Premanifest Huntington's disease : a study of early biomarkers

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Magnetisation Transfer Imaging in premanifest Huntington's disease

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Abstract

Objective: To investigate whether Magnetisation Transfer Imaging (MTI) is a useful detector of diffuse brain abnormalities in 'premanifest' carriers of the Huntington's disease (HD) gene mutation. Furthermore we examined the relations between MTI, clinical measures and CAG repeat length.
Methods: Sixteen premanifest carriers of the HD gene without motor manifestation and 14 non-carriers underwent a clinical evaluation and a MRI scan. MTI analysis of whole brain, grey matter and white matter was performed producing Magnetisation Transfer Ratio (MTR) histograms.
Results: A lower peak height of the grey matter MTR histogram in carriers was significantly associated with more UHDRS motor abnormalities. Furthermore, a lower peak height of the whole brain, grey and white matter was strongly associated with a longer CAG repeat length. MTI measures themselves did not differ significantly between carriers and non-carriers.
Conclusions: In premanifest HD mutation carriers a lower MTR peak height, reflecting worse histological brain composition, was related to subtle motor abnormalities and higher CAG repeat length. Although we could not detect altered MTI characteristics in carriers of the HD gene mutation without clinical manifestations, we did provide evidence that the MTR peak height might reflect genetic and subclinical disease burden and may be of value in monitoring further disease progression and provide insight in clinical heterogeneity.

Introduction

Huntington's disease (HD) is a hereditary neurodegenerative disorder resulting in a progressive loss of motor and cognitive functioning and changes in mood and social behaviour. The genetic defect leads to cell death especially in the basal ganglia. Recent studies demonstrated that the amount of white matter and cortical grey matter is found to be reduced as well, even early in the disease.[1-3] Improving the knowledge of brain changes in 'premanifest' carriers (further labelled as carriers) of the HD gene mutation (i.e. without overt clinical signs) is essential in the search for sensitive instruments suitable to monitoring HD onset and progression for future therapeutic trials with neuroprotective agents.

Volumetric MRI studies in carriers demonstrated smaller basal ganglia volumes even years before the onset of motor disturbances.[2,4-6] Recent voxel based morphometry (VBM), diffusion tensor imaging (DTI) or positron emission tomography (PET) studies demonstrated abnormalities in white matter and cortical grey matter as well.[3,7-9] Other quantitative MRI techniques, such as Magnetisation Transfer Imaging (MTI) may further improve our understanding of how diffuse brain changes in HD develop and how these are related to the genotype and the heterogeneous phenotype of the disease. In MTI the exchange of magnetisation between bound protons and free water is represented by the Magnetisation Transfer Ratio (MTR), which can be demonstrated quantitatively in the MTR histogram. A low peak height of the MTR histogram indicates reduced capacity of the macromolecules in brain tissue to exchange magnetisation with the surrounding water molecules, reflecting structural brain damage.[10] MTI has the advantage of being non-invasive and easy to administer, whilst having been proved to be sensitive for the first microstructural brain changes in different neurodegenerative disorders, like Alzheimer's disease (AD) and Parkinson's disease (PD), even before volumetric alterations.[11-16] In Mild Cognitive Impairment (MCI), which has become increasingly recognised as a transitional phase between normal old age and AD, abnormal MTR values of the brain parenchyma could be demonstrated without evidence of atrophy.[14]
Although MTI was initially developed to investigate white matter changes in multiple sclerosis, it was demonstrated to be sensitive for changes in tissue structure of the grey matter as well.[11,17,18] This, and the sensitivity for histopathological changes preceding atrophy, makes MTI an attractive tool in studying the earliest diffuse brain changes related to HD in both grey matter and white matter. To date, only one study investigated MTI parameters in predominantly symptomatic HD gene carriers.[19] No significant differences in mean MTR values between HD patients and controls were found. However, this study did not address the peak height of the MTR histograms, which has proven to be the most sensitive and distinctive MTI parameter for detecting brain damage in various neurodegenerative diseases.[11,20-22] Lower peak heights of the histogram could be demonstrated in AD, MCI and MS, while mean MTR values were still within the normal range.[12,14,18]

Therefore, in this explorative study, we investigated whether MTI based Magnetisation Transfer Ratios and histogram peak heights could reveal diffuse brain abnormalities in carriers in comparison with non-carriers by studying the total of brain parenchyma, the grey matter and the white matter.

It is hypothesised that MTI may provide additional information on diffuse brain pathology and its relation with the genotype and the phenotype in the premanifest phase of HD.

Patients and Methods

Seventeen carriers and 15 non-carriers were invited to participate in this study. All participants were recruited from the Leiden University Medical Center (LUMC) outpatient Neurological department. Participants had undergone gene testing according to international guidelines at an earlier time.[23] The median CAG repeat length in carriers was 42 (range 40-49) and in non-carriers 19 (range 16-24). The estimated probability of symptom onset within 5 years was determined.[24] Carriers were considered 'premanifest' in the absence of 'definite' motor signs on the Unified Huntington's Disease Rating Scale (UHDRS), as assessed during their last visit to our outpatient department. Reassessment of motor functioning during study enrolment by a neurologist blind to genetic status, resulted in the exclusion of one carrier who was rated as definite HD. One non-carrier who showed evidence of overt cerebral damage on MRI was also excluded from analysis. Ultimately analyses were performed on 16 carriers and 14 non-carriers.
The study had been approved by the local Medical Ethical Committee. Written informed consent was obtained from all subjects.

Procedure
All participants were evaluated with the UHDRS and MRI of the brain.[25] There were no more than four months between clinical assessment and MRI-scan (mean: 30 days, SD: 44 days).
From the motor part of the UHDRS the Total Motor Score (TMS) was used (max 0-124), with higher scores representing more motor abnormalities. The cognitive and behavioural sections of the UHDRS were administered by a psychologist. The Total Behavioural Score was obtained by adding the products of frequency and severity for each item.

Image acquisition
All imaging was performed on a whole body MR system operating at field strength of 3.0 Tesla (Philips Medical Systems, Best, The Netherlands). MRI consisted of a 3D-T1-weighted and Magnetisation Transfer Imaging (MTI) scan. Acquisition parameters were as follows: 3D-T1-weighted: TR = 9.8 msec; TE = 4.6 msec; flip angle = 8°; section thickness = 1.2 mm; number of sections = 120; no section gap; whole brain coverage; FOV = 224 mm; matrix = 192, reconstruction matrix = 256; 3D-gradient echo MTI: TR = 100 msec; TE = 3.7 msec; flip angle = 8°; section thickness = 7.2 mm; number of sections = 20; no section gap; whole brain coverage; FOV = 224 mm; matrix = 224, reconstruction matrix = 256. These scan parameters were chosen to minimise T1 and T2 weighting, resulting in a proton-density contrast in the absence of MT saturation pulses. Two consecutive sets of images were acquired; the first was performed in combination with the MT saturation pulse, and the second without. In the second scan a sinc-shaped saturation pulse 1100 hertz below frequency of water was added.

Image postprocessing
Images were transferred to an offline LINUX workstation. All MTR processing steps were performed using the FMRIB's software library (FSL).[26] The MTR sequence was split into an m_o dataset, which represents the signal intensity of voxels without saturation and an m_1 dataset, which represents the intensity of voxels with saturation. An MTR map was obtained by calculating the MTR value for each voxel using the formula; MTR= $\{(m_o - m_1)/m_o\} \times 100\%$.[27] To obtain segmented grey and white matter the T1 weighted scans were segmented using the segmentation tool in FSL, FAST.[28] All segmentations were eroded one voxel in plane to minimize partial volume effects on the MTI parameters. A transformation matrix was used to mask the MTR map with the segmented whole brain, grey and white matter volume from the T1 weighted scan segmentation. From the resulting MTR maps of the whole brain, grey and white matter, histograms were created and finally normalised for the size of the region of interest. From these histograms the peak height was derived. Whole brain, grey and white matter volume was measured, using an automated method, the cross-sectional version of the Structural Image Evaluation of Normalised Atrophy (SIENAx).[29] Furthermore we measured relative brain atrophy with SIENAx by accurately defining brain size with respect to skull size, normalised to a standard template, resulting in normalised brain volumes. This also reduces within group variations, making cross-group comparisons more sensitive.[30] Manual segmentation of basal ganglia volumes in these groups were described elsewhere.[6]

Statistical analysis
SPSS for Windows (release 16.0.) was used for data analysis. Group differences were analysed with parametric or non-parametric tests when appropriate. To assess differences in age, education, UHDRS scores, brain volumes and MTI parameters we used independent t-tests. Pearson correlation analysis (r) was used to investigate associations of MTI measures with UHDRS scores and CAG repeat length. The level of statistical significance was set at $p \leq 0.01$. Values of $0.01 < p \leq 0.05$ were considered as a trend towards significance.

Results

Clinical characteristics
There were no significant differences between groups for sex, age, years of education and UHDRS motor, cognitive and behavioural functioning (*table 1*).

MRI parameters
There were no significant differences between groups for whole brain, grey and white matter brain volumes and MTI parameters (*table 2*). An association between smaller brain volume and higher age, was found only in non-carriers. Since the statistical assumption 'homogeneity of regression slopes' was violated, covarying for age was not feasible. Furthermore as groups did not differ significantly in age we decided not to correct for this variable. We also investigated brain volumes normalised for head size and found a tendency towards smaller whole brain volume (p= .05), especially white matter (p= .03) in carriers compared to non-carriers.

Table 1. Clinical characteristics

	Carriers (n = 16)	Non-carriers (n = 14)
Male/ female[a]	6/10	6/8
Age in years	41.9 (10.0)	47.2 (9.2)
Education in years	12.9 (2.7)	12.6 (2.8)
CAG repeat length[b]	42 (40-49)	19 (16-24)
Probability of onset within 5 years[b,e] **(%)**	17 (0-73)	
UHDRS		
Total motor score[c,d]	3.5 (0-10)	2.4 (0-6)
Verbal fluency	38.5 (13.6)	39.0 (10.4)
SDMT	52.2 (11.8)	60.6 (11.7)
Stroop colour	72.3 (13.0)	80.9 (16.2)
Stroop word	96.6 (10.6)	100.1 (19.6)
Stroop interference	42.3 (7.7)	45.6 (6.8)
Total behavioural score[c]	12.4 (11.4)	15.8 (17.6)

Values in the table are means (SD). No significant differences were found between carriers and non-carriers, except for CAG repeat length (p= .00). Independent t-test analysis, [a]Pearson's χ^2-test, [b]Median (range). [c]Mean (range). [d]Higher scores correspond with more abnormalities. [e]A greater probability of onset within 5 years corresponds with being closer to estimated onset of disease. UHDRS= Unified Huntington's Disease Rating Scale. SDMT= Symbol Digit Modalities Test.

Table 2. Volumes and MTI parameters of whole brain, grey matter and white matter

		Carriers (n = 16)	Non-carriers (n = 14)	P value
Whole brain	Volume (cc)	1150.1 (86.2)	1205.2 (108.8)	0.13
	NBV	1492.8 (57.2)	1536.6 (59.5)	**0.05***
	MTRm	39.8 (0.6)	39.8 (0.9)	0.94
	NPH	88.8 (8.3)	90.2 (5.2)	0.59
Grey matter	Volume (cc)	654.0 (51.0)	678.7 (70.2)	0.28
	NBV	849.6 (50.0)	865.8 (62.3)	0.43
	MTRm	37.6 (0.6)	37.7 (0.9)	0.59
	NPH	87.7 (9.4)	90.1 (5.4)	0.40
White matter	Volume (cc)	496.1 (47.6)	526.5 (53.0)	0.11
	NBV	643.2 (34.3)	670.9 (29.6)	**0.03***
	MTRm	43.4 (0.5)	43.3 (1.1)	0.72
	NPH	147.6 (15.2)	155.3 (16.7)	0.20

Values in the table are means (SD). Independent t-tests were used for statistical analysis. *p<.05. NBV= normalised brain volume (normalised for skull size). MTRm= mean MTR, NPH= normalised peak height. The peak height was normalised for brain size of the region of interest (number of voxels on the peak divided by the total number of segmented voxels).

The results on the basal ganglia volumes in the studied groups have been reported in an earlier study and showed smaller caudate, putamen and globus pallidus volumes in carriers.[6]

Relations between UHDRS and MTI parameters

In gene carriers we found that a higher score on the UHDRS motor scale, reflecting more motor abnormalities, was significantly associated with a lower peak height of the grey matter MTR histogram (r= -.70, p= .003) (*figure 1*) and marginally with a lower peak height of the whole brain MTR histogram (r= -.57, p= .02). Associations remained after controlling for basal ganglia volume. From the UHDRS cognitive and behavioural assessment in carriers, a significant association was found between lower white matter mean MTR and better scores on the Stroop interference task (r= -.63, p= .009) and marginally between lower whole brain peak height and worse scores on the Stroop colour naming task (r= .56, p= .02). In non-carriers no significant associations with MTI parameters were found.

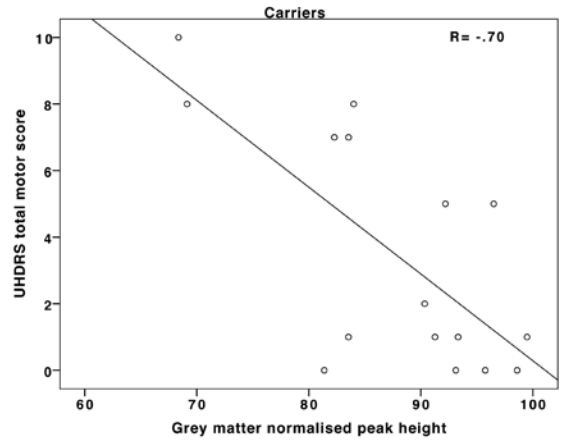

Figure 1. Significant association in carriers between higher score on the UHDRS motor scale and lower normalised peak height of the grey matter.

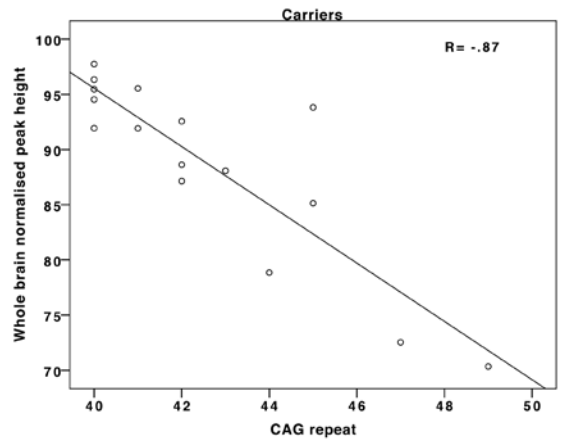

Figure 2. Significant association in carriers between higher CAG repeat length and lower normalised peak height of the whole brain.

Relations between CAG and MTI parameters

A higher CAG repeat length in carriers was strongly related to lower peak heights of the whole brain, grey and white matter (r= -.87, p= .00; r= -.86, p= .00; r= -.68, p= .004) (see *figure 2* for an example). A trend association was found between higher CAG repeat length and lower grey matter mean MTR (r= -.55, p= .03). Furthermore, a greater probability of developing symptoms within 5 years was marginally related to smaller grey matter peak height (r= -.58, p= .02).

Discussion

Regional brain changes, specifically in the striatum, have been demonstrated many years prior to the onset of clinical signs in HD.[2,6,31] The onset and progression of early diffuse brain changes in HD are imperfectly known. To the best of our knowledge, this is the first time that extensive MTI analysis has been applied to study grey and white matter in premanifest HD mutation carriers. In carriers lower MTR peak height was specifically related to decreased motor functioning and higher CAG repeat length. Although we could not demonstrate MTR differences between carriers and non-carriers, the strong associations with HD characteristics in carriers indicate that MTR values might reflect disease burden.

In line with one previous study, predominantly including symptomatic carriers, mean MTR values did not differ between carriers and non-carriers.[19] We also studied the more sensitive peak height of the MTR histogram and could not demonstrate any brain abnormalities in carriers with that parameter either. However, when volumes of the whole brain, grey matter and white matter were normalised for skull size, hereby measuring atrophy state, carriers had a smaller volume of the whole brain parenchyma and especially the white matter, indicating the presence of global brain changes in carriers.[30] This is in accordance with previous studies demonstrating early grey and white matter tissue loss in premanifest HD.[2,3]

Our data show that in these areas of reduced grey and white matter tissue MTR values were not abnormal, indicating that the integrity of the tissue was not significantly altered on a group level. It might be that the specific HD pathologic process does not result in altered tissue integrity, confirmed by the study of Mascalchi et al who could not demonstrate MTI abnormalities in basal ganglia of HD patients, despite smaller basal ganglia volumes compared to controls.[19] In other neurological diseases a reduction in MTR has been especially associated to myelin loss, axonal loss, gliosis and inflammation.[14,17,32] Since there is no unequivocal substrate underlying MTR, the technique may be less sensitive for detecting other microscopic changes, such as those in HD. Nevertheless, the remarkably strong association between lower MTR peak height and a higher CAG repeat length, indicates that the integrity of the tissue reflects underlying genetic disease burden. Furthermore, clinical relevance of the MTR was also demonstrated; lower whole brain and grey matter peak height in carriers, reflecting less cerebral homogeneity, was found to be strongly related to more motor abnormalities. Motor functioning has been linked to striatal changes in previous premanifest studies.[5,6,31] Since associations remained after controlling for basal ganglia volumes, the findings might indicate the involvement of more diffuse grey matter changes in altered motor functioning.

It can be argued that with advancing motor abnormalities and pathological burden the MTR peak heights continue to decrease and will significantly differ from controls. This is strengthened by the finding that MTR peak heights tend to be somewhat lower in carriers in this study. Furthermore, lower grey matter peak heights were associated with a greater probability of developing symptoms within five years.

Various studies in other diseases, like Alzheimer's disease and Multiple sclerosis, related MTI measurements to cognitive functioning, however results are inconsistent.[14,18,33,34] Surprisingly, we showed in carriers that a lower mean value of white matter MTR, indicating worse histological brain composition, was associated with better performance on the Stroop interference task. Since minimum variation is found in MTR values in both carriers and non-carriers this result has to be interpreted with caution. Furthermore MTR peak heights proved a better reflection of abnormal brain structure in other MTI studies of neurodegenerative diseases.[11,20-22] Van der Flier et al demonstrated a strong association between diffuse brain damage as measured with global MTR peak heights and neuropsychological test results in Mild Cognitive Impairment and AD.[35] We did show that lower whole brain peak height was related to worse scores on the Stroop colour naming task. Snowden et al. emphasised that relatively automatic speed based tasks, such as this Stroop condition, are most sensitive for HD related cognitive changes.[36]

Strengths of the current study include the automated segmentation methods for MRI data. Furthermore, we associated MTI values with the full spectrum of HD characteristics. Potential limitations include the small sample size, which might have contributed to the absence of a statistical difference in MTI characteristics between carriers and non-carriers. Also the rather thick section thickness of the MTI sequence might have contributed to less sensitivity. Furthermore, carriers seemed quite far from onset based on probability of onset estimations and on the relatively low motor scores compared to other studies in premanifest HD.[37,38] A follow up of the current cohort and a larger sample, including manifest HD carriers as well, should shed more light on diffuse MTR abnormalities and motor and cognitive correlates in HD.

In conclusion, although we could not detect altered MTI characteristics in carriers of the HD gene mutation without clinical manifestations, we did provide evidence that the MTR peak height might reflect genetic and subclinical disease burden. Whether MTI parameters are sensitive for HD related brain changes and associations with the phenotype in more advanced stages of disease remains to be investigated.

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