MRI biomarkers in Huntington's disease

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1. ABSTRACT

Huntington's disease (HD) is a devastating neurodegenerative disease affecting the brain resulting in neuronal dysfunction and neuronal loss. Since the identification of the gene responsible for HD, genetic testing has become widely available, allowing for genetic status of persons at risk for HD to be determined. For the effective evaluation of future therapeutic trials a great need exists for sensitive biomarkers. In (premanifest) HD, MRI of the brain is one of the most logical candidates as a biomarker, as opposed to clinical measures, since brain neurons are the main target of the disease. These biomarkers can facilitate early detection of disease related changes, but are also needed to monitor disease progression from the premanifest phase of HD onwards. MRI derived parameters have this biomarker potential as they have been shown to identify brain abnormalities before symptom onset. In this review the available MRI techniques of conventional MRI, Diffusion Tensor Imaging, Magnetization Transfer Imaging, Magnetic Resonance Spectroscopy and Functional MRI will be discussed and the findings will be placed into context of different HD stages.

2. INTRODUCTION

Huntington's disease (HD) is a devastating neurodegenerative disease affecting the brain through neuronal dysfunction and loss. The disease is caused by a Cytosine-Adenine-Guanine (CAG)-repeat on chromosome four in the HTT gene, resulting in a mutant huntingtin protein. The disease is clinically characterized by a triad of progressive symptoms that occur with varying severity amongst patients. First and foremost HD is regarded as a movement disorder with chorea, dystonia, rigidity, bradykinesia and gait impairments. Besides motor disturbances cognitive decline is apparent with disturbances in psychomotor speed, executive functioning and memory with relative preservation of language and speech tasks. Lastly, behavioural abnormalities are characterised by depression, anxiety, disinhibition, aggressive behaviour and a tendency to suicide (1, 2). Besides the classical triad, reports also show symptoms of weight loss, sleep disorders and autonomic nervous system disturbances (3-5).

The onset of Huntington's disease typically occurs in the third or fourth decade of life, with a large

range in disease onset, for 60-70% explained by the length of the CAG mutation (6). Since genetic testing became available in 1993 it is possible to determine the genetic status of those at risk of HD. The symptom free period of gene carriers prior to inevitable disease onset is typically referred to as the preclinical or premanifest phase of the disease and historically has always been defined by the absence of motor disturbances. However, it is been recognised for some time now that the disease can begin with cognitive or behavioural problems (7, 8).

The pathologic hallmark has always been atrophy of the striatum, with further histological findings of nuclear inclusions in neurons and atrophy both globally as well as specific brain areas (9, 10). The disadvantage of the available histological data is that it is typically only available from end-stage HD patients. For characterisation of the brain changes in the premanifest phase and early stages of HD we must rely on *in vivo* imaging techniques.

Since the early nineties reports of *in vivo* atrophy using MRI appear in the literature. These reports followed initial findings using Computerized Tomography, showing a decreased bicaudate ratio (11). Initially, MRI studies only discussed the manifest disease stages, but after the widespread introduction of genetic testing, reports have also documented the premanifest phase of HD. MRI is a non-invasive tool for imaging the brain in high resolution yielding good contrast between grey and white matter. Currently, MRI has progressed from just detecting contrast differences to the development of techniques such as Diffusion Tensor Imaging (DTI), Magnetization Transfer Imaging (MTI), Magnetic Resonance Spectroscopy (MRS), functional MRI (fMRI) and more. However, given the multitude of possibilities, it is pertinent to ask what it is we are measuring with these applications. Atrophy for instance, is the consequence or end product of the disease as this is the measurement of tissue loss. Newer techniques such as MRS can measure metabolic levels, delving further into the pathophysiological processes than atrophy alone. DTI was developed to investigate the integrity of brain tissue by quantifying the properties of tissues and their boundaries, in terms of the water molecule displacement across or alongside these boundaries. Therefore DTI is perhaps able to give insight into both deterioration itself as well as the processes underlying such deterioration. Therefore it is always important to specify the goal of an MRI study in terms of understanding of pathophysiological processes or measuring and quantifying change.

In this review we will focus on measurements and quantification of the different MRI-studies in HD as our main interest is biomarker research. The MRI approaches of conventional or volumetric MRI, DTI, MTI, MRS and fMRI will be discussed and the findings will be placed into context of the different HD stages.

3. BIOMARKERS

To measure and quantify brain changes by means of MRI is to enter the field of the biomarker research. A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to interventions" (12). In the context of HD research many different measurements could potentially qualify as a biomarker, physically (motor, cognitive and psychiatric measures), chemically, or by imaging. However, the question remains, what makes a good biomarker and furthermore, why is one needed for HD? To start with the latter it is relevant to note that currently no cure exists for HD, however there are many potential agents currently under investigation (13). Given that the symptoms of HD arise as a consequence of underlying brain changes, the search for an effective cure should embrace the premanifest period in which brain changes have started but have not yet resulted in symptom manifestation. In this phase of disease development neuroprotective therapies could be at their most effective. Currently, testing new agents in manifest HD is difficult as it is a slowly progressive disease taking up to 15 to 20 years from disease onset until death. As clinical trials are not deemed effective when a long follow up is required, a shorter set-up is preferential. To measure the effect of an agent over a short time an effective biomarker in HD must be able to detect change over this relatively short period. Subsequently once an agent is found to be effective, the next pertinent question will be when to start with the intervention? Is it most effective when applied in the period that symptoms are not noticed by gene carriers, but when brain changes have already started? If so, another issue arises, when no clinical symptoms can be measured, we must rely on other biologically based markers, hence returning to the term biomarker as a so called surrogate markers or end-point. In (premanifest) HD, MRI of the brain is one of the most logical candidates as a biomarker, as opposed to clinical measures, as brain neurons are the main target of the disease, and show the earliest disruption.

Currently the search for biomarkers in HD has focussed on medical imaging in an attempt to provide an *in vivo* overview of pathological changes in the natural course of HD. In essence, what happens to which part of the brain in progressing disease states? Aylward *et al.* (2007) discussed three criteria for a biomarker in the context of MRI and striatal volume: first it must be objectively measurable, secondly it must be able to predict known endpoints and thirdly it must be associated to known mechanisms of pathology of the disease (14). The application of these criteria can be extended, from the striatal region only, to all brain regions and other MRI techniques.

In summary, the ideal biomarker in HD would measure pathologic change over a relatively short period, these changes would be disease specific and reflect specific disease stages. Furthermore it would detect brain changes before symptoms are present and can therefore be deployed in the premanifest phase.

4. METHODS

In reviewing the literature on MRI in HD, a search was performed for all relevant articles on HD in the

PubMed and Medline databases published up to December 2010. The following search terms were used for HD: "huntington", "huntington's disease" or "HD" whether or not in combination with "Neurodegenerative Diseases". Furthermore, the terms for MRI specifically were: "mri", "magnetic resonance imaging", "mr imaging", "mrs", "magnetic resonance spectroscopy", "mrsi", "fmri", "dti", "diffusion tensor", "mti" and "magnetization transfer imaging" in combination with any of the above described HD search criteria. All papers that reported on HD with MRI outcome measures were initially included. We then categorized the papers based on the type of MRI research and only those who reported on the topics described in the introduction were included. For the section on MRS it is noteworthy we only included 1H-MRS studies as opposed to carbon or phosphor MRS. Furthermore, we limited our review to reports that included only genetically confirmed Huntington's disease patients or premanifest gene carriers.

This review will not describe the various techniques with their advantages and disadvantages, but rather focus on the outcome of these studies in terms of changing brain structures in HD.

5. STRUCTURAL MRI

Structural or volumetric MRI was the first *in vivo* imaging application to be described in HD (15). Since the start of the MRI era the analysis or post-processing techniques of these T1-weighted and T2-weighted scans has evolved significantly. Various manual segmentation techniques have been described as well as the more recent application of semi- to fully-automated techniques such as Voxel Based Morphometry (VBM), Boundary Shift Integral (BSI) and FMRIB's Integrated Registration and Segmentation Technique (FIRST) (16-18).

The hallmark of HD has always been atrophy of the caudate nucleus and putamen, which together form the striatum. MRI studies from the early nineties report atrophy of these structures in manifest HD (19-23) and every study since has confirmed this finding (16-18, 24-33). It has also become clear that atrophy is already present well before disease onset in premanifest gene carriers (16, 17, 34-42). Earlier reports only make the distinction between manifest and premanifest HD, but later reports also include a distinction within premanifest gene carriers of "far from predicted disease onset" and "close to predicted disease onset" (16, 17, 40, 43). These predictions of disease onset, or sometimes disease burden, are most regularly based on Langbehn's formula of predicted age of onset (6, 44, 45). Also many studies include correlation analysis of predicted disease onset with the amount of atrophy of the striatum in premanifest gene carriers (37, 41, 43, 46). Exactly when in the disease stages this reported atrophy of caudate nucleus and putamen shows significant difference to control subjects varies per study, but the reports suggest as early as 11-15 years before disease onset (43, 46, 47). Longitudinal analyses of atrophy of the striatum are available and demonstrate a relative stable or linear rate of decline from premanifest to moderate severe HD onwards (26, 47). Such changes in premanifest HD could not be demonstrated in a

cohort "very far from disease onset" of 18.1 years (48). The most recent longitudinal publication of the large TRACK-HD study reports atrophy rates in the premanifest group of 1.37% higher than controls and 2.86% in manifest HD of the caudate nucleus over 12 months follow up (49). Cross sectional analysis from studies examining more than just two disease phases, but rather make subgroups within (pre)manifest HD, suggest that the striatal decline seems to slow down the further the disease progresses with little or no differences than is seen in the early HD stages (16, 17). A pilot study from Aylward *et al* (2003) rated caudate atrophy in a clinical trial investigating remacemide and co-enzyme Q10 and state that these atrophy measures show great potential as an outcome measure (50). To summarize, striatal atrophy seems to begin more than a decade before disease onset, displays a rate of decline disproportionate to whole brain atrophy and volume seems to decrease substantially until at least the early HD stages.

Extra-striatal subcortical grey matter structures have been less studied than the striatum, yet there is convincing evidence that almost all other subcortical grey matter structures are affected to some degree at various disease stages. In manifest HD reports exist on atrophy of the thalamus (17, 19, 22, 31, 33, 51), pallidum (17, 25, 30, 31, 36), accumbens nucleus (17, 25, 31, 33), amygdala and hippocampus (17, 25, 31). For the premanifest phase some controversy exists for the thalamus as some studies report atrophy in this stage (35, 40, 43) and others do not (42) or report that atrophy is consistent with whole brain atrophy (17). The report which does not report atrophy is a study with a relatively small number of participants and did not discriminate between far and close to disease onset. The largest studies, e.g. the PREDICT-HD and TRACK-HD studies do report thalamic atrophy in the premanifest phase of HD. When combining the evidence it is apparent that although not as significant as in the striatum, the thalamus does show reduced volume in the premanifest phase.

Atrophy of the pallidum is also under some dispute in the premanifest phase as two reports did not demonstrate atrophy (34, 35), however more reports found support for atrophy in the premanifest phase (17, 38, 39, 42). Furthermore, the reports in favour of pallidum atrophy are again the larger and more recent trails. Therefore the same conclusion applies for the pallidum as for the thalamus. In a large sample of premanifest gene carriers also the accumbens nucleus and hippocampus were shown to show signs of atrophy (17).

Longitudinal analysis of extra-striatal structures is underreported, especially in the premanifest phase. One report found a significant decrease in accumbens nucleus and thalamus over a 2 year follow up in manifest HD, but not in premanifest HD (48). The pallidum has been found to show progressive atrophy in manifest HD by Ruocco *et al* (2008) (52), although this was not found in an earlier study (27). The two studies on pallidum atrophy in manifest HD were comparable in terms of duration of symptoms and mean CAG repeat length of the participants as well as scan interval. However the study by Ruocco *et al.* (2008) included slightly more participants and used VBM as
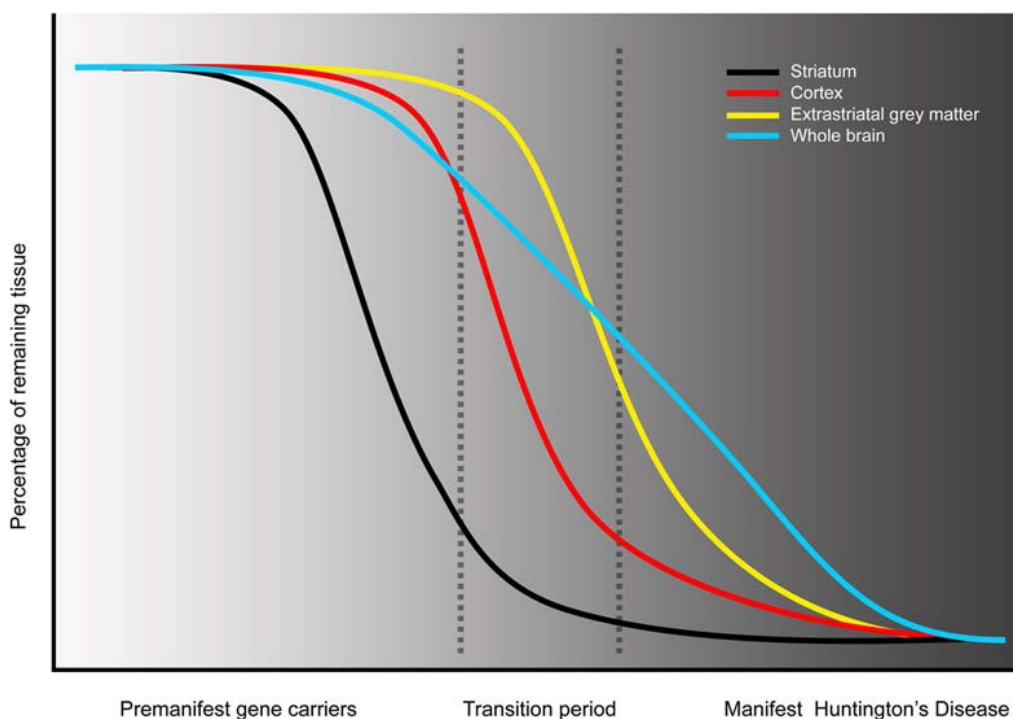
Figure 1. Atrophy of cerebral structures depicted by percentage of atrophy. GM = grey matter. The x-axis displays time, the y-axis displays percentage of remaining tissue of the different brain structures.

opposed to manual segmentation. On the basis of these two studies the rate of atrophy is difficult to assess.

Cortical grey matter has repeatedly been shown to be affected in manifest HD, as measured by VBM (18, 31, 52, 53), cortical thickness measurements (16, 54-56) or other volumetric measures (19, 33). In the premanifest phase of HD one report exists of an enlargement of cortical grey matter volume (40), however the same group did not replicate this in subsequent studies with larger populations (43). It seems apparent that cortical grey matter is affected already far (>15 years) (43) or midway (9-15 years) (16, 57) from predicted disease onset (43). The cortical areas reported to be affected in the earliest premanifest stage are the posterior frontal (16), parietal and occipital regions (57). Other studies who did not specify the number of predicted years to onset of their group, report atrophy of insular regions (38), superior temporal, precuneus, middle and pre-central frontal regions (55) and prefrontal cortex (58). Longitudinal analysis of cortical disturbances has not been reported to a great extent. Ruocco *et al.* (2008) observed in manifest HD progressive volume loss in the insula, cingulate, prefrontal, medial temporal and frontal cortex over a follow up period of 1 year (52). Hobbs *et al.* (2010) reported progressive frontal, occipital and parietal cortex loss over a 2 year period in manifest HD, but not in premanifest HD (48). When summarizing the findings it appears that regionally specific cortical atrophy appears in premanifest HD around a decade before disease onset, and spreads over almost the entire cortex in early HD stages.

Besides grey matter, also white matter demonstrates volume reductions in both manifest (19, 25,

33, 59, 60) and premanifest (38, 40, 43) HD. The earliest report of white matter volume loss is in a "far from predicted disease onset" premanifest HD group (<15 years) and the authors report this as a good predictor of disease onset (43). Longitudinal analysis demonstrated severe progressive white matter loss in early HD (48, 49), and the TRACK-HD study showed this progressive white matter loss already in the premanifest phase far from disease onset (49).

With reports on cortical grey, subcortical grey and white matter loss, whole brain atrophy is a logical consequence in premanifest and manifest HD (16, 43). This measure demonstrated significant difference from controls in "far from" (43) and "close to" (16) predicted disease onset groups. Longitudinal analysis seems to indicate this is a fairly stable and easy measure in manifest HD only, as this change is relatively severe in early HD and can be demonstrated with just a 6 month follow up period (61-63). The most recent study found a 0.20% higher atrophy rate in premanifest HD and 0.60% in manifest HD in one year follow up (49).

To capture all the literature into one overview we have constructed hypothetical models. These models are our own interpretation of the combined evidence and are therefore not to be taken as proven fact, rather as a way of providing a guide towards temporal changes in the brain of HD. Figure 1 graphically displays the hypothetical model for atrophy of different brain regions and their relation to disease stage.

6. DIFFUSION TENSOR IMAGING

Diffusion Tensor Imaging (DTI) is based upon the diffusion properties of protons in the intra- and

extracellular space, and was developed to investigate the integrity of tissue matter. During a DTI scan the preferential orientation of the diffusion as well as the strength or directionality of the diffusion of water molecules is measured throughout the brain. In highly organised tissue, such as the white matter comprising of dense axon bundles, the diffusion properties are dramatically influenced because of the 'boundaries'. In essence the axons in white matter provide a pathway for diffusion along the axonal axis, whereas diffusion is frustrated by cellular barriers perpendicular to axis of axons. DTI is not restricted for use in white matter only, but can be applied in grey matter as well. The two most widely derived measures from DTI scans are fractional anisotrophy (FA) representing the strength of the main direction of diffusion, and mean diffusivity (MD) portraying the speed of the diffusion, although Apparent Diffusion Coefficient (ADC) is also used in this regard. Sometimes TraceD is used, which is the total diffusivity, meaning the sum instead of the mean of the three eigenvalues. An FA value close to 0 is representative of equal diffusion in all directions, as is seen in cerebral spinal fluid. In contrast an FA value close to or equal to 1 represents highly directional diffusion. High MD-values represent unrestricted diffusion (for example in CSF) and low MD-values suggest restricted diffusion. The post-processing techniques used for DTI consisted of either a (manual) region of interest (ROI) segmentation technique (combined with computed tracts between ROI's) or a voxel wise statistical approach such as tract based spatial statistics (TBSS).

In manifest HD disturbances in either FA or ADC in white matter have been reported in the corpus callosum (64-67), fornix (66), internal capsula (65), external capsula (66), capsula extrema (66), frontal subcortical white matter (65,67), sensorimotor white matter pathway (67), inferior fronto-occipital fasciculus (66), inferior longitudinal fasciculus (66) and total white matter (28). The grey matter is also reported to show disturbances in the putamen (28, 65, 68-70), caudate nucleus (28, 67-70), pallidum (65, 69, 70) and thalamus (70). It is important to note that these studies report increased FA in the grey matter, whereas in white matter a decrease in this measure is apparent. Douaud *et al.* (2009) explains this as a decrease of dispersion of the fibre orientation, characterising a loss of connections from these structures (70).

In premanifest HD the findings seem comparable to the findings in manifest HD, only not as extensive. General widespread white matter (71) is reported to display disturbed DTI properties, as well as specific areas such as the corpus callosum (64, 65), external capsula (72), putamen (72) and different areas of frontal white matter (73). Furthermore specific pathways to the sensorimotor cortex (67) and the fronto-striatal connections (74) have been found to be affected. The integrity of the corpus callosum has been reported affected in a far from predicted disease onset group (>11 years) (64). The disturbances were found to correlate well to predicted time to disease onset (67, 71, 72).

Longitudinal findings from Weaver *et al.* (2009) show FA reduction in white matter throughout the brain, which increased during a 1 year follow up in manifest HD, and not in controls (75). Sritharan *et al.* (2010) examined the subcortical grey matter structures over a 1 year period using the MD instead of FA and found higher values in the putamen and caudate nucleus at both time points, however no longitudinal change was observed (76). Similarly, Vandenberghe *et al.* (2009) did not demonstrate a longitudinal change over 2 years in the striatum in a manifest population and even suggests that volumetric change is more sensitive as a longitudinal marker. When considering this conclusion it must be noted that this report only examined TraceD in the striatum and not diffusion properties in the rest of the brain (68). When combining the information from these three longitudinal studies it is evident that these studies all included relatively small samples, ranging from 7 gene carriers (mixed premanifest and manifest) in the study by Weaver *et al.* (2009) (75) to 8 manifest HD by Vandenberghe *et al.* (2009) (68) up to a maximum of 18 manifest HD by Sritharan *et al.* (2010) (76). On examination of the data it can be seen that all the measures used are changing over time in the expected direction in each study, yet none but the study by Weaver *et al.* (2009) demonstrates significant results. It is likely that the results of these studies were influenced by power issues which hindered demonstration of significant change over time.

In summary, widespread diffusion abnormalities have been demonstrated in both manifest and premanifest HD, most notably the white matter, but also in subcortical grey matter. In measuring longitudinal change of the diffusion properties of white matter seem more promising than those in grey matter. It remains the question whether FA, MD or TraceD (or some combination) is the best single measure. Furthermore the choice of post-processing technique can affect the outcome. A priori a voxelwise approach may have a greater sensitivity for detecting change as opposed to a region/tractography based method, yet how sensitive this is over time remains unclear. Noteworthy is also the introduction of radial and axial diffusivity, making use of more than only the principal diffusion direction (70, 75), as this may provide not only be a more sensitive measure, but could also shed light on pathophysiological processes. Weaver *et al.* (2009) reported larger decreases of axial diffusivity (a marker of the stability of axons), than radial diffusivity (a marker for demyelinisation), and concluded that axonal injury plays a larger role than myeline disturbances (75).

Also for DTI we have constructed the same type of hypothetical model as with the volumetric MRI data. Figure 2 graphically displays the hypothetical model for DTI changes in different brain regions and their relation to disease stage.

7. MAGNETIZATION TRANSFER IMAGING

MTI offers a way of examining tissue structure and structural components in a different way than
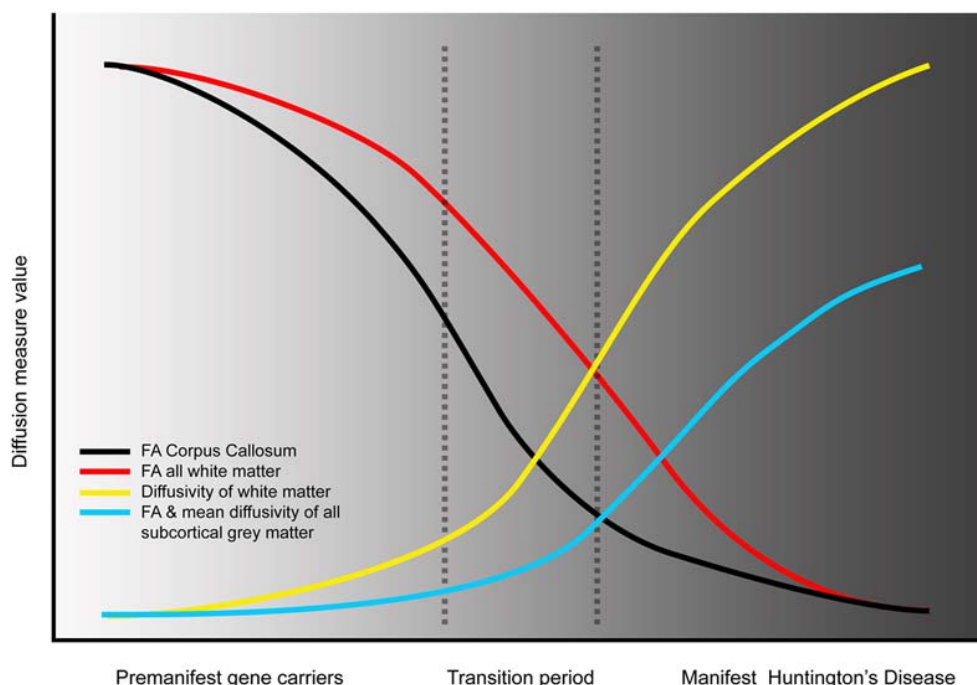
Figure 2. Diffusion tensor imaging values in relation to time. WM = white matter, CC = corpus callosum, WM = white matter, FA = fractional anisotropy, MD = mean diffusivity, SGM = subcortical grey matter. The x-axis displays time, the y-axis displays percentage of diffusion measure value.

conventional MRI. 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 Magnetization Transfer Ratio (MTR), representing the percentage of variation in the MR signal between the saturated and unsaturated acquisitions, is a measure used in clinical studies (77). MTI differs from other techniques such as DTI in the sense that it measures properties of macromolecules found in cells as opposed to the properties of water molecules. Two main outcome measures are reported, the mean MTR and the MTR peak height from histogram analysis.

In manifest HD, Mascalchi *et al.* (2004) reported no differences between a group of 21 gene carriers (of which 19 manifest HD) and 21 controls (28) examining the mean MTR. In contrast Ginestroni *et al.* (2010) found a statistically significant decrease of the MT ratio in all subcortical grey matter structures (except putamen) and in the cerebral cortex diffusely. The whole brain white matter was not significantly decreased (78). There was a close correlation found to clinical measures of motor and neuropsychological testing and disease duration (78). The difference between these studies are only obvious in post-processing methodology, Ginestroni *et al.* (2010) used the automated segmentation tools provided by FSL and Mascalchi *et al.* (2004) used manual segmentation. In terms of the patient characteristics we cannot determine if any differences exist in clinical severity due to the fact that

mainly by Mascalchi *et al.* (2004) this is not described in detail, only by a gross measure, namely the TFC (28). Based on these two studies it is not possible to draw any definite conclusions.

In premanifest HD only one study has published results examining not only the mean MTR, but also the MTR histogram peak height. There were no group differences, however there was a good correlation between this measure and subtle motor disturbances and CAG repeat length (79). Unpublished results from our own study group confirm the findings by both Jurgens *et al.* (2010) and Ginestrone *et al.* (2010) whereby group differences exist in the manifest stage of the disease only and that there is a relation to clinical measures.

In summary, there is a lack of sufficient data, but the data at hand suggests that MTI is only potentially useful as a biomarker in the manifest stages of the disease. More studies, particularly longitudinal studies, should be performed to examine MTI measures in HD.

8. MAGNETIC RESONANCE SPECTROSCOPY

Whereas conventional MRI uses hydrogen protons to characterize different tissues, MRS uses these hydrogen protons to measure metabolite concentrations. This technique therefore does not give direct structural information, rather this enables interpretation of changes in physiologically related processes. This is based on quantifications of a number of different metabolite

compounds which reflect underlying processes. The most common metabolites examined are: N-acetylaspertate (NAA), which is seen as a marker for neuronal and axonal integrity, Creatine which is regarded a marker for brain energy metabolism, Choline reflecting membrane turnover, Myo-inositol (MI) is seen as an osmolyte and astrocyte marker, Lactate which is regarded as representing interruption of oxidative processes and beginning of anaerobic glycolysis, and glutamate, with its precursor glutamine, is a neurotransmitter (80).

In manifest HD a lower NAA and creatine has been demonstrated in putamen and/or caudate nucleus (81-85) and thalamus (86). Furthermore an increase in glutamate has been demonstrated (87-89) however this is under some dispute as this is not a consistent finding in all studies (81,82), and has even been reported to be lower than in controls (81). A consideration with the reports on increased glutamate may be the fact that glutamate was reported as a ratio to creatine, however, creatine itself does not appear to be a stable metabolite in HD. Furthermore, the reports on increased glutamate were performed in the early nineties, use different methodology, have lower field strength and have a smaller sample size. This is all in contrast to recent reports. Therefore we are of the opinion that based on the reports, it is more likely that glutamate is lowered rather than increased in HD.

Increased Myo-inositol in manifest HD has been reported in the putamen and therefore a ratio between MI and NAA could be a useful marker for tracking disease pathology (81). Energy metabolism disturbances can be demonstrated with creatine measurements, but also lactate is important in this respect. Increased lactate (or ratio of lactate to either NAA or creatine) has been found in basal ganglia, cerebellum, occipital, parietal and frontal cortex (83, 84, 90, 91). It must be noted, that although quite some studies suggest impaired energy metabolism, not all studies concur, as Hoang *et al.* (1998) conclude that no evidence can be found for impaired energy metabolism using a combination on 1H-MRS and 31P-MRS (92), and furthermore Reynolds *et al.* (2005) stress the issue of heterogeneity in MRS findings in HD, stating that there is no pathognomic alteration of any metabolite for HD (93). There is substantial evidence from non-clinical studies that energy metabolism is impaired in HD (94), also the number of positive results from *in vivo* MRI-studies described above is quite substantial, therefore we feel that it is not really a question if energy metabolism is impaired, but rather how it can be measured accurately. Finally, only one report found an alteration of choline, with a decreased NAA/choline ratio in the frontal cortex (90).

Conflicting results exist for the premanifest stage of HD with regards to alterations of metabolites. As in manifest HD, lower creatine and NAA are reported in putamen and caudate nucleus (81, 85), although other studies did not find these differences (95,96). Of the other metabolites, only choline is reported to be decreased in the frontal cortex (96). There are important differences between the studies in favour and those in dispute of NAA or creatine changes in the premanifest stage, Sturrock *et al.*

used a higher magnetic field (3 Tesla as opposed to 1.5 Tesla) and had the largest sample size as well the benefit of occurring as part of a methodologically well set up TRACK-HD study (81). The higher magnetic field has a definite theoretical advantage to obtain good quality spectra. In light of the above described findings the evidence in favour of premanifest changes in the concentrations of these metabolites weighs more heavily than against the presence of such changes.

There appears to be a relationship between alterations in metabolites and measures of motor performance (81, 85), disease severity (90), neuropsychological assessment (96) and disease burden (81). Another interesting feature of MRS has been demonstrated by using MRS in the evaluation of high dose oral creatine as a treatment of HD. The brain concentrations were shown to be elevated by this therapy and even had an effect on the glutamate concentration, however no clinical improvement was shown, nevertheless it does show a potential application of this technique (97, 98).

A great lack of longitudinal MRS studies exists. Sturrock *et al.* (2010) is the only available longitudinal study as it is part of the TRACK-HD study (16). Preliminary data presented at the European Huntington's Disease Network congress did not show any longitudinal change over a 1 year follow-up period (99).

Overall, creatine and NAA are reported to be lowered in different brain regions and lactate possibly increased. Choline has not been reported as widely and it remains unclear whether or not glutamate is different in HD. The alterations of metabolites is likely to occur already in the premanifest stage, not only based on the above described studies, but also from a hypothetical point of view, where structural changes normally would occur simultaneously or be preceded by metabolic changes. Figure 3 graphically displays the hypothetical model for metabolite concentration changes and their relation to disease stage.

9. FUNCTIONAL MRI

Event related functional MRI (fMRI) uses the blood-oxygen-level-dependent (BOLD) signal to discriminate brain regions with altered activation. Simply put, this means that when a brain region is activated it will require an increase in energy resulting in blood demand, which is measurable with this method. Different functional tasks can be employed during fMRI scanning to measure activation in different brain regions or networks.

In HD research several studies are available with reports in both premanifest and manifest HD of aberrant connectivity or activation patterns. In manifest HD one case report and five studies are available. The first fMRI report is a case study with a clock reading task whereby increased parietal activation suggested a higher neuronal effort was required to perform the task (100). Subsequently several case control studies were performed: using a verbal working memory task lower activation of parietal cortex,
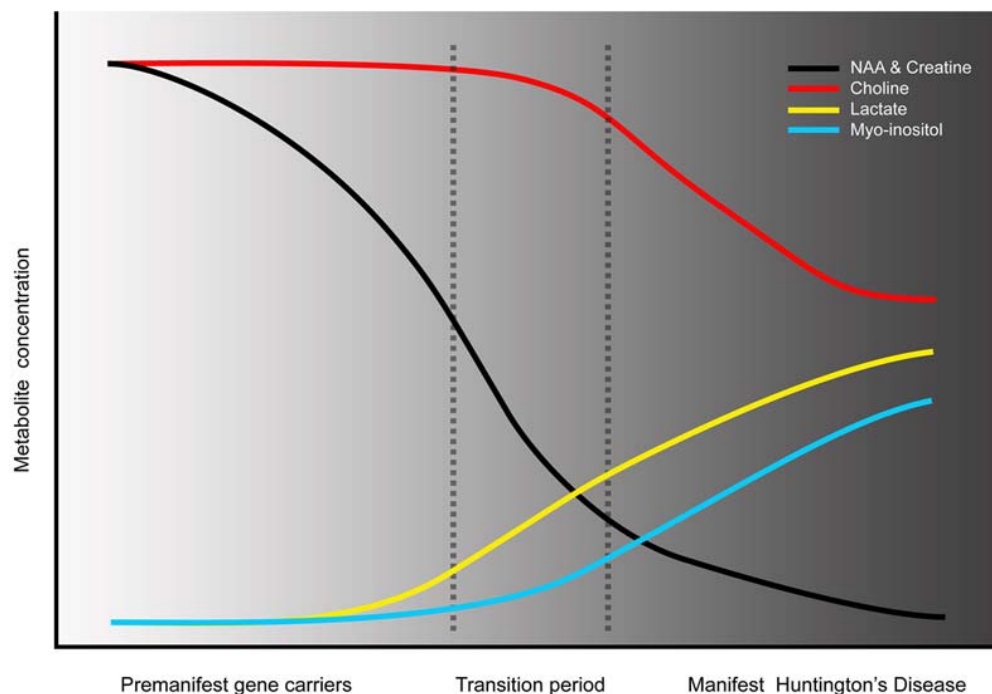
Figure 3. Magnetic Resonance Spectroscopy in Huntington's Disease in relation to disease stage. NAA = N-acetylaspartate. Glutamate is not depicted due to conflicting data in the literature. The x-axis displays time, the y-axis displays percentage concentration of metabolite.

putamen and cerebellum were shown in HD (101). Using a porteus maze task a reduced signal in occipital, parietal and somato-motor cortex and the caudate nucleus, and furthermore an increased signal in the postcentral cortex and right middle frontal gyri was demonstrated (102). Using a Simon Task, which activates the lateral prefrontal and anterior cingulate cortex, reduced functional connectivity of this region was found (103). In a mixed premanifest and manifest population, using a serial reaction time task a reduced activation of middle frontal, occipital, and precuneus regions was demonstrated (104). In slight contrast to these reports increased activation of the anterior cingulated-frontal- motor-parietal circuit was found using a Simon task (105). The two studies using the Simon task are from the same research group, whereby they differ as one examines functional connectivity (103) and the other activation patterns (105), both using fMRI. Although measuring different parameters the two studies seem at conflict with each other. However one might argue that when there is reduced connectivity the cortical recruitment of these regions can (or must) be enhanced. Currently definite conclusions on activation cannot be drawn as many different tasks have been utilised and therefore also different cortical regions have been examined. Although both increased and decreased activation have been reported, it seems that predominantly reduced activation patterns are found in manifest HD.

In premanifest HD a pattern of increased activation of several regions was demonstrated in premanifest gene carriers 'far from predicted disease onset' whereas premanifest gene carriers 'close to onset' show a reduction of activation (106,107). Reductions in activation in premanifest HD were specifically reported in areas such as left anterior cingulate cortex, lateral prefrontal cortex, parietal cortex, putamen (108-111). An interesting finding by the authors is that the change in activation pattern from far from onset to close to onset is related to the presence of atrophy in the striatum (106,107).

The use of different methodology and tasks applied during scanning complicates summarizing the results from the fMRI studies. Overall, the regions and networks shown to be affected in fMRI studies appear to widespread, confirming that HD is more than just a basal ganglia disorder, even in the premanifest stages of the disease. There appears to be mainly decreased activation in manifest HD and premanifest close to disease onset, although the study by Georgiou *et al.* (2007) contradicts this (105). The interesting finding of increased activation in premanifest gene carriers far from onset HD is hypothesized to be the result of cortical recruitment, in essence HD gene carriers need activation of more neurons and/or higher activity of these neurons in order to achieve the same task performance as controls. The pro's and con's of fMRI are discussed in a review by Paulsen *et al.* (2009) with commentary in Georgiou-Karistianis *et al.* (2009) (112,113)

10. DISCUSSION

Temporal relationships of MRI measures with HD have been extensively examined making use of the many and diverse capabilities of MRI. However, drawing
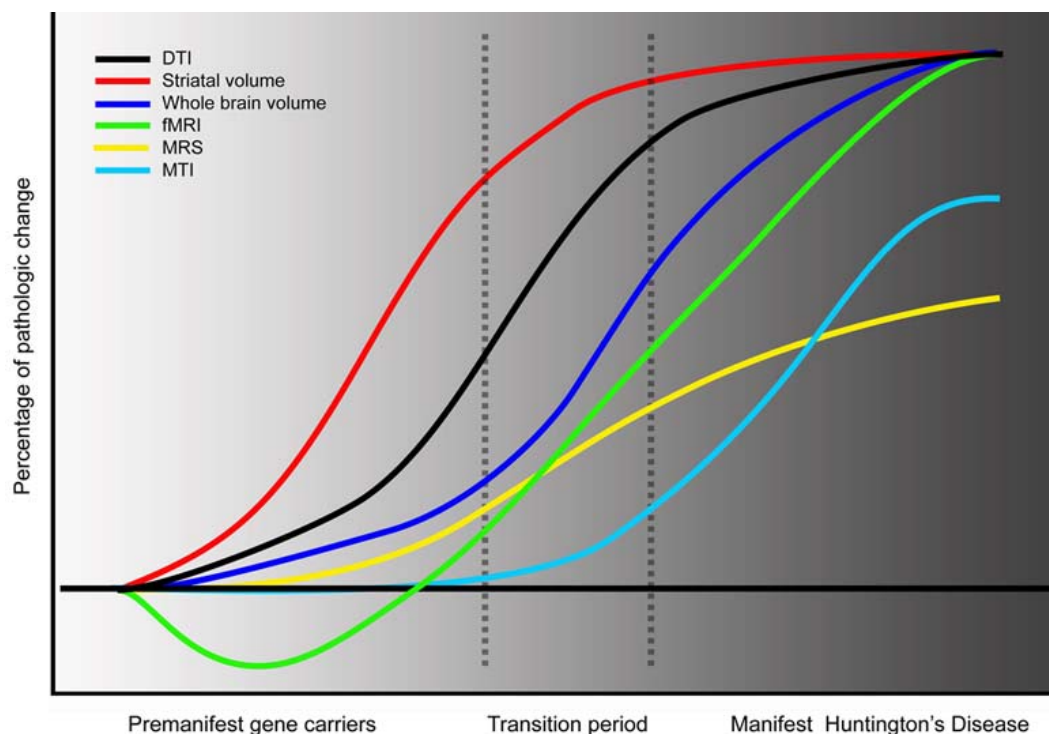
Figure 4. MRI changes in HD brains depicted as a function of time. DTI= diffusion tensor imaging, MTI = magnetization transfer imaging, MRS = magnetic resonance spectroscopy, FMRI = functional MRI. Functional MRI displays a negative curve in the premanifest 'far from onset phase' as literature suggests increased activation. This is in fact also a pathologic change, however this is displayed as opposite of reduced activation patterns in later stages. The x-axis displays time, the y-axis displays amount of pathologic change

decisive conclusions from all these studies together is challenged by a number of issues. Firstly, when a particular MRI technique is applied, many options in terms of acquisition and post-processing remain undecided, resulting in different methodology across studies. Secondly, when comparing studies, it is evident that differences in definition exist for even the most basic principles which can lead to different inclusion criteria, the most pertinent being the definition of premanifest and manifest HD. Although many studies use some form of motor examination, even when the same standard (e.g. the UHDRS motor score) is applied, different cut-off points are used for determining disease manifestation. Thirdly, MRI is ever evolving, using evermore sophisticated techniques, therefore the validity of comparing a study from 1991 versus one from 2010 can be questioned. Finally, the comparison of a study with more than 300 participants, to a study of 6 controls versus 6 manifest HD is also challenging. This is not to say that the validity of smaller studies is less than larger studies. One might in fact argue the opposite, when something can be demonstrated using only 12 participants, this can be a much stronger argument when needing 300 participants to prove a point. Despite the difficulties posed in providing an overview, this review has discussed many different studies using various techniques in an attempt to combine their findings into a hypothetical temporal model of brain changes (figure 4). This hypothetical model is constructed based on all the literature available and is our own interpretation of the data available.

When conflicts in literature exist, we have weighed the different studies as described in the specific MRI subsections. Figure 4 depicts the temporal MRI changes in relation to disease stage and to each other.

When summarising the literature it seems apparent that striatal atrophy is the first measurable brain change, starting between 10 to 20 years before disease onset (16, 17, 40, 46). DTI measures reflect signs of abnormal structure, especially in the white matter, at the same time or shortly afterwards the striatal atrophy, with reports of change in the corpus callosum being the most consistent (64, 65, 67, 75). With the combination of subcortical grey matter, cortical grey matter and white matter disturbances, whole brain atrophy is no more than a logical consequence (16, 62). The longitudinal change of whole brain atrophy seems especially apparent in the early manifest stage (49, 61, 62). MTI seems to be sensitive only in the early manifest stage onwards (78, 79). MRS demonstrates changes in metabolites before disease onset with changes in NAA and creatine (81, 85). Functional MRI seems to start with a form of enhanced activation in the premanifest stage (106,107), which is depicted by the negative curve in figure 4, and shows reduced activation in later disease stages (101,102).

This model, however is not at all without it limitations. The s-shaped curves are assumptions made on available data. The curves are, from a pathophysiological

point of view, a common form, where some minor changes occur, a certain threshold is subsequently reached and there is some form of exacerbation which slows down eventually. The use of such temporal models for biomarkers has been reported on in Alzheimer's disease (114) and seems plausible for more neurodegenerative diseases. For understanding temporal changes, longitudinal assessments are the most relevant, however cross-sectional analysis of multiple disease stages are also very relevant and also more abundant The literature is however too limited to make very firm claims. These models should therefore not be taken as proven fact, but rather to be used to test further research on and provide a framework for future clinical trials. Furthermore this model is deficient in the sense that it is limited to the most commonly used techniques and therefore not all MRI-techniques available are included and we appreciate the fact that possibly more sensitive techniques exist.

The most abundantly researched candidate biomarker technique is volumetric MRI. It seems to be quite robust and fairly easy to use. The use of T1-weighted MRI is part of routine MRI and therefore easily implemented in any type of study. However, volumetric measurements can be performed in a many different ways from simple hand-drawn measurements to newer techniques such as VBM, which also has its pitfalls (115). Nonetheless, this technique is claimed to be robust and suitable to use in a clinical trial by many studies such as the large cohort studies PREDICT-HD and TRACK-HD (14, 16, 49, 116).

DTI has been applied extensively the last decade and has the advantage that it can detect abnormalities in integrity in multiple ways, with different outcome values such as FA and MD, but also more recently applied measures such as radial or axial diffusivity, all with different proposed pathological meaning (70). The potential use of DTI measures as a biomarker in HD has been suggested by many authors (67, 69, 71, 72, 75). There is also a downside of DTI as the options available of examining DTI are plentiful in terms of not only outcome measure, but also software programme used for analysis and the choice of the examined structure, e.g. ROI-based, voxel-based or specific pathway based analysis. The most sensitive DTI marker is difficult to identify from all these studies. However, taking this into account, the number of studies combined with the rapid expansion and standard clinical use of DWI/DTI makes this technique very promising and in our view can rival with volumetric outcome measures in terms of early detection, robustness and rate of decline. More so, it has the added bonus of detecting a more pathophysiological process i.e. structural integrity, rather than only the outcome of neuronal loss, namely atrophy.

For MTI the s-shaped curve and the model itself is the one being least supported by literature, with the obvious reason of the absence of large numbers of studies. This technique has been used in many other diseases including neurodegenerative diseases (77), however the use in HD is limited. The evidence suggests that this technique

is only sensitive at later stages than volumetric or DTI measures (28, 78, 79).

MRS has some clear advantages over the other techniques described because it can be used for biomarker research, but also inevitably adds to our pathophysiological understanding of the disease as the measured metabolites represent changes in more functional processes. Every metabolite has a different substrate in the physiological processes in the brain (80), and therefore informs us about different pathologic processes. The downside of MRS lies in the fact that measuring techniques can influence the outcome severely, with the field strength used being the most apparent one. Also, the use of metabolite ratios versus absolute quantification is another important issue. For example using a ratio to creatine when creatine is reported to be lowered in HD (81, 85) is a serious issue. The absence of longitudinal reports of MRS, makes it difficult to make any firm claim regarding its use for biomarker research, although the cross sectional results are promising (81, 85).

Finally, fMRI is a technique where the use as a biomarker is clouded by the many studies using different paradigms, besides the, again, many analysis options. However, fMRI does give us a great insight which functional deficits occur, or in case of premanifest HD, compensation strategies in brains activation patterns (106, 107). The temporal changes described are all based on cross-sectional results, rather than longitudinal changes. However the potential of this technique is clear and can provide possible markers for early diagnosis and possibly disease monitoring (107, 112). Also, fMRI showed us that brain changes do not limit themselves to the frontostriatal circuitry but were more widespread (110, 111) and will provide more understanding of the brain networks in both disease and healthy states.

In general, when considering the use of MRI derived measures as biomarkers it is worth noting that scanning is non-invasive, generally well tolerated by participants and easily applied in the daily clinic as many techniques are available in a standardized form for hospital care. Claustrofobia and metal implants, however, are always of some concern and can cause patient drop out in studies. Furthermore costs can be quite high as a single MRI scanning session usually costs several hundred dollars/euro's. However, high costs are also associated with many potential biomarkers such as any biochemical or DNA marker. As MRI becomes more and more the standard, the costs will be limited and in our opinion does not outweigh the potential gain for HD patients. Furthermore, we envision MRI will not be used for diagnostic purposes but only for evaluating therapeutic interventions.

11. CONCLUSION

MRI could be used as outcome measure for clinical trials in HD. Overall a combination of MRI techniques is most appropriate with different modalities being the most suitable biomarker at different disease stages. For large multicenter trials an automated set up with

standardized scanning protocols and post-processing is essential. However for many volumetric and DTI measures the exact analysis method chosen for the specific techniques is relatively unimportant as studies consistently report the same changes. In our opinion striatal volumetric measures and white matter (DTI) integrity measures are most appropriate for the premanifest stage of HD. These measures are the best described and deemed to be robust with consistent results throughout studies. In the transition phase to early manifest HD more measures become suitable, such as whole brain atrophy, MTI and possibly MRS. The role for fMRI lays more in the understanding of brain network functioning in neurodegeneration, although early diagnosis seems plausible as well. Therefore, we recommend constructing multimodal MRI approaches and choosing the technique, or combination of techniques, depending on the disease stage being examined in order for MRI to fulfil its role as a biomarker.

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