

Cover Page



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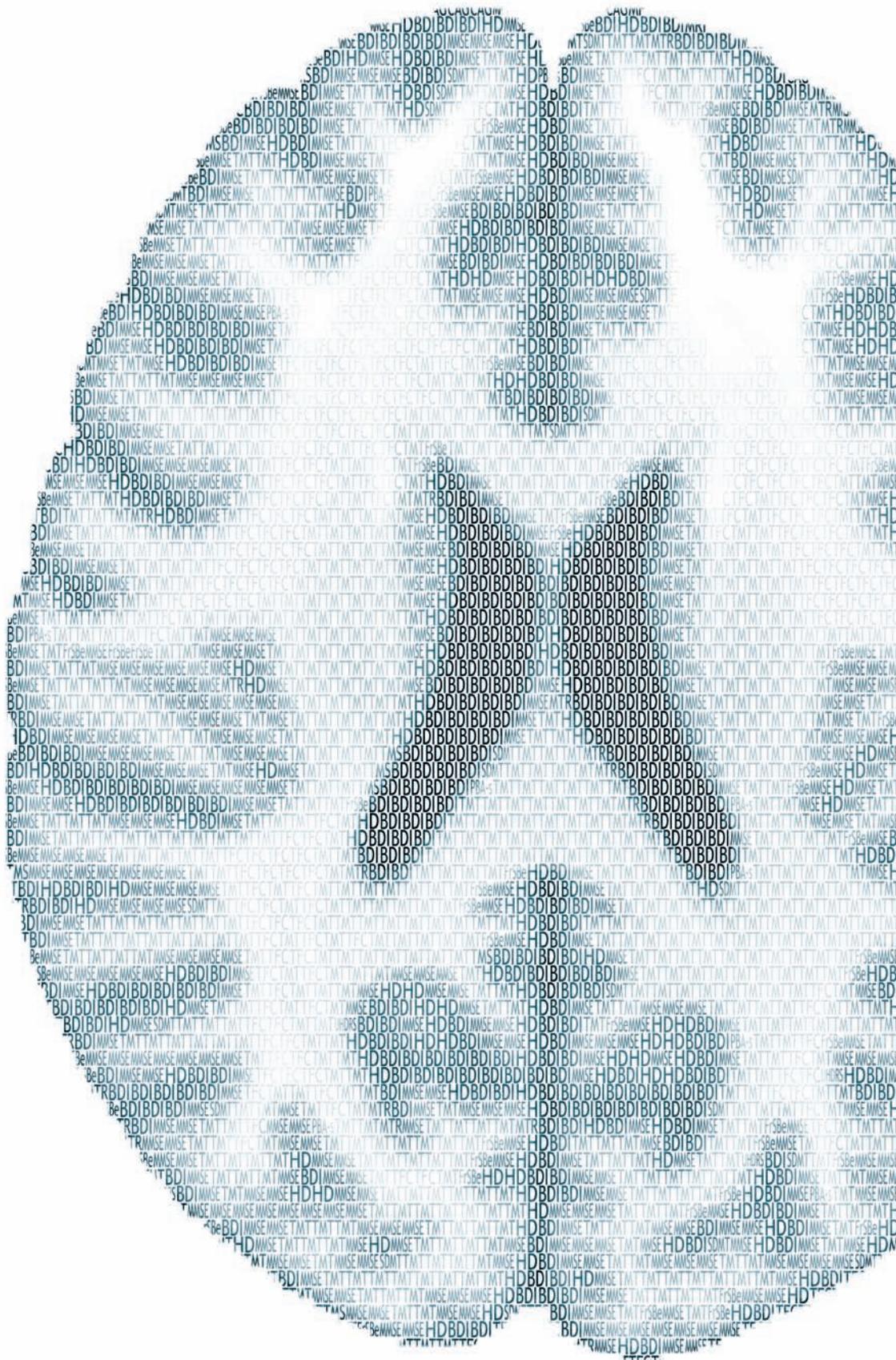


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**Title:** Huntington's disease : quantifying structural brain changes

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## Abstract

### Background and Purpose

Magnetization Transfer Imaging (MTI) has the potential to detect abnormalities in normal-appearing white and gray matter on conventional MR imaging. Early detection methods and disease progression markers are needed in Huntington's Disease (HD) research. Therefore we investigated MTI parameters and their clinical correlates in premanifest and manifest HD.

### Method

From the Leiden TRACK-HD study, 78 participants (28 controls, 25 premanifest HD gene carriers, 25 manifest HD) were included. 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 Magnetization Transfer Ratio (MTR) values were calculated from these regions and MTR histograms constructed. Regression analysis of MTR measures from all gene carriers with clinical measures was performed.

### Results

MTR peak height was reduced in both cortical grey ( $p=0.01$ ) and white matter ( $p=0.006$ ) in manifest HD compared to controls. Also mean MTR was reduced in cortical grey matter ( $p=0.01$ ) and showed a trend in white matter ( $p=0.052$ ). Deep grey matter structures showed a uniform pattern of reduced MTR values ( $p<0.05$ ). No differences between premanifest gene carriers and controls were found. MTR values correlated with disease burden, motor and cognitive impairment.

### Conclusion

Throughout the brain disturbances in MTI parameters are apparent in early HD and are homogeneous across white and grey matter. The correlation of MTI with clinical measures indicates the potential to act as a disease monitor in clinical trials. However, our study does not provide evidence for MTI as a marker in premanifest HD.

## Introduction

Huntington's disease (HD) is a progressive neurodegenerative genetic brain disorder with clinical features consisting of motor signs, cognitive impairment and psychiatric disturbances. Disease onset is typically during mid-life<sup>1</sup>. Since genetic testing became available for this autosomal dominant inheritable disease, it has become possible to identify premanifest gene carriers and in this way ascertain with certainty that they will eventually develop the disease. The disease is caused by a genetic defect on chromosome 4 which results in an expanded polyglutamine in the gene coding for the huntingtin protein<sup>2</sup>. This mutant huntingtin predominantly affects the brain, resulting in malfunction and loss of neurons. Histopathologically, the disease is characterized by cellular loss of grey matter structures, most profoundly that of the medium spiny neurons within the striatum, but also significant white matter volume loss<sup>3</sup>.

Sensitive and reliable biomarkers are needed for evaluating clinical trials in HD. The challenges in this field lay in the fact that a biomarker should be able to monitor pathophysiological changes not only in the manifest phase, but also in the preceding premanifest stage, when no overt symptoms exist. MRI characterization of brain changes is regarded as a potential source of biomarkers as previous studies have shown that atrophy of the striatum is apparent already a decade or more before symptom onset<sup>4-6</sup>. Also, abnormalities in white matter<sup>7,8</sup> and cortical grey matter<sup>5</sup> have been reported. It is likely that the underlying pathologic processes resulting in brain atrophy occur before or in concurrence with the volumetric changes.

Magnetization Transfer Imaging (MTI) has the potential to quantify the pathologic changes in central nervous system disorders in the normal appearing white and grey matter on conventional MRI sequences<sup>9,10</sup>. MTI offers a way of examining tissue structure and structural components, normally not resolvable with conventional MRI<sup>11</sup>. This allows for examination of structural integrity in a different and possibly more sensitive manner than volumetric changes alone. The technique of MTI relies on interaction between protons in free fluid and protons bound to macromolecules. The magnetization saturation and relaxation within macromolecules affect the observable signal. The MTR, representing the percentage of variation in the MR signal between the saturated and unsaturated acquisitions, is an effective and simple MTI measure to use as a clinical application. MTI has been used to characterize many different disorders, including Multiple Sclerosis, Alzheimer's and Parkinson's disease<sup>9</sup>.

This study aims to examine MTR measures in a well defined premanifest and manifest HD population, and to determine associations between MTR and clinical features of HD. By examining MTR in this sample we aim to advance understanding of the timing of pathophysiological changes in HD and we also evaluate the suitability of MTI/MTR as a potential biomarker for HD.

## Materials and Methods

### Subjects

Of the 90 participants from the Leiden TRACK-HD study, 12 did not receive MTI scanning due to either unexpected claustrophobia or time constraints of the full TRACK-HD protocol, resulting in 78 participants. The cohort consisted of three groups; 28 healthy controls, 25 Premanifest HD gene carriers (PMGC) and 25 manifest HD (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>12</sup> and absence of motor abnormalities on the Unified Huntington's disease rating scale (UHDRS), defined as a total motor score (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 total functional capacity (TFC) score of 7 or higher was required to ensure that the HD group was in the earliest disease stages. 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 from the TRACK-HD baseline paper<sup>5</sup>. Local IRB approval and written informed consent were obtained from all participants.

### Imaging sequences

All 78 participants underwent scanning on a 3 Tesla Philips whole body scanner (Philips, Best, The Netherlands) with an 8-channel receive and transmit coil. T1-weighted image volumes were acquired using an ultrafast gradient echo 3D acquisition sequence with the following imaging parameters: TR = 7.7ms, TE = 3.5ms, FA =  $8^\circ$ , FOV = 24cm, matrix size 224x224x164 with sagittal slices to cover the entire brain with a slice thickness of 1.0 mm.

A 3D gradient MTI sequence was subsequently performed with the following parameters: TR = 100 ms, TE = 11 ms, FA =  $9^\circ$ , matrix 224x180x144 mm, voxel size: 1.0x1.0x7.2 mm. Two consecutive imaging sets were acquired, one with and one without a saturation pulse. The imaging parameters are identical to those

described by Jurgens *et al.* (2010)<sup>13</sup>. Total scanning time for T1-weighted and MTI sequences was 12 minutes maximum.

### Post-processing

T1-weighted images were segmented using FAST<sup>14</sup> and FIRST<sup>15;16</sup> from FSL<sup>17</sup>. This provided individual brain masks for: total white matter, cortical grey matter, caudate nucleus, putamen, pallidum, thalamus, amygdala, and hippocampus. 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 volumes of interest (VOI). All brain masks were then registered to the MTI volumes using the transform obtained from linear registration of the T1-weighted volume with 7 degrees of freedom (FSL FLIRT). MTR is 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 voxel based MTR variations/variability within each VOI we constructed MTR histograms and calculated MTR peak height using FSL-STATs. Mean MTR and MTR peak height, normalized for the size of the volume of interest, were the primary outcome variables.

### Clinical Measures

A total measure of motor dysfunction was obtained with the UHDRS-TMS (range 0-124). Quantification of subtle motor dysfunction by measuring variability of dominant hand finger tapping and tongue protrusion force was achieved with force transducer based quantitative motor assessments<sup>18;19</sup>. The tapping and tongue measures are expressed as a logarithmic number, higher numbers representing more motor disturbances. TFC score (range: 0-13) and mini-mental state exam (MMSE) for global assessment of cognitive functioning (range: 0-30) were obtained. Cognitive scores included the total scores from the symbol digit modality test (SDMT), Stroop word reading card, trail making test (TMT) A and B and verbal fluency (VF). For the TMT a subtraction of TMT B minus TMT A was used to minimize the potential effects of motor speed on performance. The university of Pennsylvania smell identification test (UPSIT) (Sensonics, Haddon Heights, New Jersey) quantifies smell ability with a 20-item smell test, and is known to correlate to clinical features of neurodegenerative diseases<sup>20</sup>. An IQ estimate was obtained with the Dutch adult reading test (DART) (a validated translation of the National Adult Reading Test). For assessment of psychiatric disturbances the Beck Depression Inventory II, the Problem Behaviour Assessment, short version, and the Frontal Systems Behavior<sup>21</sup> were used. Predicted years to disease onset were calculated for PMGC as described in the TRACK-HD baseline paper. For a more detailed description of these clinical assessments see Tabrizi *et al.* (2009)<sup>5</sup>.

## Statistics

Statistical analysis was performed using the Statistical Package for Social Sciences (Version 17.0.2, Chicago, USA). An analysis of variance was conducted for all demographic variables. For group comparisons, all MTR values were analysed in a three group analysis of variance with post hoc analysis to determine differences between groups. Hierarchical multiple regression analysis was performed to ascertain the relationship of MTR values with clinical measures. For this analysis only gene carriers (premanifest + manifest) were included as the aim was to examine the relationship to disease progression. MTR values and 14 different clinical assessments were assessed for all regions of interest. In the hierarchical regression age and gender were entered at step 1, thus correcting for the influence of these variables. This was applied for all motor and general assessments, for the specific cognitive tasks (SDMT, Stroop word reading, VF and TMT) IQ was also entered at step 1 as IQ can have a significant impact on cognitive scores.

## Results

Demographic variables (table 1) show that there were no differences between the groups in terms of age or CAG repeat length, but that there was a significant difference ( $p < 0.05$ ) between groups for all clinical tests, except for IQ and the FrSBe-scores.

MTR peak height was significantly reduced in the MHD group as compared to either controls or PMGC in the following regions: white matter, grey matter, putamen, pallidum, amygdala, left thalamus (with a trend for the right thalamus) and the right hippocampus. No significant results were found between the control and PMGC. The mean MTR value was significantly lower between MHD and controls in the following regions: grey matter, both caudate nuclei, both thalami and right putamen. All MTR values are shown (table 2) for all regions as examined for each group.

Overall, the MTR histograms showed similar patterns for all study groups in white and cortical grey matter (figure 1) as well as all subcortical grey matter regions separately (figure 2). Whereby, in all histograms the MHD group displayed a lower and broader histogram, as compared to controls and PMGC.

The regression analysis revealed several highly significant correlations between the MTR values and clinical measures (Table 3). The disease burden score was significantly correlated with both cortical grey and white matter MTR peak height

and mean MTR. The deep grey matter structures mainly showed a correlation of MTR peak height to the disease burden except the right caudate nucleus and right putamen. The motor tests also correlated significantly with the MTR values in most regions of interest, predominantly with the UHDRS-TMS and the tapping measure and only minimally with the tongue measure. The cognitive measures showed correlations in the following regions; cortical grey matter, white matter, thalamus, left putamen, right pallidum and left amygdala. The TFC showed a correlation with white matter and the left putamen. The smell identification test was correlated to MTR values in both cortical grey and white matter as in the caudate nucleus, amygdala and thalamus. The MMSE and the measures of behavioural/psychiatric functioning revealed no correlation to any structures and are therefore not displayed in table 3.

**Table 1: Group characteristics**

	<b>Control</b> Mean (SD)	<b>PMGC</b> Mean (SD)	<b>MHD</b> Mean (SD)	<b>p-value</b> between groups
<b>N</b>	28	25	25	
<b>Age</b>	48.3 (8.0)	43.8 (8.5)	48.4 (10.9)	0.131
<b>CAG larger allele</b>	n.a.	42.72 (2.6)	43.73 (2.8)	0.182
<b>UHDRS TMS</b>	2.3 (2.3)	2.5 (1.5)	22.9 (11.4)	<b>0.000</b>
<b>TFC</b>	12.96 (0.2)	12.56 (0.8)	10.2 (2.1)	<b>0.000</b>
<b>YTO</b>	n.a.	7.06 (1.99)	n.a.	n.a.
<b>IQ</b>	104 (9)	100 (11)	99 (12)	0.260
<b>MMSE</b>	29.1 (1.2)	28.7 (1.5)	27.2 (2.6)	<b>0.001</b>
<b>Tongue Force</b>	3.56 (0.40)	3.97 (0.51)	4.88 (0.65)	<b>0.000</b>
<b>Tapping</b>	11.6 (5.8)	16.7 (8.5)	30.9 (18.2)	<b>0.000</b>
<b>SDMT</b>	50.6 (9.3)	50.7 (10.2)	35.7 (11.10)	<b>0.000</b>
<b>Stroop</b>	98.1 (14.2)	93.0 (13.7)	76.7 (20.6)	<b>0.000</b>
<b>TMT</b>	33.5 (23.5)	43.2 (26.9)	90.3 (73.6)	<b>0.000</b>
<b>Verbal Fluency</b>	26.8 (8.7)	33.3 (14.1)	20.8 (14.5)	<b>0.016</b>
<b>UPSIT</b>	15.89 (2.9)	14.6 (2.5)	12.23 (3.8)	<b>0.000</b>
<b>BDI-II</b>	4.8 (5.9)	7.0 (7.7)	11.1 (10.2)	<b>0.020</b>
<b>PBA-s</b>	6.4 (8.1)	7.6 (8.5)	14.6 (14.7)	<b>0.017</b>
<b>FrSBe</b>	78.1 (19.6)	85.9 (23.8)	87.3 (21.6)	0.259

PMGC= Premanifest HD gene carriers, MHD= manifest Huntington's Disease, CAG= Cytosine-Adenine-Guanine, UHDRS TMS = Unified Huntington's Disease Rating Scale Total Motor Score, TFC = Total Functional Capacity, YTO = Expected years to onset, IQ = Intelligence Quotient based on the Dutch Adult Reading Test, MMSE = Mini Mental State Exam, Tapping = Average speeded tapping for left and right index finger, Tongue = sustained tongue force measure, SDMT = Symbol Digit Modalities test, Stroop = Stroop word reading task, TMT = Trail making test, UPSIT = University of Pennsylvania Smell Identification Test, BDI-II = Becks Depression Inventory 2nd version, PBA-s = Problem Behaviour Assessment-short version, FrSBe = Frontal Systems Behaviour rating scale Self Report, n.a. = not applicable.

**Table 2: MTR values for all brain regions**

	Control	PMGC	MHD	P-value		
	Mean (SD)	Mean (SD)	Mean (SD)	C - P	C - M	P - M
<b>White matter</b>						
Peak height	1.080 (0.271)	1.118 (0.212)	0.860 (0.234)	0.859	<b>0.006</b>	<b>0.001</b>
Mean MTR	0.389 (0.017)	0.389 (0.015)	0.378 (0.017)	0.986	0.052	0.086
<b>Cortical Grey matter</b>						
Peak height	0.769 (0.121)	0.723 (0.118)	0.610 (0.145)	0.435	<b>0.000</b>	<b>0.010</b>
Mean MTR	0.330 (0.131)	0.326 (0.125)	0.314 (0.174)	0.687	<b>0.001</b>	<b>0.010</b>
<b>Right Caudate</b>						
Peak height	0.804 (0.209)	0.871 (0.198)	0.757 (0.198)	0.486	0.701	0.144
Mean MTR	0.349 (0.029)	0.341 (0.027)	0.325 (0.035)	0.603	<b>0.020</b>	0.199
<b>Left Caudate</b>						
Peak height	0.884 (0.203)	0.949 (0.207)	0.812 (0.231)	0.550	0.474	0.084
Mean MTR	0.367 (0.029)	0.359 (0.021)	0.346 (0.036)	0.586	<b>0.041</b>	0.330
<b>Right Putamen</b>						
Peak height	1.223 (0.388)	1.238 (0.292)	0.976 (0.288)	0.985	<b>0.029</b>	<b>0.023</b>
Mean MTR	0.341 (0.019)	0.333 (0.024)	0.325 (0.023)	0.448	<b>0.034</b>	0.410
<b>Left Putamen</b>						
Peak height	1.316 (0.329)	1.299 (0.241)	1.050 (0.290)	0.977	<b>0.006</b>	<b>0.014</b>
Mean MTR	0.358 (0.018)	0.351 (0.022)	0.356 (0.017)	0.475	0.932	0.711
<b>Right Pallidum</b>						
Peak height	1.449 (0.400)	1.587 (0.296)	1.324 (0.400)	0.404	0.480	<b>0.049</b>
Mean MTR	0.385 (0.022)	0.382 (0.014)	0.385 (0.022)	0.850	0.996	0.816
<b>Left Pallidum</b>						
Peak height	1.563 (0.369)	1.514 (0.285)	1.268 (0.332)	0.868	<b>0.008</b>	0.038
Mean MTR	0.382 (0.027)	0.384 (0.015)	0.394 (0.018)	0.951	0.098	0.197
<b>Right Thalamus</b>						
Peak height	0.995 (0.297)	1.027 (0.215)	0.864 (0.227)	0.896	0.173	0.078
Mean MTR	0.369 (0.028)	0.360 (0.025)	0.340 (0.038)	0.536	<b>0.005</b>	0.093
<b>Left Thalamus</b>						
Peak height	1.055 (0.253)	1.114 (0.241)	0.906 (0.278)	0.711	0.115	<b>0.021</b>
Mean MTR	0.380 (0.035)	0.372 (0.018)	0.355 (0.040)	0.709	<b>0.024</b>	0.168
<b>Right Amygdala</b>						
Peak height	1.299 (0.318)	1.294 (0.228)	1.127 (0.263)	0.998	0.080	0.105
Mean MTR	0.362 (0.020)	0.361 (0.020)	0.355 (0.020)	0.977	0.463	0.607
<b>Left Amygdala</b>						
Peak height	1.462 (0.343)	1.399 (0.231)	1.178 (0.280)	0.733	<b>0.003</b>	<b>0.032</b>
Mean MTR	0.373 (0.028)	0.374 (0.021)	0.368 (0.021)	0.997	0.679	0.648
<b>Right Hippocampus</b>						
Peak height	1.220 (0.334)	1.266 (0.239)	1.081 (0.195)	0.821	0.170	0.054
Mean MTR	0.369 (0.025)	0.363 (0.016)	0.357 (0.021)	0.592	0.122	0.593
<b>Left Hippocampus</b>						
Peak height	0.954 (0.273)	0.965 (0.207)	0.882 (0.230)	0.985	0.555	0.473
Mean MTR	0.390 (0.027)	0.382 (0.018)	0.383 (0.017)	0.400	0.540	0.972

MTR values for white matter, cortical grey matter and subcortical grey matter structures. Peak height represents a normalized for volume peak height. PMGC= Premanifest HD gene carriers, MHD= manifest Huntington's Disease C = controls, P = premanifest HD, M = Manifest HD

**Table 3: Regression analysis per region of interest**

	Burden	TMS	TFC	Tap	Tongue	SDMT	Stroop	TMT	VF	UPSIT
<b>WM</b>										
Peak height	<b>.204**</b>	<b>.257**</b>	<b>.074*</b>	<b>.168**</b>	<b>.110*</b>	<b>.097*</b>	.060	<b>.089*</b>	.000	<b>.151**</b>
Mean MTR	<b>.114*</b>	<b>.163**</b>	.025	.080	.034	.057	.036	.037	.004	<b>.160**</b>
<b>CGM</b>										
Peak height	<b>.193**</b>	<b>.189**</b>	.061	<b>.111*</b>	.049	.062	.063	<b>.092*</b>	.003	<b>.058*</b>
Mean MTR	<b>.083*</b>	<b>.152**</b>	.110*	<b>.098*</b>	.062	<b>.114*</b>	<b>.125*</b>	<b>.136**</b>	.034	<b>.122*</b>
<b>Right CN</b>										
Peak height	.059	.054	.019	.040	.001	.021	.061	.009	.000	.014
Mean MTR	<b>.076*</b>	<b>.153**</b>	.036	<b>.105*</b>	.008	.050	.052	.034	.003	<b>.101*</b>
<b>Left CN</b>										
Peak height	<b>.083*</b>	<b>.160**</b>	.034	<b>.116*</b>	<b>.104*</b>	.038	.070	.010	.022	<b>.104*</b>
Mean MTR	.047	<b>.111*</b>	.017	<b>.094*</b>	.037	.012	.026	.025	.000	<b>.141*</b>
<b>Right Putamen</b>										
Peak height	.055	<b>.181**</b>	.053	<b>.112*</b>	.044	.067	.067	.038	.001	.061
Mean MTR	.000	.077	.038	.013	.001	.021	.037	.005	.001	.002
<b>Left Putamen</b>										
Peak height	<b>.076*</b>	<b>.209**</b>	<b>.090*</b>	<b>.117*</b>	.060	.054	<b>.077*</b>	.035	.000	.070
Mean MTR	.025	.000	.007	.010	.002	.041	.035	.006	<b>.078*</b>	.008
<b>Right Pallidum</b>										
Peak height	<b>.110*</b>	<b>.175**</b>	.035	<b>.174**</b>	.051	.071	.048	.041	.004	.075
Mean MTR	.001	.017	.046	.003	.001	.001	.006	.019	.033	.010
<b>Left Pallidum</b>										
Peak height	<b>.104*</b>	<b>.164**</b>	.043	<b>.131*</b>	.051	.060	.070	<b>.080*</b>	.000	.045
Mean MTR	.001	.001	.026	.000	.012	.036	.053	.011	.034	.004
<b>Right Amygdala</b>										
Peak height	<b>.145**</b>	<b>.128*</b>	.023	<b>.098*</b>	.016	.037	.034	.020	.002	.116*
Mean MTR	.026	.033	.034	.058	.009	.025	.043	.044	.036	.041
<b>Left Amygdala</b>										
Peak height	<b>.252**</b>	<b>.184**</b>	.060	<b>.216**</b>	<b>.134*</b>	<b>.170**</b>	<b>.116*</b>	<b>.086*</b>	.071	<b>.172**</b>
Mean MTR	.000	.000	.007	.003	.005	.038	.047	.068	.109*	.003
<b>Right HC</b>										
Peak height	<b>.190**</b>	<b>.091*</b>	.002	.075	.002	.073	.026	.010	.001	.061
Mean MTR	.015	.041	.075	.037	.012	.043	.073	.070	.025	.034
<b>Left HC</b>										
Peak height	.095*	.062	.000	.020	.015	.022	<b>.085*</b>	.026	.039	.011
Mean MTR	.003	.006	.002	.013	.001	.015	.027	.006	.010	.008
<b>Right Thalamus</b>										
Peak height	<b>.124*</b>	.099*	.025	<b>.100*</b>	.007	.043	.055	.015	.002	.045
Mean MTR	<b>.162**</b>	<b>.215**</b>	.057	<b>.172**</b>	.033	<b>.072*</b>	.066	.061	.009	<b>.156**</b>
<b>Left Thalamus</b>										
Peak height	.154**	.223**	.065	<b>.167**</b>	<b>.122*</b>	.075	<b>.110*</b>	.030	.032	<b>.122*</b>
Mean MTR	<b>.117*</b>	<b>.156**</b>	.027	<b>.134*</b>	.046	.035	.044	.042	.004	<b>.158**</b>

Regression analysis between 14 clinical and 2 MTI measures in regions of interest. Values stated are R square changes. WM=White Matter, CGM=Cortical Grey Matter, CN=Caudate Nucleus, HC =hippocampus, TMS = Unified Huntington's Disease Rating Scale Total Motor Score, TFC = Total Functional Capacity, Tap= speeded tapping, Tongue = tongue force, SDMT = Symbol Digit Modalities test, Stroop = Stroop word reading task, TMT = Trail making test, VF = verbal fluency, UPSIT = University of Pennsylvania Smell Identification Test, \*= $p < 0.05$ , \*\*= $p < 0.005$

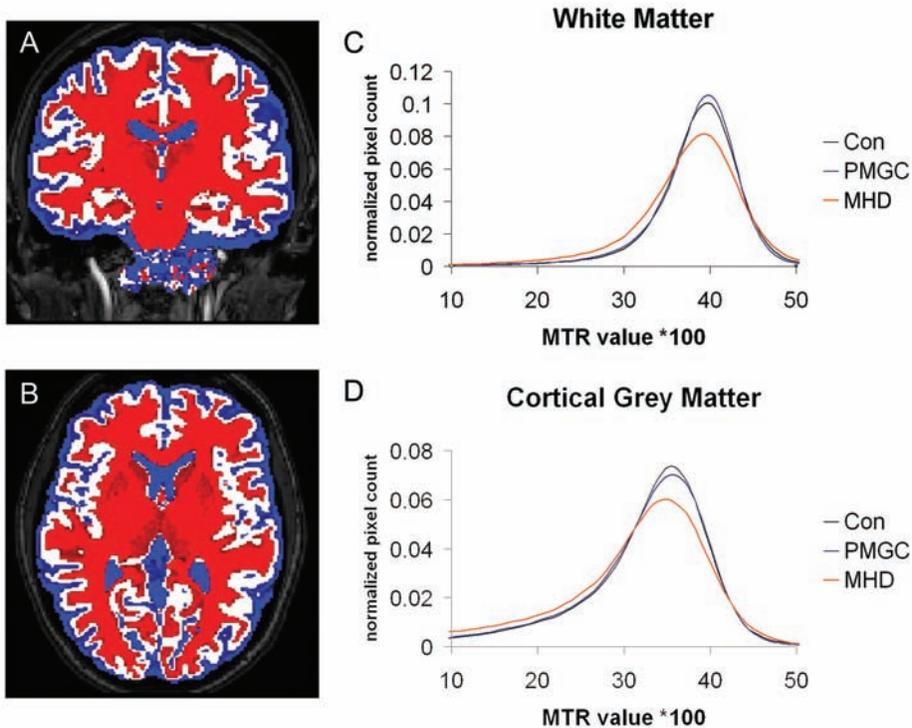


Figure 1: (A & B) example segmentations acquired with FAST software showing white matter (red), grey matter (white) and cerebral spinal fluid (blue). The subcortical grey matter structures were subtracted from these masks. Magnetization Transfer Ratio Histogram for the white matter (C) and cortical grey matter (D), corrected for volume size of the region for 3 groups.

## Discussion

MTI applied in HD reveals disturbances throughout the brain in early HD as compared to controls and PMGC. Disease burden, quantitative motor and cognitive measures have a strong correlation to MTR values, leading to the conclusion that MTI can possibly be used to track disease progression. No abnormalities are quantifiable in the premanifest stages of the disease compared to controls, which leads to suggest that MTI, although perhaps a good disease monitor, is not an early marker of the disease.

Currently, conventional structural MRI and Diffusion Tensor Imaging (DTI) are the two most widely applied methods in HD research with respect to the search for a

MRI biomarker covering all disease stages of HD. Only three reports on MTI in HD are available<sup>13;22;23</sup>. The value derived from MTI is the MTR value per brain voxel and is thought to represent structural integrity. The value quantifies the exchange of magnetization from the non-water components in the region at hand. The most frequently reported outcome measures of MTR are mean MTR and MTR peak height. Mean MTR represents the average MTR value of all voxels in a region of interest, with lower mean MTR corresponding to poorer 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. When each MTR value occurs less frequently, the histogram becomes broader, and the maximum peak height decreases. This reduction represents reduced capacity to optimally exchange magnetization over the region of interest, hence representing reduced structural integrity<sup>24;25</sup>. For example in white matter, myelin is the main component and therefore MTR is thought to relate to myelinisation or myelin integrity. To which cellular structure, whether neurons or glia cells, MTR in gray matter specifically refers is unknown.

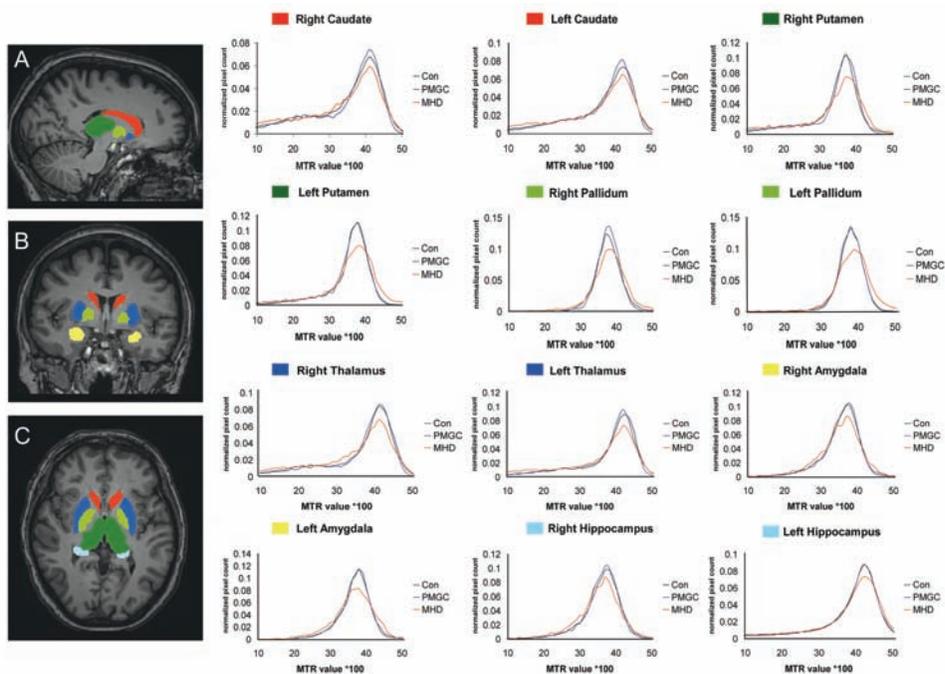


Figure 2: Magnetization Transfer Ratio histogram for 6 deep grey matter structures bilaterally, corrected for volume size of the region for 3 groups. Red = caudate nucleus, dark blue = putamen, light green = pallidum, dark green = thalamus, yellow = amygdala, light blue = hippocampus. Con = controls

In our study it does not solely reflect atrophy, as the differences found in peak height were corrected for size of the volume examined, thereby accounting for atrophy. From histopathological studies we know that medium spiny neurons in HD are most affected, making these the most likely source of the differences.

In the current study, we found that both MTR measures were significantly reduced in the manifest stages of HD in cortical grey matter, deep gray matter structures and white matter. This finding conflicts with the findings by Mascalchi *et al.* (2004), who reported no differences between a group of 21 gene carriers (of which 19 manifest HD) and controls<sup>22</sup>. The differences could be explained by the fact that Mascalchi *et al.* (2004) applied a different (manual) segmentation technique, used a lower field strength, included a slightly smaller group and did not examine MTR peak height. Mean MTR in our study does show significant results, however not in the white matter. In general we found that the peak height tended to be the more sensitive MTR measure rather than the mean MTR. The study by Jurgens *et al.* (2010) is comparable to our study with the same type of scanner and analysis. We replicate their findings as they also demonstrated a lack of group difference between PMGC and controls<sup>13</sup>. Furthermore, the clinical correlation of the MTI peak height with clinical measures in gene carriers is confirmed in our study, and this knowledge is extended from a premanifest study group to both PMGC and MHD. Ginestroni *et al.* (2010) applied a similar methodology to our study, as both used the FSL tools for segmentation<sup>23</sup>. The main difference between these two studies is that we examined an explicitly premanifest and manifest groups separately, instead of a “gene carrier group with a range of clinical severity”. The outcomes of the studies are highly comparable with reduced MTR in subcortical and cortical grey matter. The absence of white matter differences in mean MTR is similar in both reports. However we did find white matter differences with an outcome measure not examined by Ginestroni *et al.* (2010), namely MTR peak height. Finally the correlation of MTR values with clinical measures was similarly reported by both studies.<sup>23</sup>.

The finding of reduced MTR measures throughout the brain is remarkably homogeneous. This seems in contrast to the volumetric data available in HD research. Striatal degeneration is the key feature of brain pathology in HD, yet evermore evidence is building up that, although the damage starts in the striatum, HD is truly a whole brain disease, as numerous volumetric studies demonstrate widespread volumetric loss in both gray and white matter<sup>26</sup>. So the seemingly paradoxical homogeneity is really not that surprising.

The application of MTI and its relationship to clinical severity has been demonstrated in other neurological diseases such as Multiple Sclerosis and Alzheimer disease<sup>10;27;28;28-31</sup>. Our findings indicate that MTI measures in HD correlate to disease burden, specific motor tasks and cognitive measures in this study population. The finding of correlations of MTI with specific clinical parameters indicates that MTI is a good reflection of the disease status as shown by motor and cognitive measures. Furthermore the disease burden, which encompasses the CAG-repeat length, has been demonstrated to correlate with striatal degeneration and predicted time to disease onset<sup>12</sup>, therefore indirectly linking MTI outcomes to such measures.

The lack of significant group differences between PMGC and controls was unexpected. We anticipated differences on the basis of previous reports on white matter integrity loss in premanifest HD using DTI<sup>7;8;32</sup>. DTI can be used to examine protons in free water and their diffusive properties in more than one way, namely the strength of the directional of diffusivity (FA), the average amount of diffusivity (mean diffusivity), but also the amount of diffusivity in either the radial and axial direction. DTI has the potential for examining and quantifying many features of brain tissue, all captured by the terms structural integrity and/or organization. In white matter DTI is heavily influenced by axonal membranes and myelin sheaths<sup>33</sup>. In contrast MTI can be used to examine tissue structure according to the protons bound to macromolecules<sup>34</sup>. As myelin is the main component of white matter, MTI is thought to mainly represent myelin integrity. Therefore, these techniques characterize fundamentally different aspects of brain tissue, possibly explaining (part of) the differences found between DTI-studies and MTI-studies. The question remains whether DTI or MTI is more sensitive in detecting the pathologic neuronal integrity breakdown. However, answering this question was not the aim of this study.

The potential role for MTI as a biomarker in HD is apparent as there are both significant differences between groups and a clear relationship to clinical measures. However, MTI may be sensitive to a particular (early) disease state and not to all disease stages in HD. Longitudinal follow up is needed to confirm this. The biomarker role for MTI has already been suggested in Alzheimer disease by Ridha *et al.* (2007)<sup>31</sup> and the reports for using MTI as a biomarker in MS are building<sup>35</sup>, strengthening the possibility for MTI as a biomarker in neurodegenerative disorders such as HD. TRACK-HD is specifically designed for longitudinal assessment of potential biomarkers, and is therefore the ideal platform to confirm the findings longitudinally.

Limitations of our study lay in the fact that the automated segmentation technique has not specifically been validated for HD. However, we used these only for obtaining the brain regions of interest. Furthermore, we accounted for some possible incorrect segmentation and/or partial volume effects by using an eroded version of the brain masks. A limitation could also be that we have chosen a region of interest (ROI) based analysis as opposed to voxelwise analysis. However as the morphology of the structures at hand change due to the disease, registration issues could be a severe problem, not to mention that the mean MTR can remain constant while intensities do change. Therefore ROI based analysis is potentially more sensitive. Furthermore, we examined voxel based variations within structures, by representing this in histograms of each VOI. Another limitation could be that the exploratory nature of this study accounted for a high number of correlations included, which could lead to false positive results. It seems, however, that MTI measures are fairly stable in every region we examined and provides a rather uniform picture of group differences and clinical correlation outcomes. Finally, a limitation of MTI in general has been the limited reproducibility across centres due to the fact that the MT phenomenon is dependent on many technical parameters and lack of a standardized protocol<sup>9</sup>.

## Conclusions

MTI demonstrates that whole brain disturbances are apparent in early HD and furthermore, these structural integrity differences seem to be relatively homogeneous throughout the brain in early HD. The strong correlations to clinical features, especially motor and cognitive measures, suggest that there is potential for this analysis to serve as a disease monitor in future clinical trials. However, MTI does not seem to be an early marker of HD as no disturbances in MTI measures can be detected in the premanifest stages of the disease.

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