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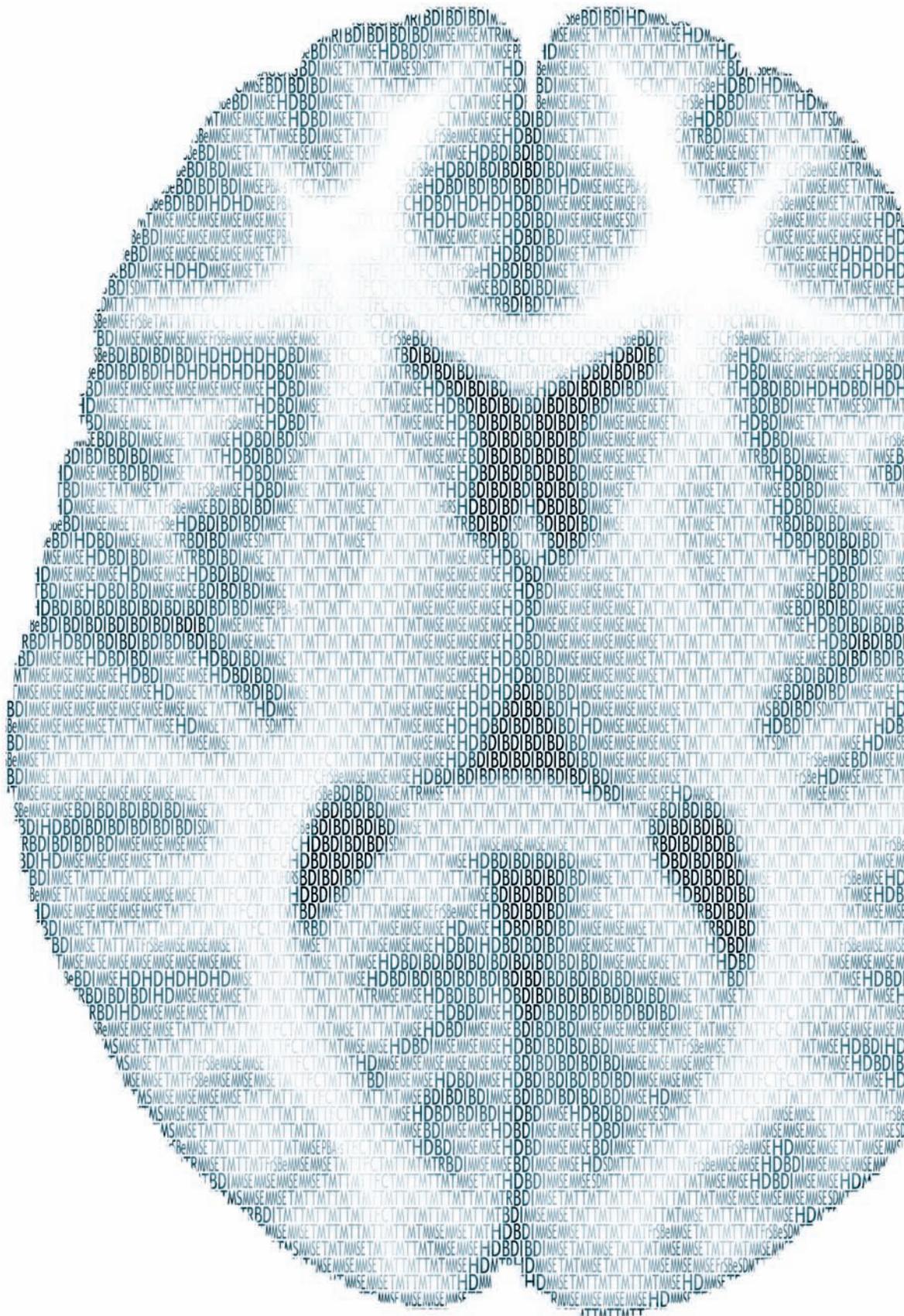


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# CHAPTER 8

## MAGNETIZATION TRANSFER IMAGING IN PREMANIFEST AND MANIFEST HUNTINGTON'S DISEASE: A 2 YEAR FOLLOW UP

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## Abstract

### Background and Purpose

Magnetization transfer imaging (MTI) is a quantitative MRI technique which has recently demonstrated structural integrity differences between controls and Huntington's disease (HD) patients. Potentially MTI can be used as a biomarker for monitoring disease progression. To establish the value of MTI as a biomarker, we aimed to examine the change of these measures during the course of HD.

### Method

From the Leiden TRACK-HD study 25 controls, 21 premanifest gene carriers and 21 manifest HD patients, participated at baseline and during a 2 year follow up visit. Brain segmentation of cortical grey matter, white matter, caudate nucleus, putamen, pallidum, thalamus, amygdala, and hippocampus was performed using FSL's automated tools FAST and FIRST. Individual MTR values were calculated from these regions and MTR histograms constructed.

### Results

In the premanifest HD group stage "far from disease onset" a significant *increase* in MTR peak height of the putamen was observed over time. During the manifest HD stage neither the mean MTR nor the MTR peak height showed a significant change during a 2 year follow up.

### Conclusion

MTI derived measures are not suitable for disease monitoring in Huntington's disease over a 2 year period as there is no decrease in structural integrity detected in any of the manifest HD groups longitudinally. The finding of increased putaminal MTR peak height in the premanifest "far from disease onset" group could relate to a pre-degenerative process, compensatory mechanisms or aberrant development, but should be interpreted with caution until future studies confirm this finding.

## Introduction

Magnetization transfer imaging (MTI) is a MRI technique which has been developed to perform structural imaging in a different, possibly more sensitive way, as it has demonstrated abnormalities in the normal appearing grey and white matter on conventional MRI in MS research<sup>1,2</sup>. The technique of MTI is based on the exchange of two pools of protons: one bound to macromolecular structures and one bound to free water molecules<sup>3</sup>. MTI has first and foremost been applied in patients with MS, however many other diseases have been studied using MTI, such as Alzheimer's disease<sup>4</sup>, Parkinson's disease<sup>5</sup> and Huntington's disease (HD)<sup>6,7</sup>.

Previous MRI studies in HD mainly focused on the search for a biomarker for monitoring disease progression. In this field lays a great opportunity to study the potential of MTI at very early stages of neurodegeneration. The genetic defect which is responsible for HD is located on the short arm of chromosome 4 and has an autosomal dominant inheritance pattern with full penetrance<sup>8</sup>. Therefore when the gene defect has been established in an individual, this inevitably leads to a certain clinical diagnosis at some point in their life. Mean disease onset is approximately 35-40 years of age with a wide range partly depended on the length of the abnormal CAG-repeat<sup>9</sup>. The ability to establish the gene defect well before any symptoms are present, gives an unique opportunity to study asymptomatic gene carriers, commonly referred to as "premanifest gene carriers"(PMGC). In previous structural MRI studies significant brain disturbances in terms of striatal atrophy<sup>10,11</sup>, white matter disturbances<sup>12,13</sup>, cortical thickness reductions<sup>14</sup> and whole brain volume reduction<sup>10,15</sup> have been described. These studies all used either conventional MRI or DTI. Some of these measures are well established, however no golden standard for disease monitoring by MRI is currently present. In essence, what technique and what region are most suitable as a biomarker? Possible problems in HD are non-linear and non-uniformly affected brain regions. The need for further research into a robust and sensitive MRI measure to monitor disease onset and progression is still evident.

Previous work in HD using MTI consists of four reports, all cross-sectional in design<sup>6,7,16,17</sup>. The main outcome measures used were mean Magnetization Transfer Ratio (MTR) and MTR peak height and are thought to represent structural integrity<sup>17-19</sup>. Mean MTR represents the average MTR value of all voxels in a region of interest, with a lower mean MTR corresponding to loss of tissue integrity. MTR peak height reflects the most frequently occurring MTR value in a region of interest when all the MTR values are set out in a MTR histogram, again with

lower peak height being associated with loss of structural integrity. Results from these previous studies show reduced structural integrity in manifest HD patients (MHD) compared to healthy controls or PMGC in multiple regions, namely the white matter, cortical grey matter, caudate nucleus, putamen, pallidum, thalamus and amygdala. The results are suggestive of the potential of MTI as a biomarker<sup>6,7</sup>. However no longitudinal reports are available.

TRACK-HD is specifically designed to determine the most valuable measures to monitor disease progression<sup>20</sup>. This study allows following both PMGC as MHD over several years. To determine the true value of any potential (MRI) biomarker, longitudinal confirmation is crucial. Therefore we aim to examine whether MTI measures change over a 2 year period during the progressive course of HD. Secondly, if any longitudinal change in a group is present, we sought to determine the correlation to clinical measures of disease progression.

## Methods

### Subjects

Participants were recruited from the Leiden site of the TRACK-HD study. From the baseline cohort (n=90) in 2008 a total of 78 participants could be included. This cohort consisted of three groups; 28 healthy controls, 25 PMGC and 25 MHD. Inclusion criteria for the PMGC consisted of genetically confirmed expanded CAG repeat  $\geq 40$ , a disease burden score (calculated as:  $((\text{CAG repeat length} - 35.5) \times \text{Age})$  of  $>250$ <sup>21</sup> and absence of motor abnormalities on the UHDRS, defined as a TMS of  $\leq 5$ . Inclusion criteria for MHD consisted of genetically confirmed CAG repeat  $\geq 40$ , presence of motor abnormalities on the UHDRS-TMS of  $>5$ . Also a TFC of 7 or higher was required to ensure that the MHD group was in the early disease stage. Subdivision of the premanifest group was made on the basis of their expected years to onset, calculated by the formula from Langbehn *et al.* (2004)<sup>9</sup>. This results in a premanifest HD group “far from expected disease onset” (PreHD-A) and premanifest HD group “close to expected disease onset” (PreHD-B). Subdivision of the manifest group was made by their staging according to the Shoulson and Fahn Scale, based on the TFC score, resulting in a manifest HD group stage 1 (HD 1) and manifest group stage 2 (HD 2). HD 1 describes a group of manifest HD in the earliest stage after disease onset, with only minor symptoms. HD 2 is the next stage in the disease with increased symptoms and impact on daily activities, but these patients are still considered “early manifest”. Healthy gene negative family members, spouses or partners were recruited as control subjects. Exclusion criteria consisted of significant (neurological) co-

morbidity, active major psychiatric disturbance and MRI incompatibility. Full details on recruitment are available in the TRACK-HD baseline paper<sup>10</sup>. Local IRB approval and written informed consent were obtained from all participants.

The same cohort was scanned 24 months later within a 6 week window of their follow up date. Of the 78 participants, 67 participants were available for follow up scanning in 2010 with the MTI-protocol included. Reasons for unavailability included too advanced disease stage, time restraints on the full TRACK-HD scanning protocol and unspecified reasons of withdrawal.

### Imaging sequences

At both time points exactly the same study protocol was enforced. The scanning protocol described in our cross-sectional report<sup>6</sup> was performed in an identical manner two years after the baseline visit. In short all participants underwent scanning on a 3 Tesla Philips whole body scanner (Philips, Best, The Netherlands) with an 8-channel receive and transmit coil. 3D T1-weighted sequences (TR = 7.7ms, TE= 3.5ms, FA= 8°, matrix size 224x224x164 mm, voxel size 1.0x1.0x1.0mm, acquisition time ~9min) and 3D gradient MTI sequences (TR = 100 ms, TE = 11 ms, FA = 9°, matrix 224x180x144 mm, voxel size 1.0x1.0x7.2 mm, acquisition time ~3min) were acquired. For the MTI sequences two consecutive imaging sets were acquired, one with and one without a saturation pulse. A sinc pulse of 25 ms with a maximal B1 of 10 uT and 2 sidelobes on an off-resonance frequency of 1100 Hz was applied. Total scanning time for T1-w and MTI sequences was maximally 12 minutes.

### Post-processing

The post-processing pipeline was identical to that described previously in the cross sectional result paper<sup>6</sup>. T1-weighted images were segmented using FAST<sup>22</sup> and FIRST<sup>23</sup> from FMRIB's Software Library (Oxford, United Kingdom)<sup>24</sup>. This provided individual brain masks for: total white matter, cortical grey matter, caudate nucleus, putamen, pallidum, thalamus, amygdala, hippocampus and whole brain. To correct for possible partial volume effects, an eroded mask of these segmentations was created by removing one voxel in plane for all above named VOI's. All brain masks were then registered to the MTI volumes using the transform obtained from linear registration of the T1-w volume with 7 degrees of freedom (FSL-FLIRT). MTR was calculated per voxel as  $M0 - Ms / M0$ , whereby Ms is the saturated image and M0 the unsaturated image. The mean MTR per VOI was calculated. Additionally, to represent the variations of voxel based MTR within each VOI we constructed MTR histograms. The MTR peak height was normalized for the size of the volume

of interest. MTR peak height and mean MTR were the primary outcome variables. For correction of the (largely unknown) influence of age on MTI values, the MTR parameters were calibrated to the control values, assuming no changes in the control subjects. This entailed scaling the individual premanifest and manifests values according to the mean MTR parameter difference between baseline and follow up of the control group, which could either be an increase or a decrease over time. In the supplementary materials (table 3) actual values of the control group on both time points are available.

To compare the biomarker potential of MTI to volumetric analysis, volumes were calculated for all subcortical regions, white matter volume and whole brain volume using the FSL tools of FIRST<sup>23</sup> and SIENAX<sup>25</sup>. The volume calculation and a correction for intracranial volume was performed as described previously<sup>11</sup>.

## Clinical Measures

A total measure of motor dysfunction was obtained with the UHDRS-TMS (range 0-124). TFC score (range: 0-13) and MMSE for global assessment of cognitive functioning (range: 0-30) were obtained. Cognitive scores included the total scores from the SDMT and the Stroop word reading card. For assessment of psychiatric disturbances the PBA-s was used. For a more detailed description of these clinical assessments see Tabrizi *et al.* (2009)<sup>10</sup>.

## Statistics

To examine the longitudinal change in clinical, MTI and volumetric values (corrected for intracranial volumes) per group, paired T-testing per group was used. Alpha was set to 0.05 to be significant. Secondly, to account for multiple testing (15 regions) a Bonferroni correction was applied, resulting in an alpha < 0.0033 for the strongest findings. For correlation analysis between significant longitudinal findings, a Pearson correlation was applied to the difference between the 2 time points of the clinical and MTI values.

## Results

Group characteristics and clinical measures on both the baseline visit as the follow up visit are shown in table 1. A significant longitudinal increase in motor disturbances is evident in PreHD-B, HD 1 and HD 2. Global functioning as measured by the TFC significantly decreases in 2 years in the HD 2 group as does the cognitive measure of Stroop word reading.

**Table 1: Group characteristics for controls, premanifest HD groups A +B and Manifest HD groups 1 and 2.**

		Controls	PreHD-A	PreHD-B	Manifest 1	Manifest 2
<b>N</b>		25	10	11	9	12
<b>Age</b>	baseline	48.3 (7.6)	45.5 (5.2)	42.9 (11.2)	47.7 (11.8)	50.9 (9.4)
<b>CAG</b>	baseline	n.a.	41.3 (1.3)	44.0 (3.1)	43.8 (3.5)	43.2 (1.9)
<b>YTO</b>	baseline	n.a.	13.8 (3.8)	8.4 (1.6)	n.a.	n.a.
<b>TMS</b>	baseline	2.4 (2.4)	2.3 (1.7)	3.0 (1.1)	16.9 (8.8)	26.2 (11.7)
	follow up	1.7 (1.4)	6.0 (6.9)	6.3 (2.5) *	23.0 (9.0) *	37.8 (14.3) *
<b>TFC</b>	baseline	12.9 (0.2)	12.7 (0.7)	12.6 (0.9)	12.0 (1.0)	8.6 (1.2)
	follow up	12.8 ( )	12.5 (0.9)	12.4 (1.2)	11.2 (2.0)	6.0 (3.3) *
<b>MMSE</b>	baseline	29.2 (1.2)	29.0 (1.2)	28.4 (1.9)	29.0 (0.9)	26.5 (3.1)
	follow up	29.1 (1.2)	29.3 (0.8)	28.6 (1.7)	28.7 (1.1)	27.0 (3.9)
<b>SDMT</b>	baseline	50.1 (9.6)	52.7 (7.4)	46.9 (12.0)	42.0 (8.1)	31.2 (11.1)
	follow up	51.5 (10.8)	52.7 (9.5)	48.3 (9.9)	39.6 (10.7)	27.9 (13.3)
<b>Stroop</b>	baseline	99.8 (13.4)	97.1 (10.5)	86.9 (16.3)	88.4 (13.6)	69.4 (21.5)
	follow up	102.7 (16.9)	92.5 (7.5)	83.4 (19.3)	89.8 (18.9)	55.6 (22.6) *
<b>PBA-s</b>	baseline	6.4 (8.4)	6.7 (8.7)	7.4 (7.1)	11.7 (11.7)	14.0 (11.8)
	follow up	6.6 (9.0)	6.1 (11.9)	7.8 (9.70)	9.7 (11.7)	22.6 (16.3)

Values are Mean (SD). PreHD-A = premanifest HD far from expected disease onset, PreHD-B premanifest HD close to expected disease onset, CAG = CAG-repeat length, YTO = expected years to disease onset, TMS = total motor score, TFC = total functional capacity, MMSE = mini mental state exam, SDMT = symbol digit modality test, Stroop = stroop word reading test, PBA = problem behaviour assessment - short version.\* =  $p < 0.005$  significant longitudinal change

All MTI-values for 15 VOI's are displayed in the supplementary material table 1 for the premanifest HD groups and in supplementary material table 2 for the Manifest HD groups. In PreHD-A five subcortical grey matter regions show an *increase* in MTI parameters, namely the mean MTR of the right caudate nucleus ( $p=0.049$ ), the MTR peak height of the right putamen( $p=0.003$ ), MTR peak height of the left pallidum ( $p=0.032$ ), mean MTR of the right thalamus ( $p=0.047$ ) and MTR peak height of the right amygdala ( $p=0.050$ ). However, after Bonferroni correction, the only statistically significant variation was the MTR peak height of the right putamen. In the PreHD-B group the cortical grey matter shows a longitudinal reduction in mean MTR ( $p=0.020$ ) and the left hippocampus an increase in MTR histogram peak height ( $p=0.037$ ). For the HD 1 group the right amygdala mean MTR decreases ( $p=0.036$ ) in the 2 year follow up period. Only the left amygdala

mean MTR shows a reduction ( $p=0.022$ ) in the HD 2 group. None of the results in the PreHD-B, HD 1 or HD 2 group are statistically significant after correction for multiple comparisons.

The statistical analysis procedure was also applied to a 3 group division instead of the 5 group division, encompassing the total premanifest group (PreHD A + B) and the total manifest group (HD 1 + 2) and the control group. This was performed in order to increase the power of the study, however in essence no additional information can be gathered from this analysis. The supplementary material contains exact details of the findings. The results also display subcortical MTR parameter increases in the premanifest group and a decrease in MTR parameters of the amygdala in the manifest HD group. The subdivision of groups is therefore more informative as it gives more exact disease stage related changes.

Correlation analysis of the significant longitudinal findings (without correction for multiple comparison) in MTI values with clinical measures resulted in two significant findings, namely in the PreHD-B group the MTR peak height of the left hippocampus correlated to TFC reduction ( $R = -.622, p= 0.043$ ) and in the HD 2 group the reduction of mean MTR correlated to the reduction in SDMT performance ( $R = -.667, p= 0.018$ ). Both findings did not survive Bonferroni correction for multiple comparisons.

All volumetric values are shown in the supplementary materials table 3. Significant volumetric decline of several VOI's was seen in premanifest (caudate nucleus and putamen) and manifest groups (caudate nucleus, putamen, thalamus, white matter and whole brain).

## Discussion

The main finding of this study is the fact that MTI parameters do not change over a two year follow up period in manifest HD. The expected decline in structural integrity in this group could not be detected with MTI. However an interesting finding in the premanifest “far from expected disease onset” was made, namely an increase in MTR value in the putamen, which possibly can be interpreted as a form of aberrant development or compensatory mechanism. There is no relationship of changing MTR parameters to increasing clinical symptoms in any of the groups. Clinical progression was evident in premanifest close to disease onset group and the manifest HD groups.

Previous cross-sectional observations with MTI in HD showed promising results for both mean MTR as MTR histogram peak height for these measures to serve as a disease monitoring biomarker<sup>6,7,17</sup>. Mean MTR was found to be lower in several brain regions in manifest HD<sup>6</sup> or in a combined cohort of premanifest and manifest HD<sup>7</sup> compared to controls. Also MTR peak height in several regions was found to be lower in manifest HD<sup>6</sup> and, although there was no group difference, the MTR peak height related to subtle motor abnormalities and higher CAG repeat length in a premanifest HD cohort<sup>17</sup>. To date no longitudinal MTI studies in HD were available. In fact there is relatively little longitudinal research of MTI values available at all. Only in multiple sclerosis, optic neuritis and systemic lupus erythematosus reports are available, suggesting a good potential for MTI to examine both grey and white matter as lesion evolution<sup>26-29</sup>. In normal aging Ge et al. (2008) described, via a histogram analysis, reduction of MTR values after the age of 40 and significant group differences after the age of 50 years<sup>30</sup>. The groups in our study span exactly this age range. We therefore calibrated our MTR values of the HD groups to the control values.

The goal of this study was to examine the potential of MTI to detect loss of structural integrity in HD to ultimately serve as an outcome measure in future therapeutic trials, as is the overall main goal of the TRACK-HD study<sup>10</sup>. This current study and the recently published 24-month analysis of the main TRACK-HD study demonstrate that volumetric MRI measures are sensitive for detecting change over a 2 year period<sup>31</sup>. In our MTI study no statistically strong changes were seen in any of the regions. Three regions did show longitudinal reduction, namely the cortical grey matter in PreHD-B and the amygdala in HD 1 and 2, however these findings did not survive correction for multiple comparison, thus should be interpreted with extreme caution. These regions are shown to be affected at these disease

stages<sup>10;11</sup>, however other regions such as the caudate nucleus or putamen or pallidum are known to be more severely affected in terms of atrophy. Noteworthy is the fact that MTR histogram peak height was normalised for volume and mean MTR is non-dependent on atrophy; only the number of voxels from which this mean is calculated could have an influence. The measures used are therefore relatively non-influenced by atrophy. We must conclude from our study, based on the group comparisons of MTI-parameters, group comparisons of volumes and the lack of correlation of MTI to clinical measures, that MTI is inferior to volumetric measures to serve as a biomarker to detect longitudinal change in a 2 year follow up. It is noteworthy to mention that clinical trials from a practical point of view will not last longer than two years, hence a longer follow up period may be of interest scientifically, but the impact on biomarker research in HD will be limited.

The interesting finding in this study in PreHD-A is that an increase in MTI values was observed. This pattern was seen in 5 subcortical grey matter structures, with the finding of increased MTR histogram peak height in the putamen, one of the most heavily involved structures in HD, surviving the stringent correction for multiple comparisons. The explanation of this increase could be sought in an earlier postulated theory by Paulsen et al. They gave two possible explanations when their group detected increased cortical volume in premanifest HD. These explanations consist of either a predegenerative process, such as swelling of tissue, or alternatively it may be a reflection of aberrant brain development or maturation<sup>32</sup>.

Furthermore, evidence from fMRI studies point towards another possible explanation. Two reports exist on increased activation in premanifest HD, which is thought to represent cortical recruitment as compensatory strategies, this was specifically true for premanifest HD far from expected onset and not in the close to onset group<sup>33;34</sup>. In our view any of these proposed explanations could be true, however we should be extremely cautious not to overly interpret the findings as the correction for multiple comparisons reduced the number and strength of the findings. This finding could however lead to broader examination of MTI and other MRI measures in the premanifest far from disease onset group as this potentially can lead to better understanding of the neuropathology in HD.

Limitations of our study lay in the fact that not all participants were retained for the follow up period. However, those lost to follow up were evenly divided over the groups; hence we do not believe this ultimately influenced our results. Furthermore the group sizes were relatively small, possibly suggesting the study could be

underpowered, however combining the groups did not result in any additional findings. On the other hand aggregated groups could be too heterogeneous to yield additional significant findings.

## Conclusion

We believe MTI derived measures are not suitable for disease monitoring in HD as there is no significant decrease in structural integrity in any of the groups over two years and there is no significant relation to clinical measures. The finding of increased MTI measures in the premanifest far from disease onset group could relate to a pre-degenerative process, compensatory mechanisms or aberrant development, but should be interpreted with caution until confirmation of these findings in future studies is made.

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## Supplementary Material

### Results

#### *Control values*

The control values displayed a significant change over time in three regions, as shown in table 4. In the left thalamus and the left caudate nucleus a significant decrease in mean MTR was seen and in the right putamen a significant increase in mean MTR was observed. To ensure that our most significant longitudinal finding, namely MTR peak height of the right putamen, was not affected by the potential influence of calibration, we also examined the difference over time of this value without calibration to controls. As expected, the MTR peak height from the right putamen in the premanifest far from onset group also displayed a very significant ( $p=0.005$ ) increase over the 2 year follow up.

#### *Premanifest versus manifest in three group division*

When the two premanifest and two manifest groups were combined there were six noteworthy increases: the MTR peak height of the right putamen ( $p=0.010$ ), the MTR peak height of the left pallidum ( $p=0.001$ ), MTR peak height of cortical grey matter ( $p=0.024$ ), mean MTR of the left amygdala ( $p=0.024$ ) and mean MTR of the right thalamus ( $p=0.048$ ). Furthermore a decrease is seen in mean MTR of the left caudate nucleus ( $p=0.030$ ) and in the manifest HD group the left and right amygdale show a decrease in mean MTR (left amygdale  $p=0.042$ , right amygdale  $p=0.025$ ). However, when the Bonferroni correction for multiple testing was applied, only the increase in MTR peak height of the left pallidum remains statistically significant.

**Table 1: MTI derived values in 14 different regions on both baseline and follow up visit for the premanifest HD A and premanifest HD B group.**

	Premanifest A					Premanifest B				
	Baseline		Follow up		$\rho$	Baseline		Follow up		$\rho$
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
<b>White matter</b>										
Peak height	1.144	0.161	1.176	0.223	0.567	1.092	0.258	1.188	0.134	0.172
Mean MTR	0.387	0.013	0.389	0.010	0.249	0.388	0.017	0.388	0.010	0.903
<b>Cortical grey matter</b>										
Peak height	0.752	0.107	0.796	0.109	0.091	0.685	0.136	0.727	0.111	0.152
Mean MTR	0.326	0.014	0.329	0.013	0.079	0.325	0.012	0.319	0.013	<b>0.020</b>
<b>Caudate Right</b>										
Peak height	0.852	0.133	0.910	0.184	0.320	0.849	0.182	0.838	0.169	0.804
Mean MTR	0.339	0.018	0.346	0.020	<b>0.049</b>	0.333	0.031	0.340	0.025	0.353
<b>Caudate Left</b>										
Peak height	0.974	0.245	1.046	0.085	0.367	0.964	0.199	1.013	0.146	0.480
Mean MTR	0.357	0.021	0.351	0.016	0.308	0.359	0.024	0.346	0.022	0.062
<b>Putamen Right</b>										
Peak height	1.132	0.235	1.375	0.206	<b>0.003</b>	1.338	0.354	1.409	0.241	0.396
Mean MTR	0.329	0.015	0.335	0.012	0.160	0.330	0.031	0.334	0.021	0.312
<b>Putamen Left</b>										
Peak height	1.291	0.199	1.340	0.181	0.614	1.298	0.314	1.433	0.295	0.091
Mean MTR	0.352	0.013	0.356	0.015	0.119	0.353	0.031	0.356	0.024	0.494
<b>Pallidum Right</b>										
Peak height	1.569	0.333	1.740	0.272	0.112	1.617	0.318	1.701	0.285	0.400
Mean MTR	0.381	0.006	0.382	0.007	0.740	0.382	0.021	0.387	0.013	0.300
<b>Pallidum Left</b>										
Peak height	1.471	0.285	1.721	0.218	<b>0.032</b>	1.528	0.310	1.709	0.223	0.086
Mean MTR	0.386	0.010	0.386	0.009	0.958	0.383	0.019	0.392	0.013	0.094
<b>Thalamus Right</b>										
Peak height	1.013	0.139	1.083	0.159	0.142	1.003	0.204	0.999	0.167	0.946
Mean MTR	0.359	0.015	0.367	0.018	<b>0.047</b>	0.352	0.028	0.359	0.021	0.398
<b>Thalamus left</b>										
Peak height	1.166	0.279	1.197	0.098	0.717	1.116	0.222	1.196	0.167	0.332
Mean MTR	0.372	0.018	0.380	0.014	0.090	0.371	0.020	0.375	0.020	0.467
<b>Amygdala Right</b>										
Peak height	1.297	0.116	1.405	0.191	<b>0.050</b>	1.259	0.327	1.279	0.240	0.837
Mean MTR	0.357	0.017	0.358	0.011	0.817	0.364	0.027	0.351	0.019	0.164
<b>Amygdala Left</b>										
Peak height	1.530	0.190	1.613	0.212	0.236	1.304	0.231	1.486	0.325	0.064
Mean MTR	0.374	0.016	0.370	0.015	0.506	0.377	0.026	0.364	0.018	0.200
<b>Hippocampus Right</b>										
Peak height	1.346	0.219	1.298	0.224	0.586	1.164	0.266	1.198	0.288	0.637
Mean MTR	0.360	0.015	0.363	0.009	0.635	0.365	0.021	0.355	0.017	0.124
<b>Hippocampus Left</b>										
Peak height	1.010	0.195	1.059	0.170	0.558	0.888	0.188	1.088	0.197	<b>0.037</b>
Mean MTR	0.381	0.022	0.387	0.018	0.347	0.380	0.018	0.373	0.013	0.317
<b>Whole brain</b>										
Peak height	0.923	0.123	0.963	0.141	0.198	0.864	0.181	0.936	0.094	0.118
Mean MTR	0.352	0.013	0.355	0.011	0.110	0.353	0.013	0.350	0.010	0.297

MTR = Magnetization Transfer Ratio. **Bold** =significant finding at  $p \leq 0.05$

**Table 2: MTI derived values in 14 different regions on both baseline and follow up visit for the manifest HD 1 and manifest HD 2 group.**

	Manifest 1					Manifest 2				
	Baseline		Follow up		<i>p</i>	Baseline		Follow up		<i>p</i>
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
<b>White matter</b>										
Peak height	0.747	0.274	0.824	0.243	0.388	0.866	0.263	0.916	0.245	0.560
Mean MTR	0.372	0.025	0.384	0.013	0.121	0.380	0.016	0.379	0.014	0.713
<b>Cortical grey matter</b>										
Peak height	0.589	0.174	0.587	0.137	0.961	0.602	0.127	0.632	0.097	0.239
Mean MTR	0.319	0.014	0.316	0.015	0.264	0.311	0.017	0.311	0.013	0.928
<b>Caudate Right</b>										
Peak height	0.726	0.234	0.704	0.182	0.762	0.777	0.204	0.703	0.200	0.371
Mean MTR	0.331	0.034	0.344	0.039	0.321	0.323	0.034	0.339	0.032	0.123
<b>Caudate Left</b>										
Peak height	0.784	0.240	0.798	0.156	0.852	0.833	0.244	0.898	0.236	0.311
Mean MTR	0.339	0.045	0.338	0.045	0.930	0.346	0.039	0.346	0.037	0.999
<b>Putamen Right</b>										
Peak height	0.877	0.326	1.018	0.287	0.152	0.961	0.282	1.082	0.334	0.221
Mean MTR	0.330	0.029	0.338	0.024	0.276	0.329	0.018	0.323	0.029	0.455
<b>Putamen Left</b>										
Peak height	0.941	0.332	1.037	0.178	0.415	1.029	0.295	1.177	0.275	0.106
Mean MTR	0.346	0.026	0.352	0.031	0.720	0.360	0.015	0.353	0.033	0.348
<b>Pallidum Right</b>										
Peak height	1.178	0.466	1.325	0.359	0.203	1.307	0.409	1.356	0.394	0.714
Mean MTR	0.396	0.039	0.389	0.018	0.565	0.384	0.021	0.394	0.019	0.146
<b>Pallidum Left</b>										
Peak height	1.138	0.366	1.240	0.211	0.321	1.237	0.364	1.370	0.282	0.268
Mean MTR	0.376	0.047	0.396	0.026	0.237	0.399	0.012	0.392	0.023	0.308
<b>Thalamus Right</b>										
Peak height	0.825	0.297	0.826	0.208	0.988	0.874	0.234	0.838	0.205	0.667
Mean MTR	0.349	0.035	0.360	0.038	0.396	0.335	0.042	0.358	0.028	0.079
<b>Thalamus left</b>										
Peak height	0.884	0.311	0.901	0.183	0.856	0.933	0.285	0.993	0.280	0.477
Mean MTR	0.346	0.054	0.367	0.045	0.230	0.354	0.044	0.373	0.038	0.214
<b>Amygdala Right</b>										
Peak height	1.076	0.366	1.100	0.336	0.875	1.131	0.277	1.080	0.325	0.536
Mean MTR	0.361	0.019	0.333	0.029	<b>0.036</b>	0.351	0.024	0.349	0.017	0.686
<b>Amygdala Left</b>										
Peak height	1.128	0.344	1.298	0.388	0.102	1.198	0.265	1.121	0.228	0.434
Mean MTR	0.377	0.042	0.345	<b>0.036</b>	0.157	0.369	0.020	0.354	0.015	<b>0.022</b>
<b>Hippocampus Right</b>										
Peak height	0.974	0.264	0.976	0.233	0.994	1.097	0.235	0.948	0.249	0.131
Mean MTR	0.372	0.036	0.344	0.022	0.073	0.354	0.027	0.359	0.018	0.576
<b>Hippocampus Left</b>										
Peak height	0.717	0.226	0.798	0.181	0.142	0.957	0.256	1.062	0.232	0.350
Mean MTR	0.394	0.045	0.364	0.029	0.150	0.389	0.017	0.379	0.020	0.284
<b>Whole brain</b>										
Peak height	0.661	0.217	0.696	0.184	0.579	0.719	0.174	0.762	0.153	0.428
Mean MTR	0.343	0.016	0.347	0.014	0.098	0.342	0.016	0.343	0.012	0.728

MTR = Magnetization Transfer Ratio. Bold =significant finding at  $p \leq 0.05$

**Table 3: Volumetric analysis of 14 brain regions in premanifest and manifest HD**

	Premanifest A						Premanifest B						
	Baseline		Follow up		p		Baseline		Follow up		p		
	Mean	SD	Mean	SD			Mean	SD	Mean	SD			
<b>Caudate</b>	Right	3.907	0.428	3.705	0.482	0.004		3.897	0.339	3.739	0.353	<b>0.001</b>	
	Left	3.797	0.395	3.651	0.406	<b>0.001</b>		3.713	0.359	3.576	0.376	<b>0.002</b>	
<b>Putamen</b>	Right	4.832	0.619	4.680	0.603	0.009		4.582	0.497	4.397	0.525	<b>0.001</b>	
	Left	4.916	0.502	4.748	0.544	0.006		4.681	0.556	4.483	0.586	<b>0.002</b>	
<b>Pallidum</b>	Right	2.056	0.173	2.089	0.207	0.555		2.009	0.155	1.952	0.142	0.067	
	Left	1.931	0.205	1.890	0.226	0.199		1.995	0.134	1.925	0.175	0.081	
<b>Thalamus</b>	Right	8.155	0.545	8.122	0.468	0.566		7.718	0.637	7.595	0.741	0.044	
	Left	8.492	0.595	8.452	0.554	0.474		7.833	0.659	7.777	0.722	0.382	
<b>Amygdala</b>	Right	1.689	0.242	1.672	0.222	0.821		1.799	0.268	1.793	0.306	0.891	
	Left	1.772	0.266	1.752	0.259	0.578		1.711	0.229	1.733	0.234	0.626	
<b>Hippocampus</b>	Right	5.519	0.385	5.411	0.431	0.033		5.204	0.470	5.252	0.619	0.497	
	Left	5.315	0.358	5.392	0.319	0.034		4.925	0.750	4.962	0.853	0.552	
<b>White matter</b>		673.9	36.2	667.2	34.0	0.028		647.2	46.2	637.0	44.4	0.014	
<b>Whole brain</b>		1486.4	38.7	1477.3	33.2	0.208		1445.7	72.9	1429.0	73.4	0.036	
		<b>Manifest 1</b>						<b>Manifest 2</b>					
	Baseline		Follow up		p		Baseline		Follow up		p		
	Mean	SD	Mean	SD			Mean	SD	Mean	SD			
<b>Caudate</b>	Right	3.290	0.530	3.165	0.621	0.046		3.302	0.495	3.223	0.624	0.219	
	Left	3.178	0.478	3.002	0.481	0.019		3.094	0.472	2.916	0.515	<b>0.001</b>	
<b>Putamen</b>	Right	4.002	0.603	3.839	0.602	0.009		3.518	0.325	3.296	0.392	0.012	
	Left	4.176	0.548	3.962	0.593	0.004		3.455	0.479	3.285	0.437	<b>0.003</b>	
<b>Pallidum</b>	Right	1.834	0.086	1.790	0.058	0.029		1.845	0.248	1.751	0.226	0.014	
	Left	1.866	0.207	1.793	0.201	0.117		1.769	0.288	1.679	0.324	0.141	
<b>Thalamus</b>	Right	7.603	0.703	7.427	0.661	<b>0.003</b>		7.285	0.593	7.207	0.435	0.352	
	Left	7.745	0.729	7.567	0.728	<b>0.001</b>		7.358	0.897	7.176	0.892	0.018	
<b>Amygdala</b>	Right	1.643	0.258	1.732	0.194	0.257		1.623	0.149	1.633	0.279	0.918	
	Left	1.654	0.311	1.676	0.357	0.707		1.638	0.106	1.594	0.115	0.295	
<b>Hippocampus</b>	Right	5.127	0.511	5.063	0.374	0.354		4.961	0.579	4.807	0.578	0.018	
	Left	5.096	0.591	5.033	0.556	0.557		4.542	0.440	4.446	0.550	0.233	
<b>White matter</b>		632.9	52.9	619.4	53.9	<b>0.001</b>		633.8	35.8	616.1	34.8	0.009	
<b>Whole brain</b>		1396.6	95.3	1371.9	93.8	<b>0.001</b>		1383.1	77.8	1350.6	81.4	<b>0.001</b>	

Volumes (mm<sup>3</sup>) of 14 VOI's on both baseline and follow up. **Bold** = significant finding after Bonferroni correction (p<0.0033)

Table 4: control values of MTR parameters

Control group (n = 25)			Mean	SD	
White matter	MTR peak height	Baseline	1.109	0.220	
		Follow up	1.125	0.231	
	Mean MTR	Baseline	0.391	0.012	
		Follow up	0.394	0.012	
Cortical grey matter	MTR peak height	Baseline	0.769	0.119	
		Follow up	0.735	0.132	
	Mean MTR	Baseline	0.329	0.013	
		Follow up	0.330	0.014	
Caudate nucleus	right	MTR peak height	Baseline	0.799	0.192
			Follow up	0.830	0.224
		Mean MTR	Baseline	0.349	0.026
			Follow up	0.343	0.025
	left	MTR peak height	Baseline	0.892	0.188
			Follow up	0.831	0.198
		Mean MTR	Baseline	0.369	0.018
			Follow up	<b>0.354</b>	0.018
Putamen	right	MTR peak height	Baseline	1.257	0.372
			Follow up	1.240	0.271
		Mean MTR	Baseline	0.340	0.019
			Follow up	<b>0.351</b>	0.017
	left	MTR peak height	Baseline	1.337	0.268
			Follow up	1.266	0.265
		Mean MTR	Baseline	0.360	0.015
			Follow up	0.357	0.016
Pallidum	right	MTR peak height	Baseline	1.494	0.343
			Follow up	1.435	0.230
		Mean MTR	Baseline	0.382	0.015
			Follow up	0.388	0.014
	left	MTR peak height	Baseline	1.601	0.301
			Follow up	1.490	0.268
		Mean MTR	Baseline	0.386	0.014
			Follow up	0.388	0.014
Thalamus	right	MTR peak height	Baseline	0.988	0.261
			Follow up	0.936	0.236
		Mean MTR	Baseline	0.369	0.021
			Follow up	0.350	0.021
	left	MTR peak height	Baseline	1.067	0.226
			Follow up	1.003	0.251
		Mean MTR	Baseline	0.383	0.020
			Follow up	<b>0.368</b>	0.016
Amygdala	right	MTR peak height	Baseline	1.329	0.295
			Follow up	1.287	0.289
		Mean MTR	Baseline	0.360	0.020
			Follow up	0.363	0.025
	left	MTR peak height	Baseline	1.475	0.307
			Follow up	1.328	0.369
		Mean MTR	Baseline	0.370	0.021
			Follow up	0.370	0.026
Hippocampus	right	MTR peak height	Baseline	1.240	0.309
			Follow up	1.194	0.273
		Mean MTR	Baseline	0.366	0.019
			Follow up	0.371	0.020
	left	MTR peak height	Baseline	0.981	0.247
			Follow up	0.929	0.222
		Mean MTR	Baseline	0.386	0.015
			Follow up	0.391	0.017
Whole brain	MTR peak height	Baseline	0.917	0.152	
		Follow up	0.906	0.156	
	Mean MTR	Baseline	0.356	0.011	
		Follow up	0.359	0.012	

MTR = Magnetization Transfer Ratio. **Bold** = significant finding after bonferroni correction ( $p < 0.0033$ )

