following steps: (1) Correction for subject motion and eddy current induced distortions;[30] (2) Correction for echo planar images based deformations due to magnetic field inhomogeneities by registration to the T1-weighted images;[31] (3) Tensor estimation using the iteratively reweighted linear least squares approach after outlier detection and removal by REKINDLE ($\kappa = 6$).[32,33]

The brain regions were segmented into WM and GM regions (Figure 1) using SPM 8 with default settings (revision 4667, 27-Feb-2012).[34] Brain regions were left/right divided with the method described by Kuijf et al.[35]

Histogram analysis

A spherical erosion filter (radius 2 mm) was applied to the brain masks (WM/GM; left/right) to minimize the inclusion of partial-volume affected voxels.[36,37] The histograms of the diffusion measures were computed from these segmented brain regions. Subsequently, histograms were normalised by the number of voxels in each subject's data set to create the group mean histograms.[38]

With histogram analysis, frequency distributions of selected DTI measures of designated voxels can be obtained. While not providing any region-specific information, this type of analysis is highly sensitive in detecting differences as the entire brain is included. Moreover, it provides a straightforward, fully automated and objective approach for interrogating imaging data. The resulting summarizing whole-brain measures are suitable for comparing diffusion between groups[29] and its value has been previously demonstrated in multiple sclerosis and CADASIL.[39-41] This type of analysis can also be applied to any given selection of voxels of interest. Given the importance of the striatum in the histopathological profile of HD, diffusion values for this structure were additionally evaluated in this study. The following diffusion features for whole-brain WM were investigated: FA, MD, AD and RD. In addition, for the whole-brain GM (including striatum) the MD, AD and RD were studied. The outcome measures were the mean and distribution peak heights of the histograms. Because two outcome measures were tested against two tissue types, p-values for omnibus F-tests were Bonferroni corrected to adjust for the increased risk of type one error and considered to be statistically significant at $p \leq 0.05/4 = 0.0125$.

Obtaining striatal masks

Striatal masks were obtained as described previously.[42] In summary, T1-weighted images were segmented with the FAST and FIRST tools from the fMRI of the Brain Software Library (http://www.fmrib.ox.ac.uk/fsl/).[43-45] This provided individual brain masks for the following structures: the caudate nucleus and the putamen, both of these forming the striatum. Figure 1 shows such a segmentation result superimposed on a T1-weighted image. To correct for potential partial volume effects, an eroded mask of these segmentations was created by removing one voxel in-plane for all the aforementioned voxels of interest.

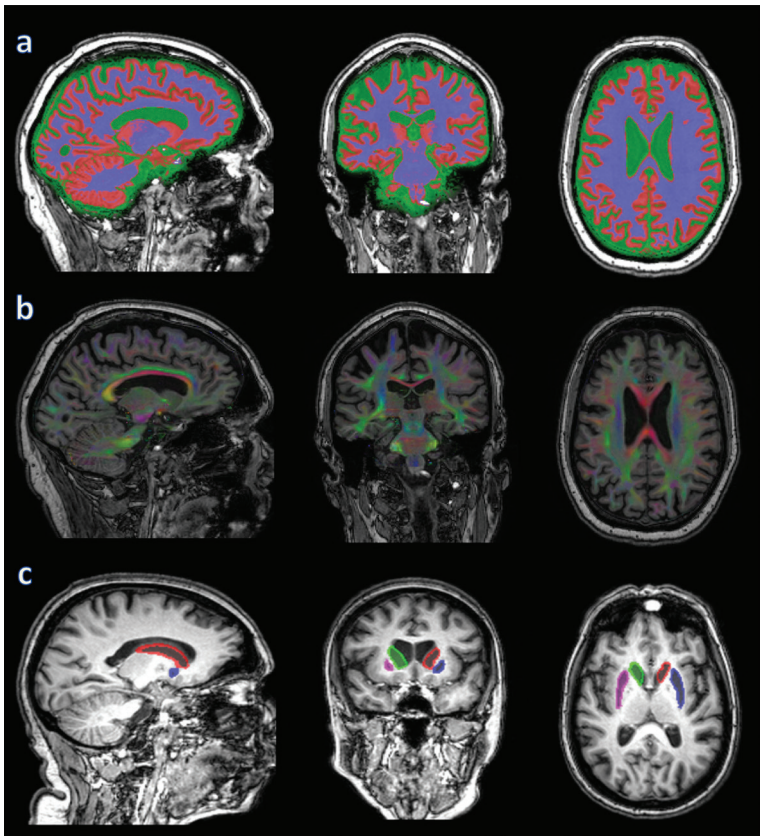

Figure 1. *From left to right: sagittal, coronal and axial images: a. brain segmentation into WM (blue), GM (red) and CSF (green); b. directionally colour encoded fractional anisotropy map; c. striatal mask: red = left caudate nucleus and blue = left putamen; green = right caudate nucleus and pink = right putamen.*

Statistical analysis

We used linear mixed models (in R version 3.0.0, R Foundation for Statistical Computing, Vienna, Austria) to model the various outcome variables with patient as a random factor to accommodate the within-person repeated nature of the data and to assess the effect of group, corrected for age at time of scanning as a co-variable. Correlations between neurocognitive measures and DTI findings were tested in the model.

Statistical analyses of group demographics were performed with SPSS (version 20, IBM, USA). Distributions and assumptions were checked. Either Analysis of Variance (ANOVA) or chi-squared tests were applied where this was appropriate. Potential longitudinal change in clinical measures between the groups was also investigated. Difference values were computed and an ANOVA was performed on these delta-scores to evaluate potential group differences. In case of a significant

omnibus F-test, exploratory post hoc analysis using Fisher's least significant difference was performed to assess which means were significantly different from each other. Differences in group demographics between preHD-A and preHD-B were compared using either independent samples t-tests or chi-squared tests, where appropriate.

Paired samples t-tests were performed to assess cross-sectional interhemispheric differences in DTI measures within the groups after excluding lefthanders. Lefthanders consisted of four control, four preHD and one manifest HD subjects. The longitudinal evolution of the interhemispheric diffusion measures was assessed with the aforementioned linear mixed model.

Table II. *Mean whole-brain DTI parameters. MD, AD and RD are shown x10³ for readability*

	Healthy controls	preHD (A and B)	preHD-A	preHD-B	Manifest HD
N	24	22	11	11	10
FA-WM	0.434 (0.008)	0.435 (0.012)	0.435 (0.014)	0.435 (0.014)	0.421 (0.014)Φ*
MD-WM	0.754 (0.010)	0.764 (0.016)	0.758 (0.017)	0.767 (0.017)	**0.783 (0.018)Φ*** **
MD-GM	0.767 (0.004)	0.777 (0.010)	0.768 (0.024)	0.778 (0.024)	**0.805 (0.012)Φ*** **
AD-WM	1.123 (0.005)	**1.140 (0.011)¥****	1.131 (0.012)	**1.149 (0.012)ə*** **	**1.172 (0.013)Φ*** **
AD-GM	0.924 (0.013)	0.934 (0.019)	0.923 (0.025)	0.938 (0.025)¤	**0.965 (0.021)Φ*** **
RD-WM	0.560 (0.011)	0.566 (0.017)	0.562 (0.019)	0.568 (0.019)	**0.589 (0.019)Φ*** **
RD-GM	0.702 (0.004)	0.711 (0.010)	0.706 (0.012)	0.716 (0.012)ˆ	**0.736 (0.012)Φ*** **

Data is shown as mixed model-based estimates of the group means corrected for age (S.E.)
*Φ significantly different from controls and preHD, ¥ significantly different from controls and HD, ə significantly different from controls, preHD-A and HD, *p ≤ 0.05 **p ≤ 0.01 ***p ≤ 0.001, bold values indicate sustained significant difference following Bonferroni correction (p ≤ 0.0125), ¤ p = 0.08, ˆ p = 0.07.*
FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity; WM = white matter; GM = grey matter.

Results

Group characteristics and clinical scores

The groups did not differ significantly in terms of gender, handedness, level of education, intelligence quotient or body mass index. A trend toward a difference in age between the groups was found (p = 0.06), with premanifest subjects being generally younger compared to both controls and subjects with manifest HD. No statistical difference was found in CAG repeat count between preHD and manifest HD subjects. The between-scan interval was not significantly different between the groups.

At baseline, significantly lower scores for subjects with manifest HD were found in TFC, SDMT and SWR when compared to both controls and preHD subjects. Higher scores for subjects with manifest HD were found for UHDRS-TMS and BDI-II when compared to both controls and preHD

subjects. For the preHD group, a significantly lower baseline score compared to controls was found for SWR (Table I).

Repeated assessment after 2-year follow-up revealed similar score differences between the groups. Progression of five of the 22 preHD subjects to the early manifest stage during the follow-up period gave rise to a significantly higher UHDRS-TMS when compared to controls. The only significant difference in longitudinal change of clinical scores was found in higher UHDRS-TMS, both when considering the preHD group (including those progressing to the early manifest stage) and the manifest HD group. Other scores showed no significant longitudinal differences in this cohort (Supplementary Table I).

Comparing the preHD-A and preHD-B groups, no significant cross-sectional score differences were found during the first visit. At the second visit, the preHD-B group showed a significantly higher UHDRS-TMS and lower SDMT score compared to preHD-A.
Significant longitudinal change was found only in the UHDRS-TMS, where the difference was higher in preHD-B relative to preHD-A (Table I; longitudinal change data not shown).

Diffusion tensor imaging histogram measures

Diffusivity values of whole-brain white matter
At baseline, all whole-brain WM diffusivity measures in the manifest HD group differed significantly from both controls and preHD subjects (Table II): FA values were reduced and MD, AD and RD were increased. Upon applying Bonferroni correction for multiple testing, all these differences remained statistically significant except for the difference in FA (see Supplementary Figures 1 and 2 for group and visit histogram plots of WM FA, including separate plots for the left and right hemisphere). Elevations in MD, AD and RD were all highly significant ($p \leq 0.001$) (see Figure 2 for histogram plots of WM MD).

Only AD in the preHD group differed significantly from both controls and subjects with manifest HD and was lower for the controls and higher for subjects with manifest HD, even after applying Bonferroni correction ($p \leq 0.01$). No statistically significant differences in FA ($p = 0.83$), MD ($p = 0.10$) or RD ($p = 0.33$) were found between controls and preHD subjects.

Dividing the preHD group in preHD-A and preHD-B revealed higher AD values only in the preHD-B group compared to both preHD-A and controls, even after Bonferroni correction ($p \leq 0.001$). No significant differences were observed in any of the diffusivity measures between controls and preHD-A (Table II). No significant longitudinal differences were found in the degree of whole-brain WM diffusivity change in any of the measures between the groups (without correction for multiple testing).

Results of histogram peak height comparison of whole-brain WM are provided in Supplementary material.

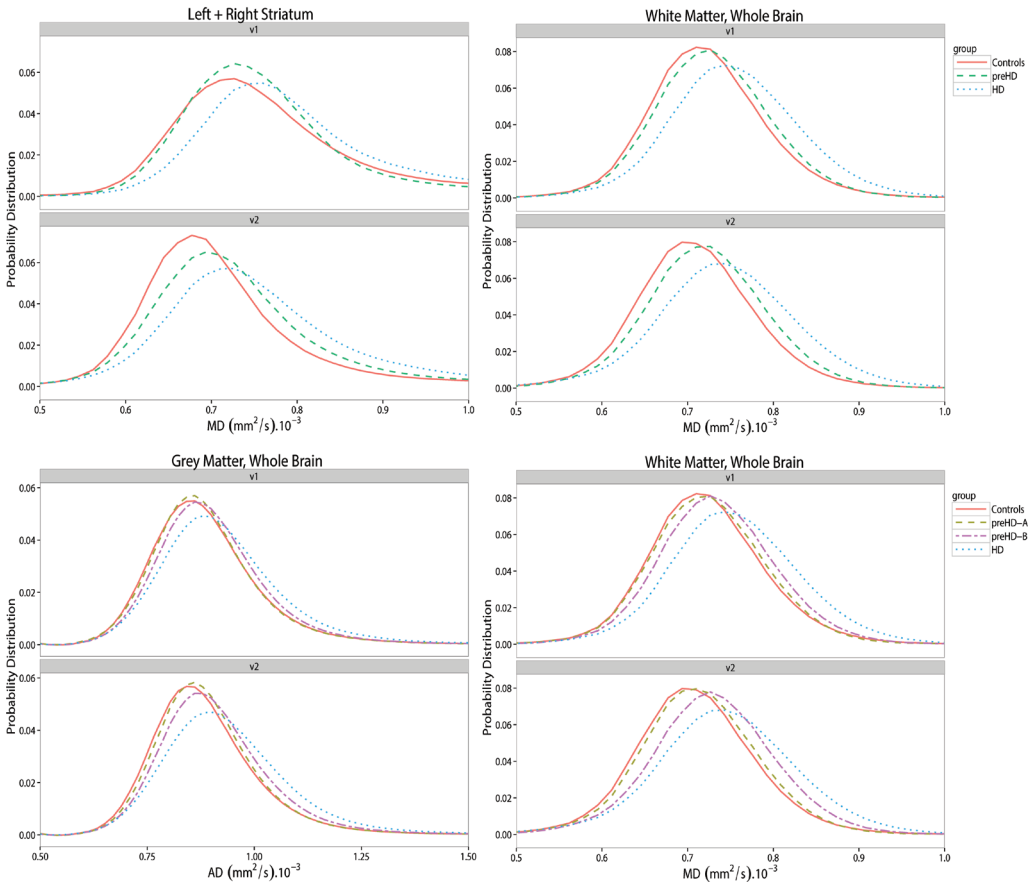

Figure 2. Histogram plots of MD (= mean diffusivity) and AD (= axial diffusivity) in whole brain white, grey matter and the striatum. Group diffusivities are plotted against the visits. v1 = visit 1, v2 = visit 2.

Diffusivity values of whole-brain grey matter and striatum

At baseline, MD, AD and RD values of whole-brain GM were significantly higher for the manifest HD group compared to both controls and preHD subjects ($p \leq 0.001$; Table II). This remained the case after Bonferroni correction for multiple testing. Figure 2 shows histogram plots for whole-brain GM AD.

No significant differences in whole-brain GM diffusivity measures were found between preHD subjects and controls. Upon dividing the preHD group in preHD-A and preHD-B, a trend was found in the preHD-B group toward higher values of AD and RD compared to controls ($p = 0.08$ and $p = 0.07$, respectively; Table II).

Baseline MD, AD and RD values in the striatum of subjects with manifest HD were significantly higher compared to both controls and preHD subjects (Table III). Upon applying Bonferroni correction for multiple testing, these differences remained statistically significant except for RD. See Figure 2 for group histogram plots of striatal MD. Separate plots for MD of the left and right striatum are shown in Supplementary Figure 3.

Table III. *Mean striatal DTI parameters. Values are of left and right striatum together. MD, AD and RD are shown x10³ for readability*

	Healthy controls	preHD (A and B)	preHD-A	preHD-B	Manifest HD
N	24	22	11	11	10
MD	0.686 (0.075)	0.695 (0.037)	0.648 (0.044)	0.758 (0.043)	**0.816 (0.045)Φ****
AD	1.130 (0.093)	1.177 (0.027)¤	1.127 (0.032)	**1.227 (0.031)Ǝ****	**1.235 (0.034)Φ****
RD	0.658 (0.108)	0.641 (0.039)	0.595 (0.048)	0.684 (0.047)	0.764 (0.049)Φ*

Data is shown as mixed model-based estimates of the group means corrected for age (S.E.)
*Φ significantly different from controls and preHD, Ǝ significantly different from controls and preHD-A, ¤ p = 0.08 (compared to controls), *p ≤ 0.05 **p ≤ 0.01, bold values indicate sustained significant difference following Bonferroni correction (p ≤ 0.0125). MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity.*

No significant baseline differences in striatal diffusivity measures were found between preHD subjects and controls, only a trend toward a higher AD in the preHD group (p = 0.08). Upon dividing the preHD group in preHD-A and preHD-B, a significantly higher Bonferroni corrected striatal AD value was found in preHD-B only, compared to both controls and preHD-A ($p \leq 0.01$; Table III). Exploratory analysis to assess whether this effect was more prominent when assessing striatal substructures separately, revealed a trend towards AD elevation in the caudate and a significantly higher AD in the putamen in preHD-B (caudate: p = 0.06; putamen: p = 0.02) compared to both controls and preHD-A. This result was therefore less sensitive than the combined assessment of both substructures ($p \leq 0.01$), and would not have survived Bonferroni correction. No significant longitudinal differences were found in the degree of whole-brain GM or in striatal diffusivity change in any of the measures between the groups (without correction for multiple testing). Results of histogram peak height comparison of whole-brain GM and striatum are provided in Supplementary material.

Neurocognitive and diffusivity measures

In Table IV, significant correlations between neurocognitive measures and baseline whole- brain diffusivity measures are shown (correlations with peak heights are not shown). As no specific group effects were found on correlations between diffusion parameters and neurocognitive measures, the following applied to all participants included in the study with a CAG repeat expansion irrespective of their group. The SDMT score was found to predict WM FA ($p \leq 0.01$): the higher the SDMT score, the higher the FA (Supplementary Figure 4). The SDMT score was also found to predict WM MD ($p \leq 0.01$): the higher the SDMT score, the lower the MD (Figure 3).

The SWR score was found to predict GM MD ($p \leq 0.05$): the higher the SWR score, the lower the MD (Supplementary Figure 5). The SDMT score was found to predict peak height in GM MD ($p \leq 0.05$): the higher the SDMT score, the higher the peak height. The SDMT score was also found to predict peak height of WM AD ($p \leq 0.01$): the higher the SDMT score, the lower the peak height. Both SDMT and SWR scores were found to predict GM AD ($p \leq 0.05$): the higher the score, the lower the AD. The SDMT score was found to predict peak height of GM AD ($p \leq 0.05$): the higher the SDMT score, the higher the peak height.

The SDMT score was found to predict WM RD ($p \leq 0.01$): the higher the SDMT score, the lower the RD. In the striatum, the SDMT score alone was found to predict AD ($p \leq 0.05$): the higher the SDMT score, the lower the AD (data not shown).

Interhemispheric differences in diffusivity measures

In Supplementary Table III, baseline differences in diffusivity measures of the left minus right hemisphere are shown, both for WM and GM. Only right handed subjects were included for this analysis. Many small, though significant interhemispheric differences were found. The magnitude and direction of these differences were similar in all groups (controls, preHD and manifest HD) with no statistical significance in these differences between the groups.

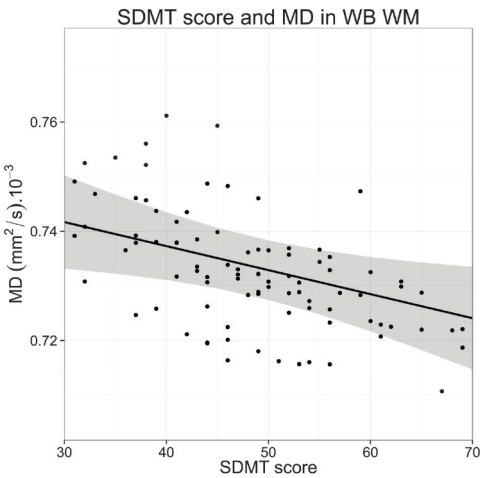

Figure 3. *Relationship plot of Symbol Digit Modalities Test (SDMT) score and whole brain (WB) white matter (WM) mean diffusivity (MD). Data points shown are mixed model-based estimates.*

No significant interhemispheric longitudinal differences between the groups were found in the degree of change of any of the diffusion measures of the WM, GM and the striatum, neither in the means nor histogram peak heights (without correction for multiple testing).

Table IV. *Mean whole-brain DTI parameters and neurocognitive measures correlations (corrected for age)*

	Diffusion parameter	SDMT score	*P*	Diffusion parameter	SWR score	*P*
FA-WM	↑ 1.2%	↑ 10 points	≤ 0.01	/	/	/
MD-WM	↓ 0.7%	↑ 10 points	≤ 0.01	/	/	/
MD-GM	/	/	/	↓ 0.4%	↑ 10 points	≤ 0.05
AD-WM	/	/	/	/	/	/
AD-GM	↓ 0.5%	↑ 10 points	≤ 0.05	↓ 0.4%	↑ 10 points	≤ 0.05
RD-WM	↓ 1.0%	↑ 10 points	≤ 0.01	/	/	/
RD-GM	/	/	/	/	/	/

This table is valid for all participants with a CAG repeat expansion included in the study, as no specific group effects were found on correlations between diffusion parameters and neurocognitive measures.
↑ = increase, ↓ = decrease, / = no significant correlation.
FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity; WM = white matter; GM = grey matter.

Discussion

The major findings from this study were significantly higher MD, AD and RD values in both WM and GM in subjects with manifest HD compared to preHD and control subjects. In preHD subjects, only WM AD proved to be a sensitive measure to differentiate between the study groups. This finding remained valid only in preHD-B upon dividing the preHD group according to the median predicted years to onset. Another significantly different finding in preHD subjects was observed again only in preHD-B in a higher AD of the striatum compared to both controls and preHD-A. No significant longitudinal differences were found in any of the diffusivity measures between any of the groups, neither in the means nor peak heights. Finally, significant relationships between neurocognitive and diffusivity measures were demonstrated.

Findings of increased MD, AD and RD values in subjects with manifest HD are in line with results from previous reports.[46-48] Although a reduction in WM FA in manifest HD was found, this finding did not maintain significance after correction for multiple testing, rendering it a far less sensitive marker for disease state in HD. This finding of individual diffusivities providing more sensitive measures for revealing pathologic microstructural brain alterations compared to FA, was in line with findings from a previous study in HD and Alzheimer's disease.[16,48] The results presented here are also in agreement with previous findings by our group, where MD was reported to be a more sensitive measure than FA in distinguishing HD subjects from controls.[17] Just as in the Alzheimer's disease study of Acosta-Cabronero et al.,[16] changes found in this study were more prominent in AD than in RD, yet not enough to substantially influence FA. This provides a possible explanation for the seemingly discrepant findings of FA alterations in HD research, as the proportions of eigenvalues could be more specifically altered in studies of distinct WM regions giving rise to a modified FA.

The presence of an increased AD in whole-brain WM and in the striatum of preHD-B, provides evidence for ongoing neurodegeneration prior to disease manifestation, a finding that is echoed by results from previous MRI volumetric investigations in preHD.[4,6-8,49] Higher AD in preHD has been previously reported by Stoffers et al.,[6] although in that study this finding was highly localized and accompanied by more pronounced and widespread increases in RD, a finding which was not replicated here. Furthermore, in the study of Stoffers et al., RD seemed to correlate with the predicted years to disease onset, while AD lacked such correlation.[6] This stands in contrast to our findings of lack of significant increases in RD irrespective of preHD group stratification and higher AD being found primarily in preHD individuals who are closest to predicted years to disease onset. The discrepancy in these findings could very well be attributed to the differing methodologies applied in analysing the data and possibly due to the difference in scanner field strength used. In GM, no significantly different diffusivities were present between preHD subjects and controls, except for the above-mentioned higher AD in the striatum of preHD-B, which is a deep GM structure. The differences found in peak heights were only present in subjects with manifest HD, not in the preHD group, alluding to a less sensitive measure in detecting differences between manifest HD, preHD, and controls.

Exploration of the longitudinal evolution of diffusivity measures, without correction for multiple comparisons, provided no significant group differences. Results from previous longitudinal DTI studies in HD are heterogeneous. In the study of Weaver et al.,[19] significant longitudinal decreases in WM FA and AD were reported over a one year period. That study consisted of seven controls, four preHD and three manifest HD subjects, where the seven (pre)manifest subjects were compared to the controls. In another study by Sritharan et al.[20] with 17 controls and 18 manifest HD subjects, no longitudinal change in the MD of the caudate, putamen, thalamus and corpus callosum could be demonstrated over a one year period, while baseline MD was significantly higher in the caudate and putamen of subjects with manifest HD compared to controls. A similar finding in MD was reported by Vandenberghe et al.[18] in eight manifest HD subjects over a two year period. Results from the present study are in agreement with findings from the latter two studies, with significant cross-sectional differences found in combination with a lack of significant longitudinal differences in the evolution of these measures within the 2-year study-period. The lack of longitudinal differences in the diffusion profile between the groups in this study could be due to a low sensitivity of this approach in detecting small changes over time or due to a true absence of observable significant alterations of this profile using DTI in the 2-year time frame.

Relationships between neurocognitive and diffusivity measures were demonstrated in our study. The SDMT and SWR scores were associated with some diffusivity measures, where the SDMT seemed to be more readily associated with WM diffusivity measures, while SWR showed associations only with GM AD and MD. The only exception to this pattern in the whole-brain analysis, was the inverse relationship found between SDMT scores and GM AD values. These findings are important in light of selecting the most suitable cognitive measures to assess, depending on the prime target of a treatment intervention. The SDMT, considered to be a measure for information-processing speed and working memory, has also been found to be

more associated with white than grey matter lesions in multiple sclerosis.[50] In the current study, the SDMT provided for the best predictive value for baseline diffusivity measures, as reflected by both the magnitude as well as the statistical significance of these associations. As was the case in the recent study by Poudel et al.,[51] we found a significant inverse relationship between SDMT and WM RD in HD. Our results did not, however, reproduce their finding for the same inverse relationship with SWR. In the striatum, an inverse relationship was found only between the SDMT score and AD. This finding is reinforced by the recent morphometric analysis report in preHD by Harrington et al.,[52] where the SDMT score was found to be positively associated with putaminal volume.

Additional findings from our interhemispheric subanalysis of diffusion parameters revealed very small, though highly significant interhemispheric differences in diffusivity measures within the groups. There were, however, no indications for a preferential degeneration to the dominant hemisphere in (pre)HD subjects, as no significant group differences were found in interhemispherical diffusion parameters. To the best of our knowledge this is the first study exploring this hypothesis using DTI in (pre)HD subjects. Interhemispheric variations in diffusivity measures in the healthy human brain have been previously reported.[53,54]

It should be stressed that inferral of underlying alterations to biological substance through changes in eigenvalues is not trivial, especially in GM.[55,56] As such, it is quite challenging to draw solid conclusions about underlying neuropathology based on diffusion parameters. The progressive histopathological features of HD are numerous. Disturbed membrane systems of neurons, with derangement of all membranes that form the cell were found in a histological study by Tellez-Nagel et al.[57] Loss of small spiny neurons in the caudate and putamen with subsequent astrocytosis,[58] and decreased neuronal densities with increased oligodendroglial densities,[59] the latter found already in preHD,[60] have been described. The primary role of the oligodendrocyte is providing myelin to neuronal axons. In HD mouse models, inhibition of the peroxisome-proliferator-activated receptor gamma coactivator 1 α in oligododrocytes by mutant huntingtin was found to be responsible for abnormal myelination.[61] WM atrophy due to myelin breakdown is supported by histological and imaging examinations in HD subjects.[62] Significantly reduced total brain, GM and WM volumes through atrophy have been demonstrated through a post mortem study in seven HD brains.[63] These various, diverse changes could result in a competing influence on the diffusion tensor model based on the individual contributions and timing of each change. In a DTI-histological study of the quinolinic acid rat model of HD, Van Camp et al.[64] demonstrated that DTI was more sensitive in detecting subtle changes in the affected structures compared to histology. In that study, increases in MD, AD, and RD were detected six weeks after neurotoxin infusion as compared to the sham injected control group, with histological findings of necrotic cells involvement with shrunken cytoplasm and spongiosis.

In this study, the pattern found in the manifest HD group of higher MD, AD, and RD values without substantial changes to FA, likely reflects an increase in tissue permeability, extra- cellular space fluid and interaxonal spacing due to neural tissue loss,[65,66] allowing the three eigenvalues to grow

proportionally due to faster diffusion of water, hereby effecting only the size of the tensor without influencing its shape.[16] This pattern of diffusivity changes, which has been associated with chronic WM degeneration,[67,68] has previously been reported in HD[48] and other neurodegenerative disorders, such as amyotrophic lateral sclerosis[69] and hereditary spastic paraplegia.[70] Findings from the histologically verified DTI study of the quinolinic acid rat model of HD, suggest that this pattern could point to cytoplasmic alterations and spongiosis.[64] In our complete preHD cohort, only WM AD showed a significantly raised value compared to controls. Increased AD may indicate WM axonal atrophy and was suggested to be useful in identifying early changes in persons with a high risk at developing Alzheimer's disease, prior to cognitive decline.[71] Taken together, these findings suggest that both axonal degeneration as well as demyelination play an important role in WM pathophysiology of HD and are present throughout the entire brain. Given that the earliest detected abnormality is represented in the WM AD in preHD subjects, this could indicate that axonal degeneration precedes myelin abnormalities in WM at this stage of the neurodegenerative process, reinforcing findings by Hobbs et al.[48] and further supporting this hypothesis. The GM diffusivity findings presented here suggest that tissue boundaries become less well defined in the cortical ribbon and the striatum in HD.[55]

Strengths of this study include the longitudinal design which has the advantage of evaluating the evolution of diffusivity measures in a well-defined study group with a similar between-scan interval. All scans were acquired on the same scanner using the same protocol, which keeps test-retest variation in DTI to a minimum.[72] Exploration of the full tensor behaviour is a further strength, as demonstrated by the better sensitivity in revealing differences between the groups in this study relative to FA characteristics. For the whole-brain analyses we applied an automated histogram analysis, which reduces user error and provides a more suitable standardized analysis method in multicentre study settings. The limitation presented with whole-brain analysis is the loss of topographic information. Also, proper interpretation of the underlying biological causes to alterations found in the diffusion profile remains restricted, as many different fiber orientations are found in diffusion images of the brain.[73] That does not, however, preclude the ability of assessing the value of this type of analysis for identifying biomarker potential and tracking disease-related modifications to the diffusion profile in time. This limitation was nonetheless addressed by applying this analysis specifically to the striatum. A further limitation was the relatively low number of manifest participants. This was mainly driven by disease progression in the cohort, where longitudinal scans or the ability to comply with study protocol deemed impossible, leaving the outcome measures presented here to more likely be an underestimation of the true extent of diffusion disturbances in the HD brain.

To conclude, alterations in cross-sectional diffusion profiles between manifest HD subjects and controls were evident both in whole-brain and striatum. In preHD, only AD alterations were found, a finding that applied only to preHD-B upon group stratification. This suggests that AD may be a sensitive marker for early change in HD gene carriers prior to disease manifestation. The individual diffusivities proved to be more sensitive in distinguishing pathologic microstructural alterations to the HD brain than FA characteristics. This study showed no longitudinal differences in any

of the diffusivity measures between the groups. Larger study samples could provide additional information on the longitudinal biomarker potential of DTI measures. However, based on the results presented here, this potential is expected to be limited.

Acknowledgments

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Supplementary material

Peak heights of whole-brain white matter

At baseline, peak heights of whole-brain WM histograms were generally lower in the manifest HD group compared to controls and preHD subjects (Supplementary Table II). In the manifest HD group, significantly lower peak heights were found for MD and RD. These differences remained significant upon Bonferroni correction (both at $p \leq 0.01$).

In the preHD group, histogram peak heights were similar to controls. Dividing the preHD group in preHD-A and preHD-B revealed a significantly lower value in peak height of the RD of the preHD-B group compared to controls ($p \leq 0.05$). This difference did not survive Bonferroni correction.

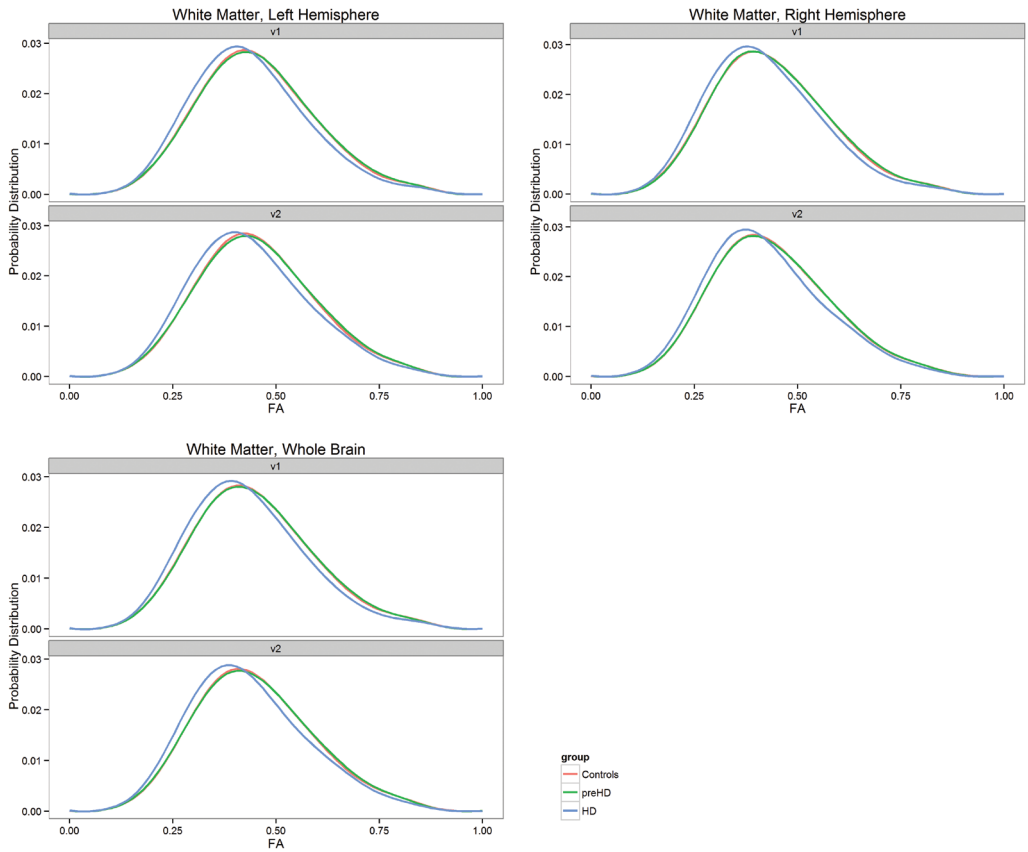
No significant longitudinal differences were found in the degree of whole-brain WM peak height change in any of the measures between the groups (without correction for multiple testing).

Peak heights of whole-brain grey matter and striatum

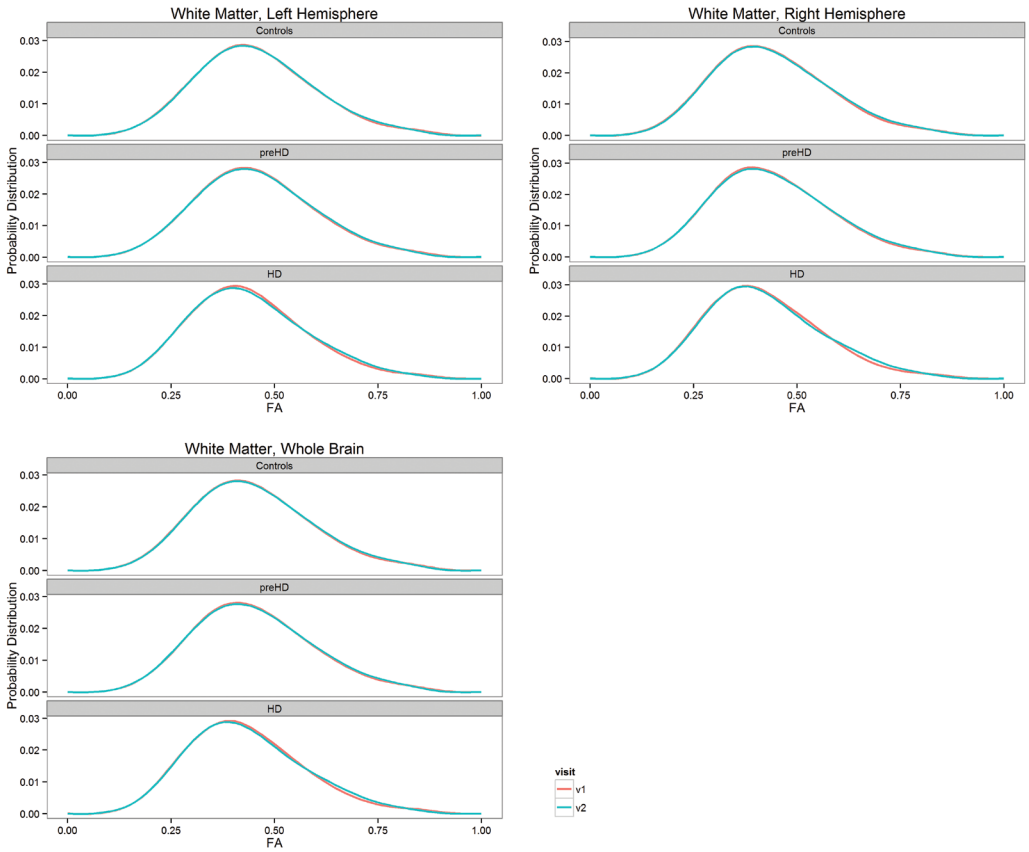
At baseline, histogram peak heights of whole-brain GM MD and AD in the manifest HD group were significantly lower compared to controls and preHD subjects (Supplementary Table II; striatal data not shown). The difference in MD peak height did not survive correction for multiple testing, while AD peak height remained significant ($p \leq 0.001$). There was a trend towards a lower peak height of RD in manifest HD compared to controls and preHD ($p = 0.08$).

No significant baseline peak height differences were observed between preHD subjects and controls in whole-brain GM. Dividing the preHD group in preHD-A and preHD-B revealed a significantly lower value in AD peak height only in preHD-B compared to controls ($p = 0.05$), not surviving correction for multiple testing.

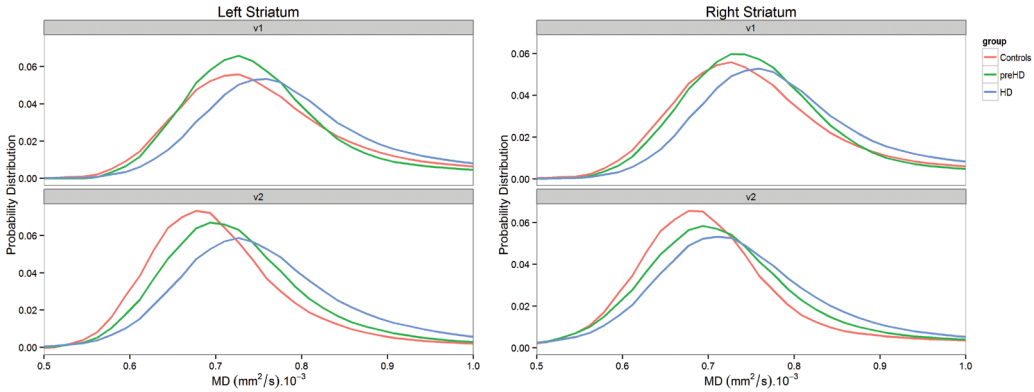
Baseline histogram peak heights of striatal MD and RD in subjects with manifest HD were significantly lower compared to controls and preHD subjects ($p \leq 0.01$ and $p = 0.03$, respectively). No significant peak height differences were observed between preHD subjects and controls in striatal diffusivity measures. Dividing the preHD group in preHD-A and preHD-B did not alter this result. No significant longitudinal differences were found in the degree of whole-brain GM nor in striatal histogram peak height change in any of the measures between the groups (without correction for multiple testing).



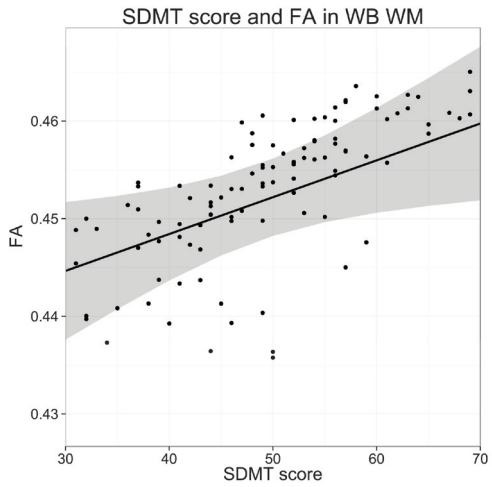
Supplementary Figure 1. *White matter fractional anisotropy (FA) histogram plots of the groups, per hemisphere and of whole brain, plotted against the visits. v1 = visit 1, v2 = visit 2.*



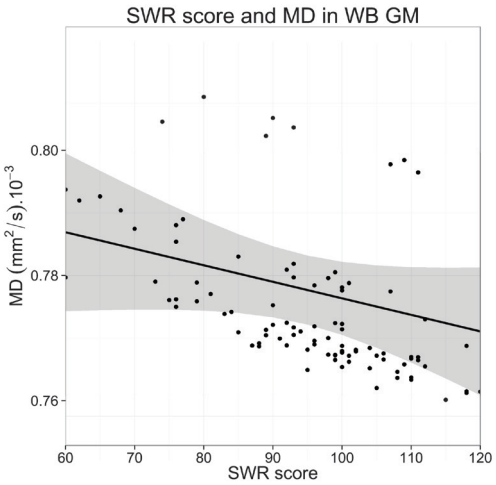
Supplementary Figure 2. *White matter fractional anisotropy (FA) histogram plots of the visits, per hemisphere and of whole brain, plotted per group. v1 = visit1, v2 = visit2.*



Supplementary Figure 3. *Separate plots for left and right striatal mean diffusivity (MD) histograms of the groups, plotted against the visits. v1 = visit 1, v2 = visit 2.*



Supplementary Figure 4. *Relationship plot of Symbol Digit Modalities Test (SDMT) score and whole brain (WB) white matter (WM) fractional anisotropy (FA). Data points shown are mixed model-based estimates.*



Supplementary Figure 5. *Relationship plot of Stroop Word Reading (SWR) task score and whole brain (WB) grey matter (GM) mean diffusivity (MD). Data points shown are mixed model-based estimates.*

Supplementary Table I. *Longitudinal change in clinical scores†, mean difference*

	Healthy controls	preHD (A and B)	Manifest HD
N	24	22‡	10
Total functional capacity, mean (SD)	-0.1 (0.5)	-0.2 (0.8)	-0.7 (1.7)
UHDRS-TMS, mean (SD)	-0.5 (2.3)	3.3 (4.5)*	8.4 (5.9)Φ
SDMT, mean (SD)	1.5 (5.8)	0.6 (6.3)	-2.0 (5.5)
SWR, mean (SD)	1.9 (8.7)	-4.1 (8.2)	-1.3 (10.9)
BDI-II, mean (SD)	-0.3 (3.7)	-1.4 (2.6)	-2.0 (4.1)

N = number of participants, SD = Standard deviation, UHDRS-TMS = Unified Huntington's Disease Rating Scale-Total Motor Score, SDMT = Symbol Digit Modalities Test, SWR = Stroop Word Reading task, BDI-II = Beck Depression Inventory-II.

*Significance at p ≤ 0.05 level: * significantly different from controls, Φ significantly different from controls and preHD.*

† Longitudinal change denotes scores from visit 1 subtracted from scores from visit 2.

‡ Including five subjects progressing to the early manifest stage during the two year follow-up period.

Supplementary Table II. *Mean DTI whole-brain peak height (shown $x10^3$ for readability). Data is shown as mixed model-based estimates of the group means corrected for age (S.E.)*

	Healthy controls	preHD (A and B)	Manifest HD
N	24	22	10
FA-WM	30.6 (1.2)	30.1 (1.6)	31.4 (1.7)
MD-WM	106.4 (7.3)	103.6 (10.0)	**96.8 (10.7)****
MD-GM	65.1 (5.1)	64.9 (6.7)	60.7 (7.1)*
AD-WM	31.1 (2.2)	34.6 (4.5)	34.3 (4.7)
AD-GM	64.4 (4.6)	62.5 (6.0)	**57.5 (6.4)*****
RD-WM	62.3 (3.0)	60.2 (4.1)	**58.6 (4.4)****
RD-GM	58.0 (4.7)	58.0 (6.1)	54.8 (6.5) (p=0.08)

**p ≤ 0.05 **p ≤ 0.01 ***p ≤ 0.001, bold values indicate sustained significant difference following Bonferroni correction (p ≤ 0.0125).*

FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity; WM = white matter; GM = grey matter.

Supplementary Table III. *Interhemispheric differences in DTI measures from visit 1; values shown as left minus right hemisphere. Differences in MD, AD and RD are shown x10³ for readability. Only right handed subjects are included*

	Healthy controls		preHD (A and B)		Manifest HD	
N	20		18		9	
	Mean (SD)	*P*	Mean (SD)	*P*	Mean (SD)	*P*
FA-WM	0.017 (0.006)	≤ 0.001	0.017 (0.006)	≤ 0.001	0.016 (0.006)	≤ 0.001
MD-WM	-0.006 (0.006)	≤ 0.001	-0.005 (0.007)	0.018	-0.010 (0.007)	0.003
MD-GM	-0.006 (0.008)	0.008	-0.003 (0.009)	0.205	-0.004 (0.011)	0.272
AD-WM	0.007 (0.010)	0.003	0.009 (0.012)	0.005	0.002 (0.009)	0.605
AD-GM	0.001 (0.010)	0.681	0.001 (0.010)	0.631	0.004 (0.014)	0.377
RD-WM	-0.013 (0.006)	≤ 0.001	-0.012 (0.006)	≤ 0.001	-0.016 (0.008)	≤ 0.001
RD-GM	-0.009 (0.007)	≤ 0.001	-0.008 (0.010)	0.003	-0.008 (0.016)	0.174

FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity; WM = white matter; GM = grey matter.