Huntington's Disease

Functional and Structural Biomarkers

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Cover: Each brain on the cover represents one of the ninety participants that took part in the TRACK-HD research in Leiden.

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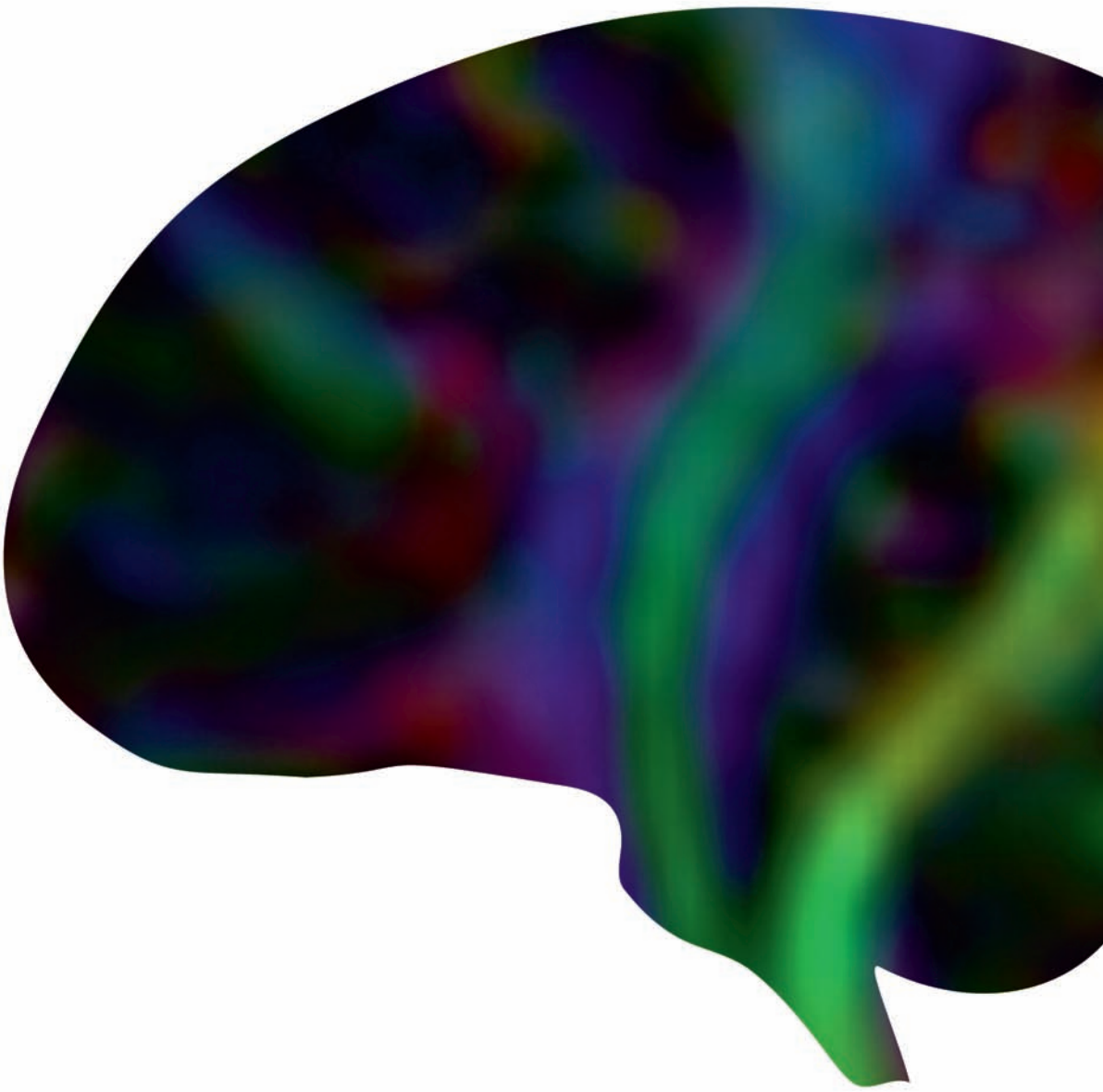
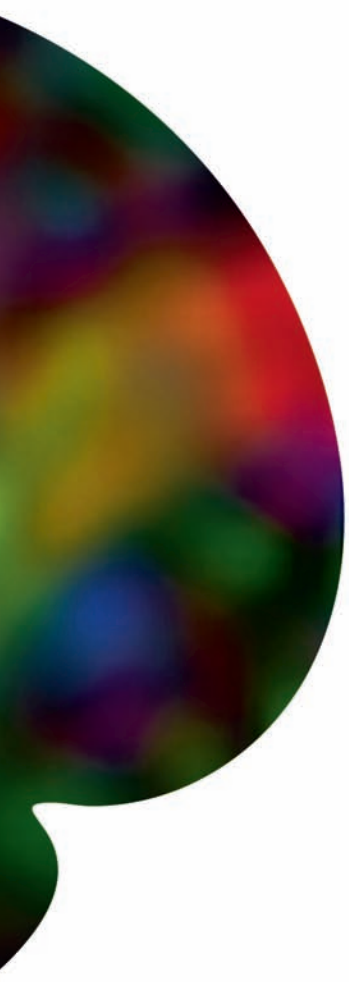
for my family
voor mijn gezin

Contents





Chapter 1

Introduction

Introduction and aims of the study

Huntington's disease (HD) is an autosomal dominant inherited disease, determined by a mutation in the *Htt* gene coding for the protein Huntingtin. The occurrence of an expansion of the cytosine adenine guanine (CAG) repeat on chromosome 4 leads to destruction of brain neurons[1]. At an unknown point in life, pathogenic processes give rise to gradually progressing disturbance of motor function, cognitive ability, and behaviour. Other symptoms include weight loss and sleep disturbances. Patients typically display the first symptoms between 35 and 45 years of age and die 15 to 20 years later[2].

Genetic testing allows for the identification of individuals that carry the HD gene. A unique research situation arises from the ability to identify individuals who do not show symptoms but will certainly do so in the future. These so called 'premanifest gene carriers' play a valuable role in understanding underlying processes of HD prior to the appearance of symptoms. Commonly, after disease onset, 'manifest' HD patients demonstrate gradual cognitive decline, motor dysfunction and behavioural abnormalities[3]. The occurrence and severity of such symptoms vary per individual, even so, four successive disease stages can be determined. Stages 1 and 2 are classified as 'early HD' and 3 and 4 as 'late stage HD'.[4] The clinical aspects of the disease have been studied extensively since the first description of the disease by George Huntington in 1872[5]. Also, the cellular and tissue changes of HD have been documented. However, this has not resulted in a cure for the disease. Symptom suppression is the only treatment option currently available.

Currently, the complex disease process of HD is not understood. It is not possible to exactly predict how and when symptoms will arise, or how the disease will develop. However, in order to progress towards therapeutic interventions clinical research requires consensus about the measures that could be used to objectively reflect the status and progression of the disease. This thesis focuses on the determination of early biomarkers in premanifest gene carriers and patients with early HD. The identification of one or more suitable biomarkers would allow for future clinical interventions to be accurately monitored.

The ideal biomarker would closely reflect the disease state of HD gene carriers, be non-invasive and objective. All clinical functioning domains have the potential to deliver biomarkers that can meet these criteria. However, due to focus on motor behaviour, cognitive decline was overlooked as an important domain in the HD symptom spectrum for many years. Recently, cognitive deterioration has received more attention[6;7]. This has resulted in the recognition that cognitive deterioration can be part of the course of HD from its earliest disease phase onwards[8]. Diminished executive functioning and psychomotor speed have been shown in premanifest gene carriers[9], however, reports on memory functioning remain inconclusive. In manifest HD memory, psychomotor speed

and executive functioning have been implicated[10]. However, it is unclear which cognitive (sub)domains demonstrate the most consistent deterioration and which areas of cognitive functioning could deliver a cognitive biomarker. Therefore, we aimed to provide an overview of the (sub)domains of cognitive functioning in HD per disease stage, and to indicate which domains have the highest biomarker potential (Chapter 2). Furthermore, we aimed to examine one of the cognitive subdomains to further understand the cognitive process (Chapter 3).

The search for biomarkers extends into the field of brain imaging. The widespread application of magnetic resonance imaging (MRI) has allowed for in vivo exploration of the HD brain[11]. From the first autopsies performed on HD patients in the twentieth century there has been evidence that brain changes are apparent in HD[12]. Especially cell loss in the caudate nucleus and putamen has been observed[13]. The extent of atrophy in the other subcortical grey matter structures in the brain is not well established. Therefore, we aimed to quantify atrophy of the subcortical grey matter structures to determine their involvement in HD pathology (Chapter 4).

Atrophy of the caudate nucleus and putamen has been shown to occur prior to clinical changes. Therefore, atrophy of these regions is a strong candidate biomarker. However, given that cell loss is the final outcome of a sequence of pathological events, it is not unlikely that other changes prior to widespread atrophy could be detected. With this in mind, magnetic resonance spectroscopy (MRS) has been applied in HD, and has demonstrated metabolite disturbances in large brain regions[14]. Quantifying metabolic changes in individual brain structures, especially prior to disease onset, is desirable due to the localised nature of profound brain changes in HD. Application of in vivo MRS has the potential to measure metabolite concentrations present in individual brain structures in HD and thereby give insight into pathofysiological changes. Therefore, we aimed to quantify brain metabolites by applying MRS in subcortical brain structures and to assess the potential of metabolites as biomarkers (Chapter 5).

Inherent structural or functional changes of the subcortical grey matter structures may not be the only cause of their dysfunction in HD. The connectivity of the subcortical structures with other areas of the brain may also play a role in their disturbances. Diffusion tensor imaging (DTI) provides insight into the structural integrity and structural connectivity of brain tissues. DTI has previously been applied to determine the integrity of subcortical structures in HD and has shown diminished integrity of the caudate nucleus, putamen and overall white matter[15;16]. Examining specific white matter pathways to and from brain structures important to HD, may provide further insight into early brain changes. The quantification and integrity of major white matter pathways using DTI in premanifest and early HD is described (Chapter 6).

Autopsy, has shown iron accumulation in the basal ganglia HD brains[17]. The processes and timing of iron accumulation in the course of HD are not fully understood. Furthermore, the relationship between atrophy and iron accumulation has not been examined. Magnetic field inhomogeneities were assessed for quantification of iron levels in premanifest and manifest HD (Chapter 7). Understanding whether excessive iron accumulation is related to cellular loss, or whether this occurs prior to such loss will not only determine the independence of such disease processes but will give insight into iron as a potential biomarker.

In an effort to bridge the gap between the structural brain changes and the functional deterioration, insight is required into the functioning of the brain. Studying potential changes in functional connectivity networks using resting state fMRI, may add to the knowledge of functional changes in HD. We explored the nature and timing of functional disturbances in the HD brain at rest and described the potential of resting state fMRI as a biomarker for HD (Chapter 8).

In the final chapter (Chapter 9) the conclusions are summarised and discussed. Recommendations for future research are given.

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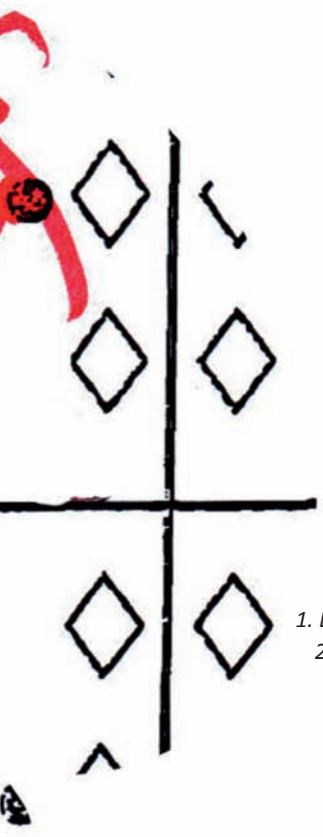

Chapter 2

A review of cognition in Huntington's Disease

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Abstract

With the prospect of potential treatments for Huntington's disease (HD), non-invasive markers of disease progression are needed. Cognitive impairment has long been recognised as one of the core symptoms of HD. The first aim of this review is to provide insight into the onset and nature of cognitive loss in the progressing stages of HD. The second aim is to provide an overview of the cognitive functions that have been examined in an attempt to identify those areas that have the most potential to yield a cognitive biomarker. Literature, consisting of 110 studies, since the implementation of genetic testing until the beginning of 2011 has been included in this review. The clinical features of premanifest HD include deficits in psychomotor speed, negative emotion recognition and to some extent in executive functioning. The clinical profile of manifest HD includes impairment in memory, psychomotor speed, negative emotion recognition and executive functioning. Furthermore, potential candidate biomarkers should be most expected from such domains as working memory, psychomotor speed, recognition of negative emotions, attentional and visuospatial executive functions.

Introduction

Disturbance in cognitive functioning eventually ending in dementia is a core symptom in Huntington's Disease (HD), and was referred to in the first report by George Huntington when he discussed 'insanity' and 'impairment of the mind'[1]. An expanded cytosine adenine guanine (CAG) repeat on the short arm of chromosome four eventually causes neuronal loss in the brain. As a consequence of these brain changes disturbances in motor functioning, behaviour and cognitive functioning are the most frequently reported clinical symptoms. Despite HD classically being regarded as a disease of motor impairment, cognitive decline as an early symptom has increasingly been recognised. A recent report of the symptom type with which HD manifested, found that 8.4% of a group of 615 patients in Europe were rated by a clinician as having first disease symptoms of a cognitive nature. An additional 13.2% had a mixed onset of motor and/or cognitive and/or psychiatric symptoms[2]. In a group of 1238 patients, over a period of two to ten years after receiving the diagnosis, companions reported intellectual decline and memory loss[3]. Self-reporting of cognitive abilities has proven to be problematic in HD, as patients have been shown to demonstrate impaired awareness, which was found to relate to problems with executive functioning, memory and global cognitive functioning[4]. For this reason the need for objective assessments, e.g. formal neuropsychological testing, has become apparent. Although it has become clear through numerous reports of formal examination that cognitive decline occurs and worsens in the course of HD[5-9], the progression of decline over the stages of HD is not well-established.

With the growing prospect of potential treatment for HD, the need for accurate, sensitive and non-invasive biomarkers of disease progression has become clear. Since the discovery of the HD CAG repeat expansion in 1993, genetic testing has become widely available for both patients and family members[10]. Since then, at-risk individuals could undergo genetic screening. If found to carry the gene and with no overt symptoms, these individuals are referred to as premanifest gene carriers of HD. However, this test result gives little indication of how and when the disease will start, or in which disease stage patients are in. The ideal biomarker for HD would objectively pinpoint the start of the disease and/or current disease phase of a gene carrier. Any improvement (or stabilisation) as a result of an intervention would then accurately be reflected by this measure. Such a measure could thereby serve as an outcome measure in future clinical trials. To date, no single (cognitive) measure is generally accepted as a sufficiently sensitive measure to serve in such trials. Currently, the most frequently used outcome measures are such measures as the Total Functional Capacity Score (range 1-13) or the Mini-Mental state examination (range 0-30), however, these are often insensitive to small changes in function. The search for an adequate biomarker has given rise to many observational studies of all domains of the disease, including invasive, non-invasive, wet and dry biomarkers[11-14]. Cognitive research has attempted to identify candidate cognitive biomarkers for a long time and

much research has been performed to assess feasibility. However, again no single measure is currently accepted as a sufficiently sensitive marker of current or changing cognitive functioning.

This review has two aims, firstly, to provide insight into the onset and nature of specific and global cognitive loss in the successive stages of HD, secondly, to provide an overview of results from the functional (sub)domains that have been examined in an attempt to identify a cognitive biomarker.

Cognitive Domains and stages of Huntington's disease

From the start of research into cognitive functioning in HD in 1974 there have been many attempts to categorise the deficits seen into domains of cognitive functioning[15]. This has been done for two main reasons, first to try to grasp the nature of cognitive decline for diagnostic and treatment purposes, and secondly to identify cognitive biomarkers as a means for tracking disease progression. For the purpose of this review, the results of the reports that have been reviewed have been categorised in accordance with the above mentioned goals. Firstly, in accordance with the clinical diagnosis of cognitive decline and dementia, the five domains set out in the Diagnositic and Statistical manual of Mental disorders, fourth edition (DSM-IV)[16] were used as a starting point for classification. These are; amnesia, aphasia, apraxia, agnosia and executive dysfunctioning. As there is no evidence to suggest disorders in praxis in HD, rather only in motor functioning affecting psychomotor speed, we chose to collect all results under the classification *psychomotor speed*. The same is the case for agnosia, which in this review has been relabelled as *emotion recognition*, as this was found to be the most prominently examined function found to show deficiencies.

Although the division of functions exists, cognitive processes are complicated and complex and therefore it is often very difficult to pinpoint just one specific function that is responsible for the correct performance of a task. As a result the DSM-IV discusses a number of functions related to, or collected under, one of the main umbrella terms of the five domains. This sub-specification will also be discussed throughout this review. Specifically under the domain *memory*, we will address declarative, non-declarative, verbal, visual, working and general memory. Under *emotion recognition* the emotions happiness, surprise, sadness, fear, disgust, anger and general negative emotions will specifically be addressed. Under executive functions both general and the specific functions of attention, categorisation, verbal and visual-spatial executive functioning will be considered. Additionally, in terms of the search for a biomarker, an additional specific domain has proven to be of interest and will be used as a means to categorise experimental findings, namely global cognitive functioning.

Methods

All literature relating to cognition in HD between the discovery and application of direct genetic testing in the mid nineteen nineties and January 2011, was included. Literature searches were performed in four databases. The searches were performed with the following terms in Pubmed, ("huntington disease" (Majr) OR huntington (ti) OR huntington's (ti) OR huntingtons (ti) OR huntington* (ti) OR huntingtin* (ti)) AND (cognition OR cognitive OR "Cognition Disorders" (Mesh:noexp) OR psychology OR neuropsych* OR Neuropsychological Tests), Embase, (*Huntington Chorea/ OR huntington$.ti OR *Huntingtin/ OR huntingtin$.ti) AND (exp Cognition/ OR exp Cognitive Defect/ OR exp Psychology/ OR exp Psychological Aspect/ OR exp Neuropsychology/ OR Neuropsychological Test/ OR exp Learning disorder/ OR (cognition OR cognitive OR psychology OR neuropsych* OR Neuropsychological Test* OR learning).mp), Web of Science, ti= (huntington* OR huntingtin*) AND ts= (cognition OR cognitive OR psychol* OR neuropsych*) and PsycINFO, (exp *huntingtons disease/ OR huntington*.ti OR huntingtin*.ti) AND (exp Cognition/ OR exp cognitions/ or exp cognitive ability/ or exp cognitive assessment/ or exp cognitive impairment/ or exp cognitive processing speed/ OR exp memory/ or exp memory decay/ or exp memory disorders/ or exp memory trace/ or exp memory training/ OR exp Psychology/ OR exp Neuropsychology/ OR exp Neuropsychological Assessment/ OR exp learning/ or exp learning ability/ or exp learning disabilities/ or exp learning disorders/ OR (cognition OR cognitive OR memory OR psychology OR neuropsych* OR Neuropsychological Test* OR learning).

Of the approximately 1000 papers that were found with this search strategy the majority (± 75%) purely referred to cognition in HD without having objectively examined cognitive abilities or having an aim related to cognition and were therefore removed. The remaining ± 25% was examined and only included if they fulfilled the following criteria: were written in the English language, had examined human gene carriers or patients with directly confirmed presence of the HD gene and, for cross-sectional reports, had directly compared the cognitive performance of the HD participants to control subjects. Papers were excluded if: the study was performed prior to the implementation of the genetic test, data was collected as part of a clinical intervention trial, if it was not defined how HD was determined or tested, if patient data were compared to data from other patient groups or to norm data only[17]. This approach yielded 110 strictly selected papers, which is comparable to the report by Stout *et al.* (2011) of approximately 150 reports of "neurocognitive function" since the identification of the HD gene[18].

Each report was examined and the results categorised based on the domains of cognitive functioning as described above. For the majority of results it was evident how they should be categorised and sub-categorised as the authors had indicated how they had done so.

For other results where the described test had not been categorised by the authors, a categorisation was made based on the cognitive abilities required and/or how this had been categorised by other studies. The categorisation of the Verbal Fluency Test or the Controlled Oral Word Association Test (letters F-A-S) from the Unified Huntington's Disease Rating Scale (UHDRS), proved more complicated. In neuropsychological manuals it is often listed under language abilities[19], however in the majority of HD research it is regarded as a test of executive functioning[13,20,21]. To correspond to the majority of studies in the HD field, results from this specific test were categorised under executive functioning. For each domain the absence or presence of a significant difference to controls for the patient groups was noted. A study was classified as finding a difference between HD mutation carriers and controls when the authors had stated that this was the case, based on the statistical criteria they had described, and not on the basis of a significance cut-off point. This was done as many different statistical approaches have been taken, rendering direct comparability unfeasible.

Further subdivision in results per disease stage was achieved with the following. For HD patients a distinction was made between those with early or mild HD versus patients with moderate/severe or late stage HD, most often related to the disease stages defined by Shoulson and Fahn[22]. Where it was possible to ascertain the stage of severity of the disease from the text the presence or absence of a cognitive defect in this domain was noted only for that group. This same approach was taken to studies of premanifest HD. Numerous studies classified premanifest gene carriers based on the number of years to estimated disease onset[23] as either far from – or close to – disease onset. In practical terms this yielded a split on average around 10 years from estimated disease onset[11,13,24]. In cases whereby it was not clear in which phase or stage of the disease the participants fell, a notation of difference to controls was noted for both participant groups (ie. both premanifest groups, or both HD patient groups). From these notations it was possible to indicate the number of times that a difference to controls was or was not found, for each disease stages, for the cognitive domains. These data were used to construct graphical displays of the presence and staging of cognitive defects (figures 2-10).

Anmesia/Memory

In the domain memory, all functional tests related to the recall of previously learnt information or to the learning of new information were classified. This included sub-types of long-term memory such as declarative memory, with subdivision of semantic memory (factual information) and episodic memory (situation specific information related to a persons life). Also non-declarative memory was specified and related to all tasks testing skill related or automated procedural memory. Furthermore, in the subtype of short-term memory, working memory was included, referring to storage, retrieval and application of information that is required only briefly. In addition, two other sub-categories of short-

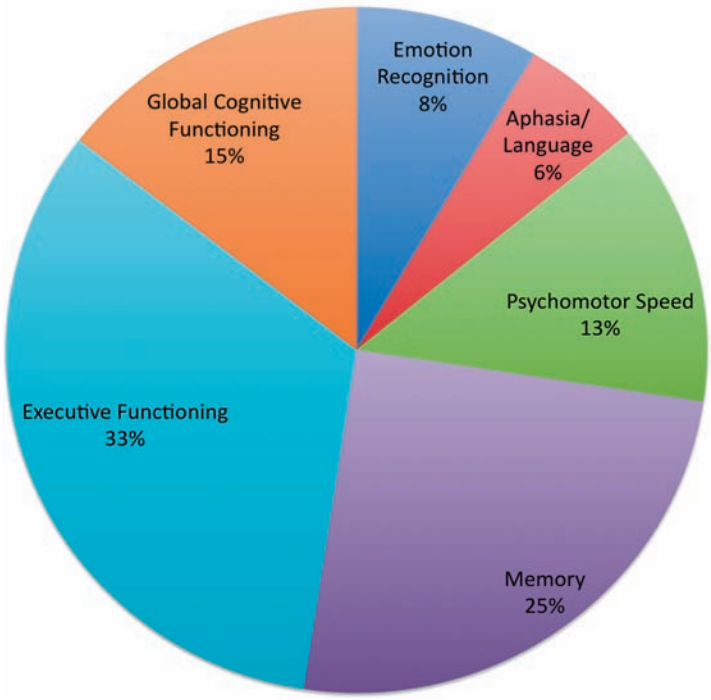

Figure 1. Proportion of research performed per separate domain of cognitive functioning.

term memory were recognised, namely verbal memory and visuo-spatial short-term memory[25]. In HD research a quarter of the research into cognition in HD has been related to memory (figure 1). The distribution of positive and negative findings in regards to memory research over the different stages of HD is shown in figure 2.

In manifest HD, cross-sectional findings in the various domains of memory provide a fairly homogenous profile of impaired memory functioning in both early and late stage HD[8,11,26-44]. Longitudinal studies found both evidence for the presence of the specific types of amnestic disorders in manifest HD[5,38,45-47], and against such deficiencies in the same or other subdomains of memory functioning[5,6,45,46,48]. The results from the smaller studies (n = 20-40) often (just) failed to reach significance for the majority of memory measures, and often found just a limited number of measures show significance. For example, a group of measures of memory approached statistical significance (p<0.10) over one- and two-year follow-up periods[48]. In other studies visual memory was found to decline over annual visits[45] and over 16 months[46]. This was also found by a moderately sized study which had reached significant levels (p< 0.05) for visuo-spatial memory. However, in this same group verbal learning, just failed to reach significance (p between 0.06 and 0.09) over 3 annual visits[5]. A larger study (117 early HD patients vs. 119 controls) did not find differences with a computerised visual working memory task over a one year period in patients with stage 1 HD, but did find this in patients in stage 2[6]. These findings do suggest that overall memory decline does occur in manifest HD and that for specific sub-domains there is a relative consensus in terms of decline in visual memory, and more uncertainty about verbal memory.

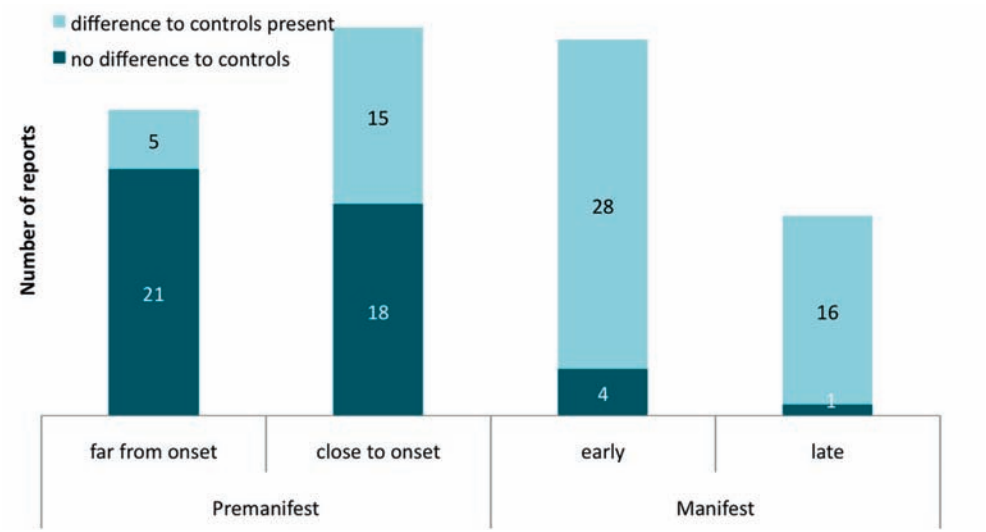

Figure 2. For the cognitive domain: Memory, the number of positive and negative findings over disease stages.

Memory functioning in premanifest gene carriers is not as clear cut as that of manifest subjects. Cross-sectionally, many different types of memory have been investigated and results show that in some studies evidence is found for a defect, albeit sometimes not in all, but in a limited number of subdomains[11,18,30,49-59], whereby for poorer working memory the most consistent findings were present (Figure 3). The larger studies (n>100) did show difference to controls with strong significance levels[11,18]. This was similar to smaller studies (n<30), who found differences in other sub-domains, such as prospective and visual memory[49] and explicit motor sequence learning[30]. Examination of the timing of onset of such deficits reveals that the majority of these findings apply to premanifest gene carriers close to onset, as is shown in figure 2. In contrast, there is also a substantial body of evidence to suggest that cross-sectional memory functioning in some domains, such as verbal memory, is equal to that of controls[21,32,38,42,60-68].

Longitudinal studies in premanifest gene carriers found that memory related tasks showed more decline over longer periods of time (120 and 30 months respectively) than in controls[9,47]. However, others, including shorter follow-up periods (12 to 24 months), found that there was no difference in task performance over time[6,64,69,70]. These results suggest that memory decline is a slow process in premanifest gene carriers.

The interpretation of studies of memory functioning is complicated by the many domains that have been investigated. All types of memory functioning are complex and thought to recruit numerous different brain regions. Therefore although one type of short term memory, say working memory is found to be poorer in HD, this does not mean that the sub-types of long-term memory are equally affected.

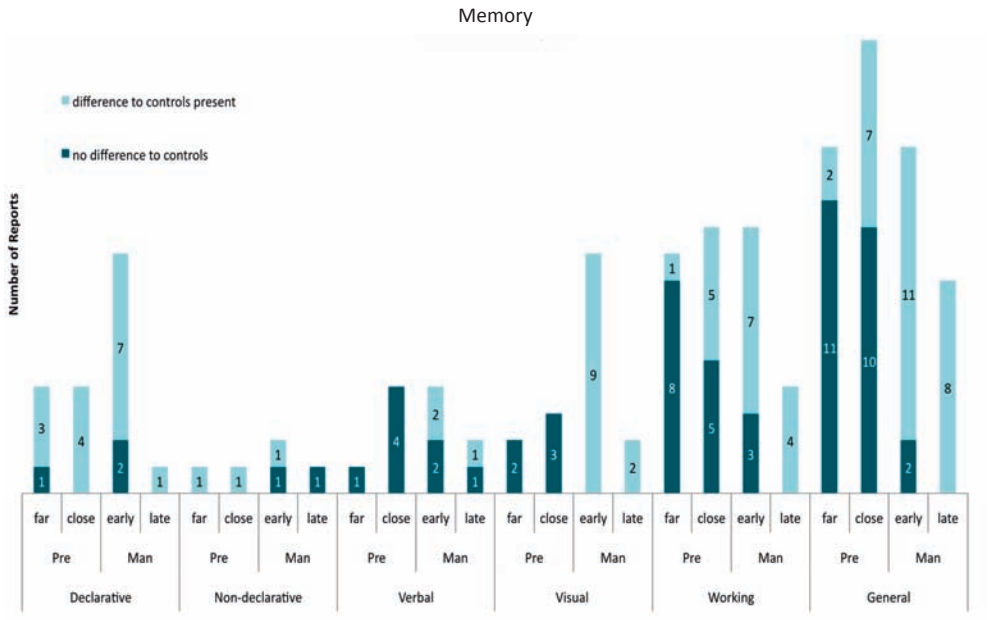

Figure 3. Memory research divided into sub-domains of memory functioning. The number in each section of a bar represents the number of positive versus negative findings.

For this reason it is preferable to examine the subtype of memory that is impaired, figure 3 shows the distribution of research findings over some domains of memory functioning (where subtype information was provided in the literature). From this graph it becomes clear that there is not one single pattern of memory impairment that is valid for all subdomains, rather that the impairment pattern is unique to each memory subtype. When taken together the findings from the cross-sectional studies strongly suggest that there are deficits present in (sub)domains of memory functioning that differentiate premanifest gene carriers from controls, however the limited evidence for further longitudinal decline may suggest that the rate of decline of memory functioning is not so pronounced. Of the subdomains of memory, the clearest findings related to problems with working memory, therefore this may be the most appropriate memory based candidate for biomarker selection.

Aphasia / Language

Aphasia relates to all language producing or understanding functions. An important issue arises when examining language abilities in HD as the motor impairment can also cause dysarthria, which could be mistakenly be regarded as a language problem. The presence of slurred or poorly comprehendible speech does not relate to the cognitive function required for speech production or understanding. Therefore when examining language abilities it has proven vital to distinguish between the content and the practical impairment. The language abilities that were collected under this domain were: spontaneous speech, ability to repeat words or phrases, comprehension, naming, reading and writing[19].

Of all cognitive domains the least amount of research has been performed into language functions in HD (Figure 1). In manifest HD, decline in language functions has been reported both cross-sectionally[38,71,-74], and over a number of years[38,45,47]. In premanifest gene carriers, except for one study[18], no differences in language function were found either cross-sectionally or over time (Figure 4)[38,47,61,66,73].

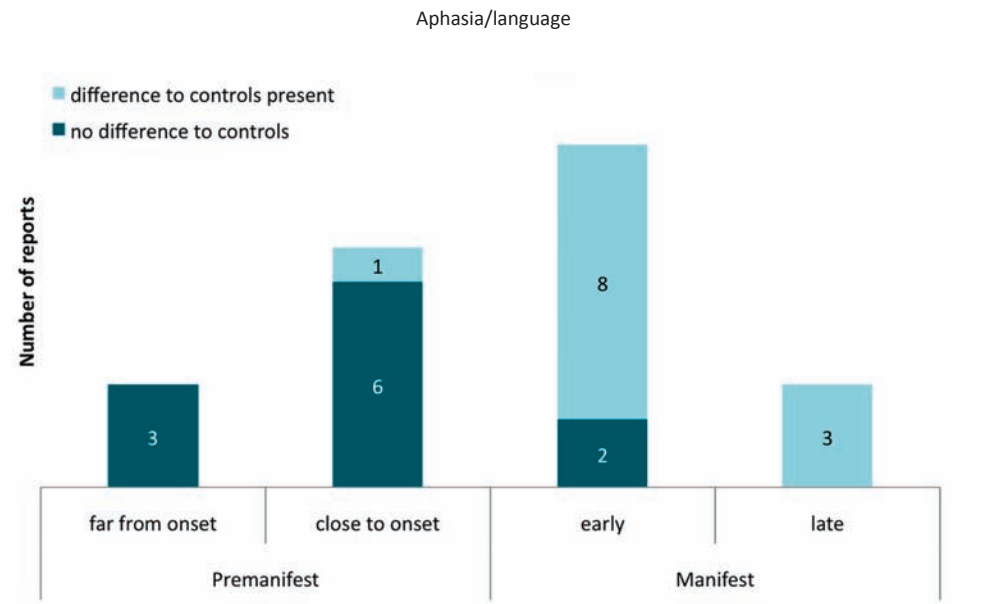

Figure 4. For the cognitive domain: Aphasia/language, the number of positive and negative findings over disease stages

Psychomotor speed

The speed of thinking and acting often referred to as psychomotor speed, has proven important in HD literature. Patients are known to have deficits in their motor abilities, one result of which is bradykinesia. However, the slowing of brain processes related to task performance is also an important measure of functioning and should ideally be separated from poor motor performance alone. Understandably, in clinical practice this can prove to be difficult. The speed and/or strength with which motor movements are performed, although not strictly responsible for the correct performance of a motor procedure, is also a domain often examined as part of neuropsychological testing. The deterioration of psychomotor speed often reflected by slowed performance of a task is a frequently reported phenomenon in HD.

In manifest HD all findings show the presence of a deficit in psychomotor speed[8,33,34,37,43,75-79], also longitudinally[5,6]. Given that the gene carriers are labelled as manifest based on the existence of motor deficits, the impact of motor impairment on cognitive functioning is to be expected. This was further investigated by Aron *et al.*, (2003) and differentiated between reaction time and movement time during a cognitive test. The separate analysis of these two constructs showed that the most purely motor based parameter, motor time, was not different between patients and controls, but that only the more cognitively related reaction time was different between the groups[75]. This suggests that despite their motor impairment, the HD patients are most slowed by their cognitive processes rather than their actual hand movements. That not all differences in cognitive performance can be explained by the negative influence of motor impairment was also demonstrated by Lawrence *et al.*, (1996) when they studied patients in the early stages of HD with impaired psychomotor speed. The influence of this slowing was examined in relation to the performance on other cognitive tasks in which psychomotor speed was incorporated. Even when psychomotor speed was accounted for, slowing did not explain the differences in visuo-spatial functioning[37].

The majority of cross-sectional findings in premanifest HD point towards slower psychomotor speed especially when the group premanifest gene carriers close to onset is examined (Figure 5)[18,49,50,52,55,60,65,77,78,80,81]. This was also confirmed longitudinally[6,9,69,82,83]. However, as seen in other cognitive domains not all reports found differences in psychomotor speed between premanifest gene carriers and controls, either cross-sectionally[21,60,61,64,66,84] or longitudinally[64,70]. Nonetheless, given that the largest body of evidence both from smaller and larger studies has repeatedly demonstrated this deficit, with sometimes highly significant results, psychomotor dysfunction does seem to be present prior to disease onset.

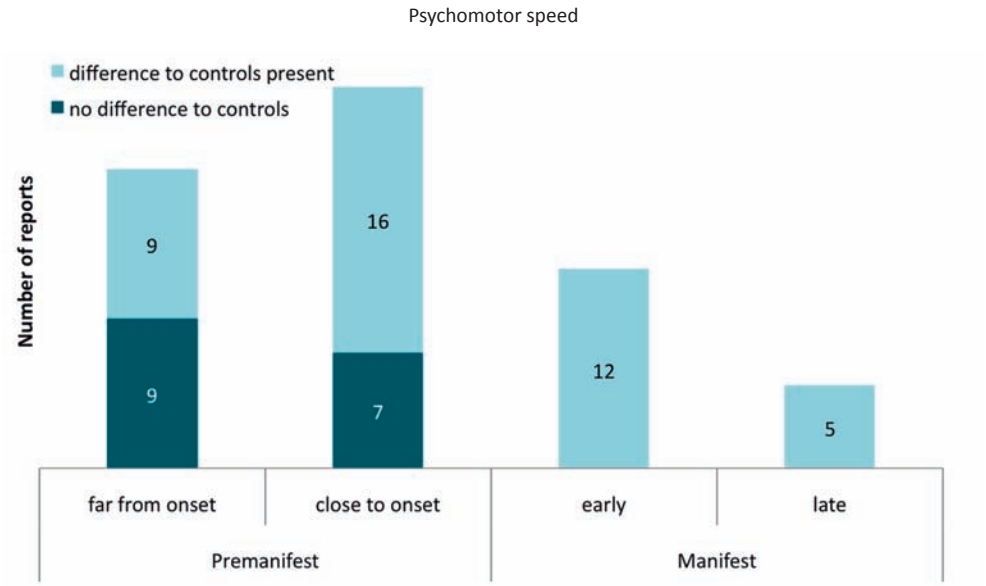

Figure 5. For the cognitive domain: Psychomotor speed, the number of positive and negative findings over disease stages

Emotion Recognition

Research into problems with recognition in HD has been largely limited to the recognition of odor, faces and emotions (figure 6), with by far the most research performed on the latter. Recognition of emotion has been extensively researched in both premanifest gene carriers and patients with HD. This function could be categorised under memory, however, those with deficits of emotion recognition do remember what each emotion type means, only cannot recognise it upon presentation, therefore this is discussed as a separate domain.

In manifest HD the recognition of certain emotions was found to be different between patients and controls in almost all studies[11,67,73,85-91]. However, this does not apply for all emotions, with negative emotions most affected and no evidence for deficits in the recognition of positive emotions such as happiness and only one report of diminished surprise[89]. This extensive deficit of negative emotion recognition in manifest HD was also supported by evidence from longitudinal reports[6,91]. Figure 7 shows the distribution of findings in regards to specific emotion types and shows that there is the most evidence for problems with disgust, followed by fear. To gain more insight into these deficits Hayes *et al.* (2007), assessed patients with seven tests of emotion recognition and found consistent results with impairment in multiple types of disgust recognition[88]. In a subsequent study they examined patients in early to late HD and found that their impairment of anger,

fear and sadness recognition was correlated to a decline in general cognitive functioning, however the impaired recognition of disgust was not. Furthermore in the majority of patients disgust was the most poorly recognised emotion[89]. This suggests that disgust recognition is related to HD pathology and the recognition of other emotions maybe related to general cognitive ability.

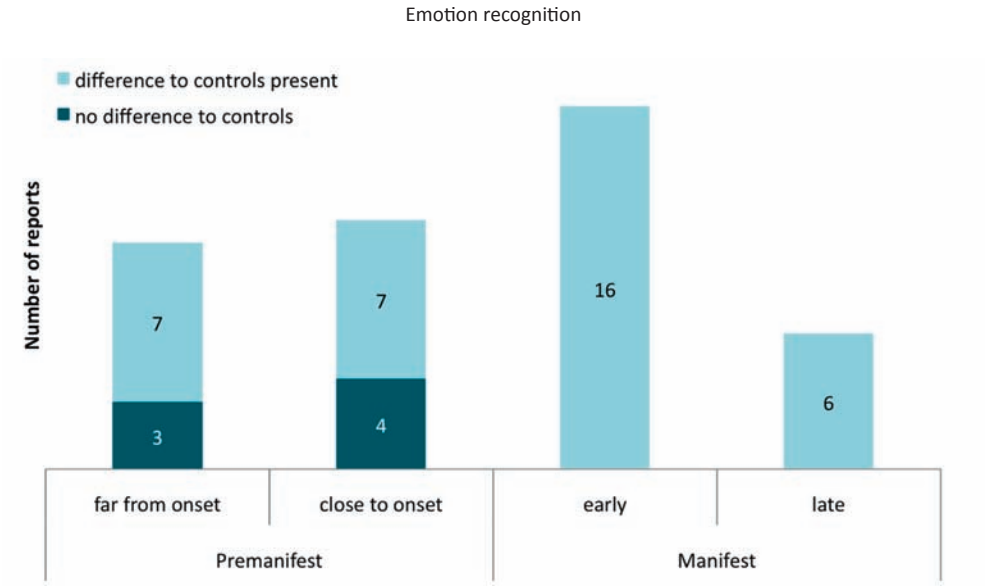

Figure 6. For the cognitive domain: Emotion recognition, the number of positive and negative findings over disease stages

In manifest HD there has been some evidence found for impaired odor recognition[11,92-94] and general recognition[76]. Odor recognition was not found to be different in premanifest HD to controls[93].

The first detectable emotion recognition deficits in premanifest HD appears to be poorer recognition of one or more of the negative emotions[67,85,91,95] also in gene carriers more than 12 years from disease onset[11,18]. Only one report found that premanifest gene carriers were worse at recognising a positive emotion, namely happiness[90]. A minority of studies report no differences in emotion recognition cross-sectionally[73,96]. However, even these studies demonstrated trends towards significance for emotions such as fear[73] in a group of 20 gene carriers, and to a lesser extent, in a small study of 13 gene carriers, disgust[96]. The only longitudinal study in a large sample size did not find decline over a one year follow-up[6].

Overall these findings do suggest that the recognition of negative emotions starts early on, and does not decline at a rapid rate initially but more so after disease onset. Although such longitudinal findings should be replicated, there does seem to be conclusive evidence that

recognition of negative emotions is impaired in both premanifest and manifest HD, which suggests potential as a biomarker.

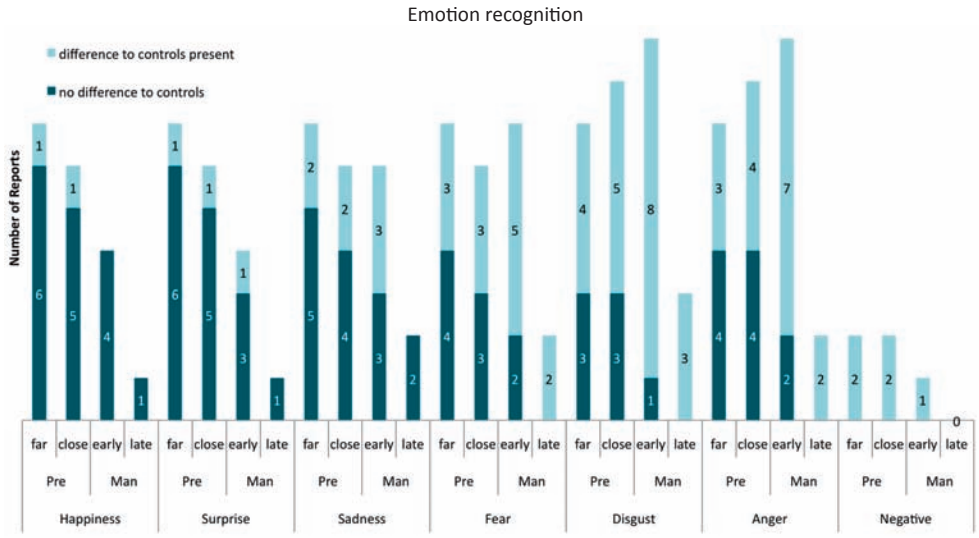

Figure 7. Emotion recognition – per emotion research divided into sub-domains of memory functioning. The number in each section of a bar represents the number of positive versus negative findings.

Executive (dys)functioning

The most commonly assessed area of cognitive functioning in HD research is that of executive functioning. All reports of higher order functions of attention, planning, categorising, sequencing and abstracting were collected under executive functioning. These are regarded as the most complex of behaviours and are needed to be able to adapt in flexible manner to many daily life situations, whereby conceptualisation of the task at hand, planning, action and evaluation of the performed task are required. Many different types of complex functioning shelter under the term executive function, these include, attention, task-switching, categorisation abilities and cognitive flexibility. As executive functioning requires so many integrated cognitive functions many factors can confound correct performance[19].

Early reports found problems in executive functioning, so much so that tests of this function were implemented in standardised assessment batteries, of which the Unified Huntington's Disease Rating Scale is the most frequently applied[97]. Motor, behavioural and cognitive functions are assessed with this tool. The three cognitive tests including in this rating scale are the Symbol Digit Modalities Test (SDMT), the verbal fluency or Controlled Oral Word Association (FAS,) and the Stroop Colour naming, Word reading and Interference cards (Stroop). Within HD research, findings regarding these three tests are often referred to as reflecting executive functioning.

Almost all cross-sectional reports demonstrate differences between manifest HD and controls[8,26,27,29,34,36-38,40-45,72,73,75,77-79,93,98-113], and in the few cases where this was not demonstrated this was always in early HD patients and not later stage HD (Figure 8)[58,114,115]. Impairment of executive functioning in manifest HD was also found longitudinally on numerous occasions[5,6,9,38,41,45,47,48,113,116].

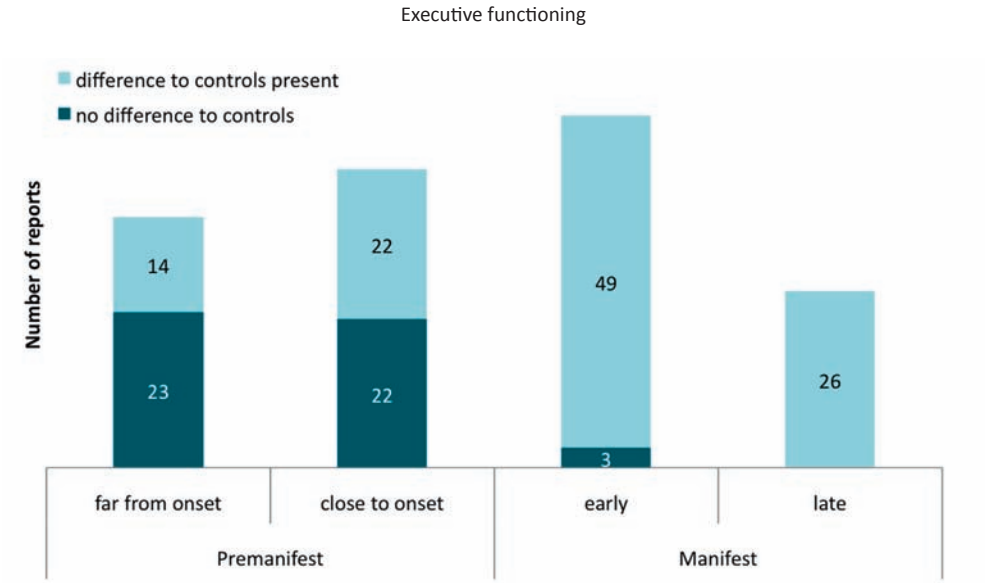

Figure 8. For the cognitive domain: Executive functioning, number of positive and negative findings over disease stages.

The reports on executive functioning in premanifest gene carriers find almost equal support for and against the presence of a dysfunction. A number of studies regard their findings as evidence for the presence of subtle cognitive changes many years prior to the onset of motor symptoms[18,49,78] and some suggest that this domain would represent a good biomarker[50,77]. Those papers that find support for executive dysfunction in premanifest HD[38,52-54,65,73,81,96,101,117-121] include both studies with smaller and larger sample sizes (up to 700+ premanifest gene carriers). However just as many negative findings have been demonstrated[21,42,53,55,58,60-62,64,66,67,84,93,100,102,105,110-112,122] generally by studies with lower sample sizes.

Longitudinal research into executive functioning also provides a mixed view on whether or not this domain is effected in premanifest HD. Reports confirming the decline of executive functioning are present over 120 months in 43 premanifest gene carriers, over 12 months in 12 gene carriers and over 30 months in 38 gene carriers respectively[9,38,69] as are reports against the presence of a dysfunction over 24 months in 22 gene carriers and 36 months in 33 gene carriers[64,70].
From all these reports the conclusion can be drawn that executive functioning is impaired

in manifest HD. There is mixed evidence for executive dysfunction in premanifest HD, but that the majority of reports points towards a dysfunction, albeit with a slowly progressive decline. Lemiere *et al.*, (2002) suggested that some tests of executive functioning may be suitable for demonstrating differences between premanifest gene carriers and controls at one point, but not for showing evolution of disease progression over time[38]. It may be that premanifest gene carriers are worse at some times, stable over longer periods and that sudden drops in ability are related to staging within the disease. Furthermore, some but not all tests of executive functioning showed difference to controls, therefore it is important to identify which subtypes of executive functioning are affected. Noted however, that not all reports specified the subdomains of executive functioning examined. Figure 9 gives an overview of those reports that specified if their results pertained to sub-types of executive functioning, namely, attention, categorisation, verbal, visual or unspecified or to general executive functioning. The graph shows that there is little evidence for problems with categorisation, attention or verbal executive functioning in premanifest HD, and that there is evidence for early disturbances in visual and more general or mixed types of executive functioning. In premanifest HD, the impairment of visual-spatial executive function is seen as in manifest HD. Furthermore, in manifest HD the evidence for attention impairment is apparent, both in early and later stages.

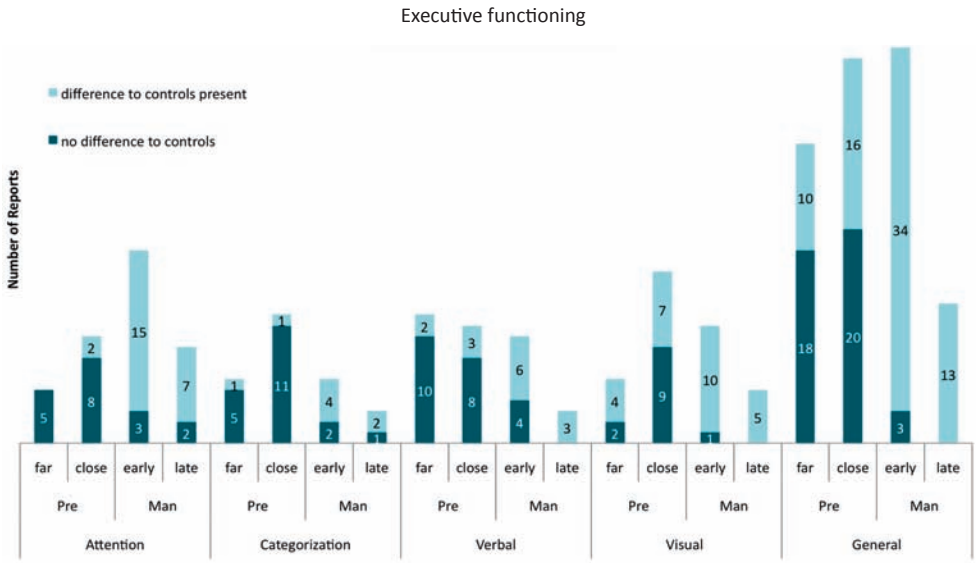

Figure 9. Executive functioning research divided into sub-domains. The number in each section of a bar represents the number of positive versus negative findings.

Global cognitive (dys)functioning

A number of different measures have been used to examine global cognitive functioning in both premanifest and manifest HD. The most commonly used have been the MMSE, various versions of the Wechsler Adult Intelligence Scale (WAIS), and the Mattis Dementia Rating Scale. More recently the National Adult Reading Test (NART) has been widely applied and, as an estimate of premorbid IQ, it is a very brief measure to administer as opposed to the many hours it can take to administer the WAIS or other IQ tests. These and other measures of multiple cognitive domains such as the Cambridge examination for mental disorders of the elderly (CAMCOG) have proven crucial in clinical settings for dementia screening purposes.

In patients with manifest HD the findings of impaired global functioning have been mixed (Figure 10). A number of studies clearly find differences between patients and controls[29,39,41,42,45,72,78,79,93]. However the stage at which this occurs is not yet entirely clear. It appears that there is less evidence for the early HD stage than there is evidence for the advanced HD group by both cross-sectional[114,115] as longitudinal design[38,41,45,47]. A study found poorer performance on the MMSE by HD patients. However, when the group was broken down into early and late HD, the early stage HD patients reached borderline significance and the result was mainly created by the late stage HD patients[115]. However, there is also evidence that these measures are not sensitive to change as reports of similar global cognitive functioning in manifest HD as controls are also available[93,107,110,116,123]. It must be noted however that of these reports, the majority are in early manifest HD. These reports of comparable functioning were also confirmed longitudinally by two studies[5,116].

As depicted in Figure 10, it does not seem as if measures of global cognitive functioning are sensitive to the subtle changes reported in premanifest HD but that, as the disease progresses in early and certainly later stages of HD, a broad deficit is measurable. As with the other domains different tests have been applied, and a clear sensitive measure is not apparent. Recently, a comparison study of the MMSE and the more recently developed Montreal Cognitive Assessment (MoCA) as screening tools for cognitive deficits in HD was performed. Both global cognitive functioning and subdomains of the two tests were examined in HD patients as compared to controls. Patients performed worse in every domain examined in at least one of the two tests, if not on both. The MoCa has the additional benefit to the MMSE in assessing the domains known to be affected, such as executive functioning. Overall on the basis of Reciever Operator Characteristic curves (ROC) the MoCA achieved higher sensitivity, and may be a better general screening measure due to its broader coverage of cognitive domains[72].

Global cognitive functioning

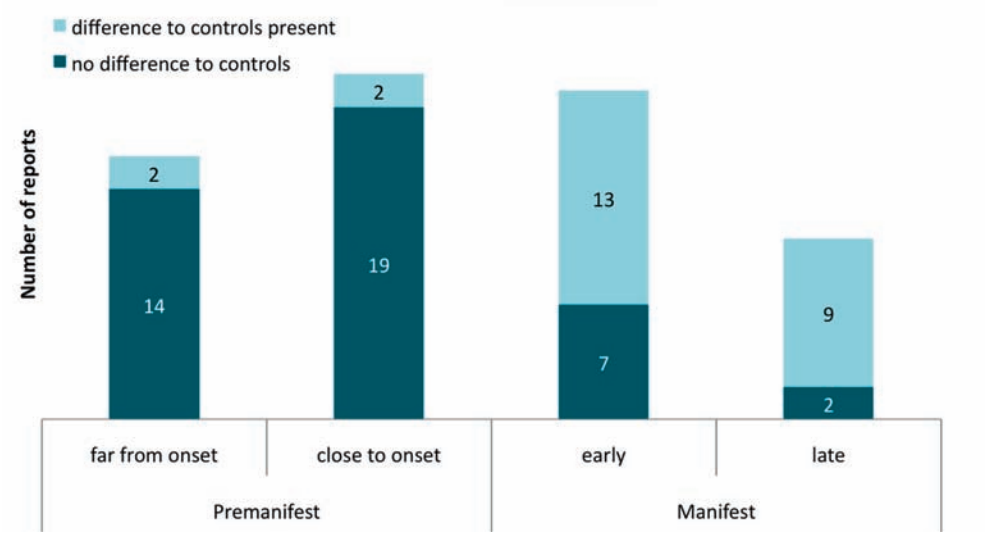

Figure 10. For the cognitive domain: Global cognitive functioning, the number of positive and negative findings over disease stages.

Findings in premanifest gene carriers were more homogeneous than those of the manifest groups. Overall the majority of studies did not find differences between premanifest HD and controls either cross-sectionally[42,50,58,60,61,66,93,95,110,123-126] or longitudinally[38,47,69,70,125].

A few reports do suggest changes in global functioning prior to disease onset, with such findings as premanifest gene carriers far from expected onset not being significantly worse than controls for global measures, but that premanifest gene carriers near to onset were worse[78]. The WAIS-R, for example, was used to assess the effect of proximity to disease onset on generalised measures of intelligence. Premanifest gene carriers close to onset (n=15) showed significantly lower total, performance and verbal IQ as opposed to healthy controls. Premanifest gene carriers far from onset only demonstrated lowered performance IQ. The authors regard these findings as support for a linear model of cognitive decline in premanifest HD, whereby not all functions decrease at the same time[49].

Discussion

This review aimed to provide information on the profile of cognitive functioning over the course of HD. Secondly we aimed to indicate which domains of functioning could provide the best candidate biomarkers for research purposes.

Amnesia/ memory

Memory deficits in premanifest HD are not clear cut, furthermore there is evidence that memory tests are susceptible to re-tests or learning effects[45]. More research is needed to confirm the presence of learning effects in memory tests, however if this is the case then such assessments are not suitable for tracking disease progression over time, both clinically or from a research point of view. Memory functions do seem impaired in manifest HD, and for this reason, memory functioning has been suggested as suitable state marker, namely a feature at some point during the course of the disease, rather than a trait marker, a feature present regardless of the course of the disease[33]. In terms of suitability of memory tasks for a biomarker of cognition in HD, the most promising candidate subdomain for delivering such as task seems to be working memory.

Aphasia /language

Language deficits in HD generally only occur after disease onset and are limited in their prevalence or severity. The literature on this subject has focused on many specific aspects of language functioning. This very specific approach indirectly demonstrates that global language deficits are not a main feature of the disease symptoms. For this reason language deficits do not seem to belong to the cognitive profile of HD. Furthermore, this domain does not seem to lend itself for application as a sensitive biomarker, especially not in premanifest gene carriers.

Psychomotor speed

Changes in psychomotor speed are suggested to be among the first changes in premanifest gene carriers, and this is regarded as support for the hypothesis that subtle cognitive changes occur 10 years or more prior to disease manifestation[6,49,50]. Furthermore cognitive slowing may be a good target for a cognitive biomarker. Evidence for this can be found in results from a large group of premanifest gene carriers that were compared to healthy controls on a battery of cognitive tests, which cross-sectionally revealed differences in tests of memory, executive function and psychomotor speed. The surprising element of these findings lays in the higher sensitivity of low demand cognitive tests, as opposed to more complex tests. These low demand tests all had a psychomotor timed element to them, which indicate suitability of such tests[52].

Emotion recognition

Diminished recognition of negative emotions can appear very early in the disease process, and may be pathologically linked to HD. Currently the most likely candidate for a cognitive biomarker is disgust recognition. However, due to the negative longitudinal findings over periods of three and twelve months, a longer longitudinal follow-up is desirable to understand the potential rate of change of this deficit and its suitability as a sensitive biomarker. The reports on this domain have examined in detail different emotions, so to further validate and understand this construct, gain could be achieved by combining

emotion recognition assessments and functional MRI scanning.

Executive (dys)functioning

Deficiencies in executive functions are part of the cognitive profile in HD, with some subtle changes detectable prior to motor onset of the disease. Executive functioning has also long been regarded as a good candidate biomarker for cognitive functioning in HD, however this statement may be too broad as not all reports of executive functioning showed difference to controls. The sudden rise in evidence for attention dysfunction in manifest HD as opposed to the lack of findings in premanifest HD may suggest that attention is an appropriately sensitive marker of disease state. Distinguishing between the subtypes of executive functioning may be vital in identifying the most appropriate biomarker. That this distinction may be important in premanifest HD was also noted by Lawrence *et al.*, (2000), who discussed that in depth analysis of visual and spatial functions may be relevant to understanding the early cognitive changes in HD[36]. Future research should focus on attention and visual sub-types of executive functioning as the other potential biomarker candidates.

Global cognitive (dys)functioning

The overall evidence for impairment in global functioning is not persuasive, with little to no evidence of impairment in premanifest HD, and with almost equal numbers of reports for and against the presence of a deficit in early manifest HD. For this reason it may be relevant to establish premorbid IQ levels from a clinical point of view at some point during diagnostic or treatment procedures, but not to measure IQ over several time points as the longitudinal evidence is insufficient. In our opinion such measures are not sensitive enough to be considered as a biomarker.

Limitations and considerations

Reviewing the cognitive literature was complicated by a number of issues, and some limitations have consequences for the conclusions drawn. The literature search was limited by the choice of search terms used. In compiling the figures we did not take into account size of the groups studied. However, in drawing our global conclusions, we did to some extent take into account the sample sizes and other methodological issues when appropriate. The study sizes discussed varied from numerous smaller studies with 10 or 15 participants and moderately sized studies of 30 to 100 participants to a limited number of studies with more than 500 subjects in a group. The increase in sample size positively affects the chance that significant differences between groups will be found. This is positive when trying to investigate subtle changes that may be overshadowed by inter-subject variability, however if a difference is demonstrated with a small study size this may indicate that the sensitivity of this deficit is very high. This same concept applies to the presence and length of follow-up studies. For successful biomarker evaluation longitudinal

assessment of the results is essential. However, how long should this follow-up ideally be? The longitudinal studies discussed in this review varied in length from three months to ten years. This issue could not entirely be taken into account when compiling the results or when drawing conclusions. This may have affected the conclusions drawn. However, there is no golden rule that can be followed as to under which circumstances we feel a deficit is proven. These differences in length of follow-up restrict the potential for attempting to understand in great detail the nature of cognitive changes in HD. However, this issue is not entirely restrictive as the presence of positive findings from studies of all sizes has allowed us to draw conclusions on important functional domains as well as general assumptions on when the deficits become apparent or seem to be most prominent.

A further problem was posed by the manner in which groups were defined. Although all studies based their disease assessment on the absence or presence of motor symptoms, there was large variation in how 'motor symptoms' were defined. Some reports did not specify how this was determined. Others defined premanifest HD in a varied manner either using a definition of low Total Motor scores on the UHDRS with predefined cut-off points to the use of diagnostic confidence ratings[97], also with different cut-off points. In this review we have approached the findings based on the manner in which the authors describe the groups. However this can potentially seriously impact the conclusions drawn in regards to the stage at which a deficit is present. This variability makes it complicated to interpret numerous findings, and therefore it would be advisable for the HD research community to construct guidelines for study design, so that future communications are directly comparable.

Reports of dysfunction in a particular cognitive domain may be clouded or over-reported due to the majority of papers only investigating sub-domains of a function, this was taken into account as much as possible but it remains challenging to draw global conclusions. Furthermore, in the later disease stages, other disease aspects can have great impact on test performance. Such results can be confounded by medication, severe movement disorder, behavioural issues and other functional limitations. Depression, apathy and anxiety, as some of the most frequently occurring (30-60%) behavioural issues, and these can directly effect motivation and test performance, as well as the treatment given for such conditions[127,128]. It was not possible to take all this into account when comparing results.

Highly sensitive measurement techniques have proven useful in the detection of early changes. Devices such as oculomotor eye trackers have been used to register responses in a memory task in premanifest HD and found that these outcomes measures were sensitive to subtle differences to controls[69] Furthermore, although performance on memory tasks was not different between premanifest gene carriers and controls, measurement by EEG

recording[63] and fMRI scanning[68] did show differences in brain reactions using memory tasks. For this reason it is advisable, where possible, to make use of such technological enhancements. The use of physiologically based measurement tools such as MRI in combination with cognitive assessments may prove most sensitive. This is especially so when investigating the subtle changes associated with premanifest HD, because the results are then supported by other objectively quantified measures with known correlations to brain changes in HD.

Despite its limitations, this review also has its strengths, which lie in the comprehensive nature of the study and the objective assessment of the articles. Furthermore this review tries to discuss both the division of disease stage according to premanifest and manifest, but also their subdivision, in an attempt to provide information on the staging of deficits. Prior to this review no single paper had addressed cognitive functioning in all disease stages. The strict selection criteria imposed on the papers included allow for conclusions to be drawn that can have relevance from both a clinical and scientific point of view.

Conclusions

Drawing conclusions for clinical purposes from research papers should be approached cautiously. The results and conclusions discussed above are based on group differences and can never be projected onto a personal basis without considering the individual at hand. Having said this, the overall profile of cognitive disorder in HD can be summarised as follows. In premanifest gene carriers there are typically no to little deficiencies in memory, language or global cognitive functions. Differences can be found in tasks assessing the functions of psychomotor speed, negative emotion recognition, and to some degree in executive functioning. In manifest HD adequate functioning appears to remain intact for the longest periods for language and global functional domains, however as a result of the progressing cognitive decline resulting in dementia, during end stage HD, these functions can also show marked deterioration. During the progression of the disease, impairments can be expected in memory (especially visuo-spatial), psychomotor speed, negative emotion recognition, and executive functioning. For this reason we suggest that these four functional (sub)domains should be recognised when clinically diagnosing substantial cognitive decline or dementia due to Huntington's Disease.

The most promising domains and sub-domains for providing a cognitive biomarker appear to be working memory, measures of psychomotor speed, recognition of negative emotions (in particular disgust), attention and executive functions, because measures of these functions seem to detect early changes that progress during the disease. Having said this, the importance of longitudinal investigation of such candidates must be reiterated. A biomarker will only prove useful when it is sensitive to change over time, preferably not only in manifest but also in premanifest HD.

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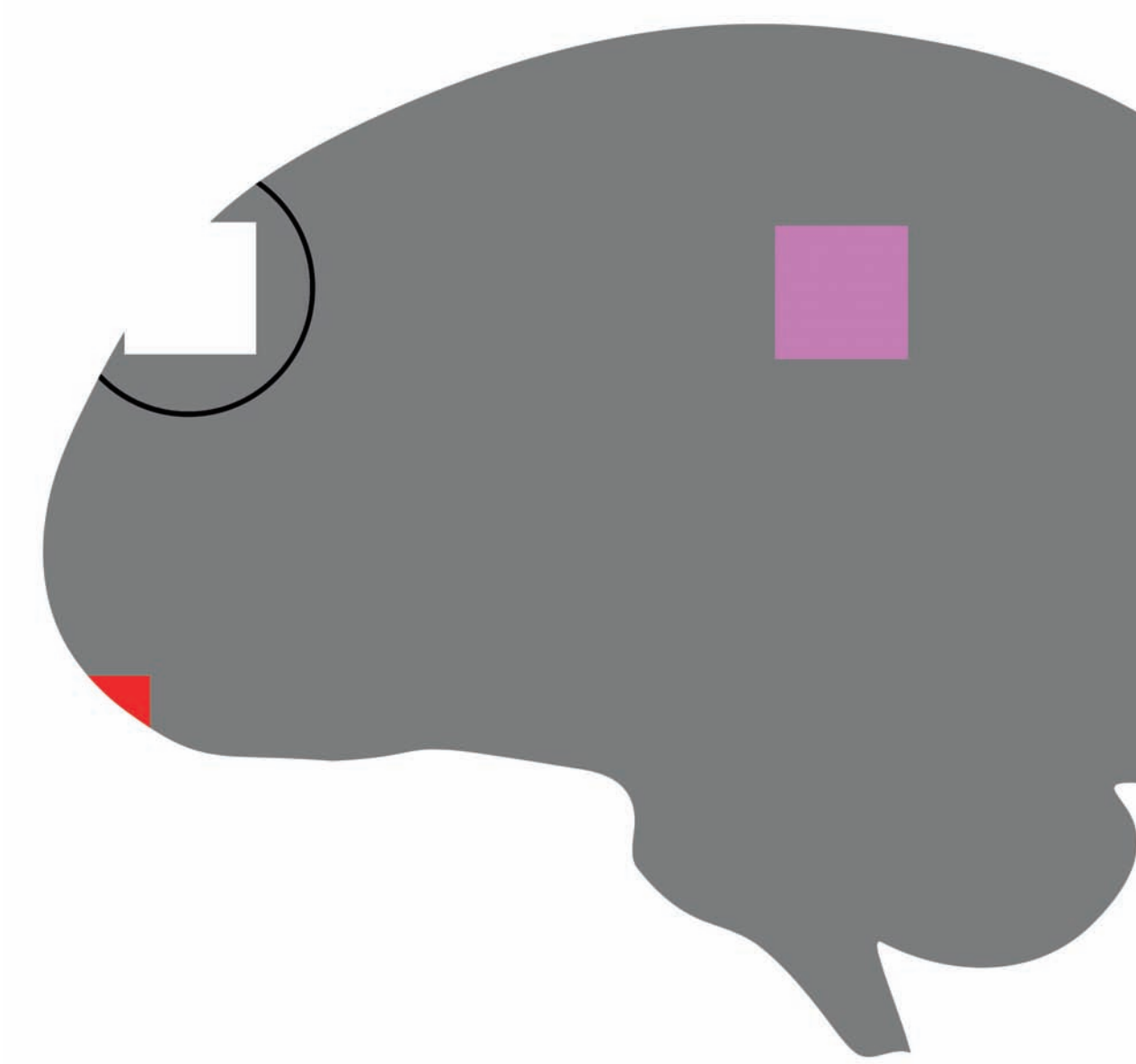

Chapter 3

Working memory impairment in premanifest gene carriers and early Huntington's disease

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Abstract

Objective

Working memory deficits have been found in Huntington's disease (HD) and in a small group of premanifest HD gene carriers. However, the nature and extent of these deficits are not known. We aimed to determine, in a large cross-sectional and 12-month longitudinal study, the degree of visuospatial working memory dysfunction across multiple disease stages including both premanifest and early HD. We also examined the relationship between visuospatial working memory and motor dysfunction.

Method

We examined 363 participants from the TRACK-HD study, including 62 premanifest gene carriers far from estimated disease onset (preHD-A), 58 premanifest gene carriers close to disease onset (preHD-B), 77 stage 1 HD patients (HD1), 44 stage 2 HD patients (HD2), and 122 healthy controls. For the visuospatial working memory test, participants performed 64 simple and moderately difficult trials at baseline, and 64 moderate and difficult trials after 12 months.

Results

Cross-sectionally, differences in visuospatial working memory capacity were seen in PreHD-B and in the two HD groups when compared to the controls. Longitudinally, only patients in HD stage 2 showed a reduction of visuospatial working memory capacity. Speed and accuracy were positively correlated, but only in the HD groups.

Conclusions

Impairment in visuospatial working memory is detectable cross-sectionally in both premanifest and manifest stages of HD, but declines in visuospatial working memory at 12 months were only significant in HD stage 2. Furthermore, in manifest HD there is evidence for a "worse-worse phenomenon", whereby reductions were present in both motor speed and accuracy.

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease, which is characterised by progressive motor, psychiatric and cognitive symptoms and signs. The mean age of disease diagnosis is between 35 and 45 years. Individuals at risk of carrying the HD gene can be tested. Those who are found to have the gene but not to have clinical disease signs are referred to as premanifest gene carriers. Many studies investigating cognition in HD have demonstrated progressive cognitive decline resulting in dementia[1;2]. Cognitive decline is also detectable in the premanifest gene carriers across a number of domains, including executive functions, memory, emotion recognition and psychomotor functions[3-5].

Working memory is a topic of recent attention as a possible marker for disease state in HD[6;7]. Many day-to-day activities require retention, integration and manipulation of either verbally or visually presented information, referred to as verbal or visual (or visuospatial) working memory[8]. Poorer working memory has been described as part of the disease course of HD. In particular, several cross-sectional studies have demonstrated that HD patients show poorer spatial or visual working memory in comparison to controls[6;9;10]. Longitudinally, visual working memory span was found to decline over a period of 3.6 years in 22 patients with HD[11]. Verbal working memory has also been found to be impaired cross-sectionally in HD[10;12].

Studies of premanifest gene carriers have identified mild-to-moderate cognitive deficits in a range of domains, including attention, memory, psychomotor speed and executive functioning, which are among the first cognitive functions to show decline in the premanifest phase[13-19]. With regard to working memory, the evidence in premanifest HD is unclear. Some studies have reported a decline in both verbal and visual working memory[18;20]. However, others have suggested that premanifest HD do not differ from controls in either visual or verbal working memory[14;21]. Limited longitudinal evidence is available regarding working memory in premanifest HD. However, in one study of 12 premanifest gene carriers who were tested with an extensive neuropsychological battery 3 times over a period of 2.5 years, it was suggested that working memory, in particular visuospatial working memory, may be among the first cognitive functions to show decline in the premanifest phase[7].

Working memory is implemented in complex brain networks which integrate signals received by the parietal cortex, and then project these integrated signals onto the frontostriatal brain circuits which subsequently drive motor responses[22-24]. In healthy adults, evidence from functional magnetic resonance imaging (fMRI) demonstrates the involvement of the parietal cortex in working memory. For example, short-term memory

capacity is correlated with parietal cortex blood oxygen level dependent (BOLD) activity levels[25;26]. The underlying brain regions associated with working memory are also among the primary regions implicated in the cognitive dysfunction observed in HD, namely the caudate nucleus and putamen, which lie within the frontostriatal brain circuits[12;27-30]. The brain regions which are involved in working memory overlap considerably with those regions shown to be affected structurally and functionally in both premanifest and diagnosed HD[31-33]. Measures of brain functioning during performance of working memory tasks, such as fMRI or electroencephalography (EEG), have shown that patterns of the underlying brain processes are different in premanifest gene carriers than those of controls, even in the absence of differences in working memory task performance[12;34;35]. Brain atrophy develops prior to disease diagnosis in these premanifest gene carriers, and progresses during the disease course, with the most profound and earliest changes found in the deep grey matter structures such as the caudate nucleus and putamen[31;32;36;37]. The integrity of the white matter is also affected in HD[38;39]. Given that both brain atrophy and decline in objectively assessed clinical measures have been observed more than ten years before estimated disease onset[13;15;31;37], it can be expected that deficiencies in visual working memory would develop as these brain regions deteriorate.

Motor functioning overlaps with cognitive functioning, in that both are implemented in brain structures such as the basal ganglia[40], and cognitive performance is measured through motor outputs such as verbal or button-based responses. The most sensitive assessments of early cognitive changes in HD are those with a substantial psychomotor speed component[15;41-43]. Therefore, to better understand how HD affects cognition, it is important to distinguish, where possible, the impact of motor functioning on cognitive measures. Also, as we move toward treatment-focused studies in HD, it is necessary to understand the progression of cognitive deficits in relation to motor dysfunction. This is important since patient groups are often defined in terms of their level of motor deficits. The distinction between premanifest and manifest HD is made based on the level of motor abnormalities. For premanifest groups, stringent exclusion of motor deficits can facilitate distinctions between motor and cognitive disease effects, although subtle motor changes are not eliminated by this approach. Cognitive tasks that require minimal motor responses are also desirable in this respect.

The background presence of motor slowing also complicates the interpretation of cognitive testing in HD. One approach to disentangling the motor and cognitive roles is to examine the relationship between performance accuracy and response times. This relationship is often observed as a 'speed-accuracy trade-off', which refers to a strategy whereby participants use a slower, more cautious approach to ensure the accuracy of their performance. Conversely, faster responses may be less accurate due to being less careful or cautious. We hypothesized that HD gene carriers may slow their responses as a compensatory strategy in order to maintain satisfactory cognitive performance. Because

we wanted to examine whether speed-accuracy trade-offs would appear in relation to working memory performance in HD, we selected a task in which these two aspects of performance could be examined separately, allowing their relationship to be studied in the context of HD.

The aim of this study was to determine, using a large cross-sectional study, the degree of visuospatial working memory dysfunction across multiple disease stages including both premanifest gene carriers, and those in early stage HD. Furthermore, we wanted to examine visuospatial working memory function in HD across different levels of task complexity. We expected to find evidence of visuospatial working memory decline in early HD and also in the premanifest phase, especially given the progressively widespread grey and white matter brain changes known to occur in HD. We also wanted to distinguish between cognitive and motor influences in order to clarify whether working memory itself, rather than just the motor expression of this cognitive function, is affected in HD. By addressing these aims, we can obtain evidence regarding the possibility that a working memory task may be suitable as a marker for cognitive deterioration in early diagnosed or premanifest HD.

Methods

Participants

Three hundred and sixty-six subjects were studied as part of the TRACK-HD longitudinal observational study. Of these, 123 were premanifest gene carriers, defined as genetically confirmed but without clinically evident symptoms, 120 were patients with stage 1 and 2 HD, and 123 were age- and sex-matched healthy controls. Participants were recruited from four study sites: London (UK), Paris (F), Vancouver (CAN), and Leiden (NL). Premanifest participants were included only if they did not have substantial motor signs as indicated by total motor scores of ≤ 5 points on the Unified Huntington Disease Rating Scale (UHDRS), and if they had Disease Burden Scores of at least 250[44]. For each premanifest gene carrier, we computed an estimate of the proximity (in years) to predicted disease onset based on CAG repeat length and current age[45]. Then, using a median split (10.8 years to expected onset) we divided the group into a *further from estimated onset group* (PreHD-A, > 10.8 years to estimated onset) and a *closer to estimated onset group* (PreHD-B, < 10.8 years to estimated onset). For early stage HD participants, we used Total Functional Capacity (TFC) scores from the UHDRS to differentiate between patients in HD stage 1 (HD1, TFC scores 11-13) and HD stage 2 (HD2, TFC scores of 7-10) groups. Participants were studied annually, and in the current report we include baseline cross-sectional data on a visual working memory task, the *Spot the Change task* (SPOT), as well as data from the first longitudinal (12-month) visit. For information on the full cognitive assessment battery, additional examinations and detailed inclusion criteria see Tabrizi *et al.* (2009)[5].

Spot the change task

The Spot the Change task (SPOT) was based on the previously described visual array comparison task[46;47]. Using a Lenovo Vantage Thinkpad tablet PC (IBM, New York), participants viewed an array of coloured squares (250 ms), followed by a blank display (1000 ms), and then second array of coloured squares in which one of the squares had been encircled. The position of the squares was unchanged between the two presentations. Participants were then asked to indicate if the colour of the encircled square had changed from the first to second display. Using a mouse mounted on a stabilising wooden platform, the response "same" could be given using the thumb of the dominant hand or "different" using the non-dominant thumb. The mouse platform included labels for the "same" and "different" responses to remind subjects which thumb corresponded to which response. Answers could be given up to 8 seconds after the beginning of the second display. Prior to starting the task, instructions and a minimum of four practice trials were given to ensure task comprehension. Three levels of difficulty, based on the number of coloured squares contained in the array, were used. Specifically, at baseline, the easiest level included three coloured squares (set size 3) and the harder level included five coloured squares (set size 5). At the 12-month visit, set sizes 5 and 7 were used. The easiest level, set size 3, showed a ceiling effect at baseline and was dropped from the test battery and replaced by set size 7 for the 12-month visit. This design yielded cross-sectional data on set sizes 3, 5 and 7, and longitudinal data at 12 months for set size 5. Thirty-two trials of each set size were randomly mixed, yielding a total of 64 trials at each visit. Both accuracy and response time were recorded and analysed separately for each of the set sizes.

Non-response trials were recorded when a participant did not respond within the given 8 second time frame, which occurred 168 times across both visits and study groups (0.38% of the trials). In an additional seven trials, responses were given within 100 ms of the stimulus; these were considered to be 'pre-cognitive' or accidental responses and were excluded from the analysis. Accuracy measures were corrected for guessing by the calculation of *k*, a measure of working memory capacity as described by Cowan (2001), computed as $k = \text{set size } \underline{n}$ ([number correct hits/number of trials] + [number correct rejections/number of trials] -1)[46]. A *k* or working memory capacity value close to the set size (e.g., 3, 5 or 7) indicates good working memory capacity, whereas working memory capacities close to or less than zero represent performances closer to chance.

Of subjects that attended the visits, only a small number of participants failed to complete the SPOT, which was nearly always due to time constraints. The SPOT was completed at the baseline visit, the 12-month visit, or at both visits by a total of 363/366 (99%) of the participants (with 1 control and 2 HD2 participants not completing the SPOT at any of the visits and were thus excluded from the analysis). The baseline visit had a total of 355 of 366 who completed the task (97%), yielding missing data for 3 controls, 5 HD1, and 3 HD2.

Three hundred and twenty five of 355 (92%) of baseline visit participants returned for the 12-month visit, with an additional 8 completing the task who did not do so at baseline. Therefore, 333 out of 366 (91%) completed the task during the 12-month visit, yielding missing data for 9 control, 1 PreA, 5 PreB, 6 HD1 and 12 HD2 during this visit.

Statistical analysis

All working memory capacity (*k*) data were analysed in a single regression model incorporating data from all levels of difficulty and both visits. Working memory capacity, *k*, was the outcome variable. The main predictors were group (controls, PreHD-A, PreHD-B, HD1 and HD2) and set size at each visit (set size 3 and 5 at visit 1; set size 5 and 7 at visit 2).

Response times (RT) were considered separately for correct (correct recognitions and correct rejections) or incorrect (incorrect recognitions and incorrect rejections) trials. The distributions of RTs were highly skewed and therefore log transformed prior to analysis to improve normalisation of these variables for statistical analysis. Similar to the analyses for working memory capacity, all RT data from both visits and from all three set sizes were analysed in a separate single regression model with RT as the outcome. The main predictors were group (Controls, PreHD-A, PreHD-B, HD1 and HD2), response accuracy (correct or incorrect) and set size (set sizes 3 and 5 at the baseline visit; set sizes 5 and 7 at the 12-month).

Age, gender, education level and study site were included as covariates for both the working memory capacity (*k*) and RT models. The regression models used generalised estimating equations, which have a working assumption of exchangeability and robust standard errors[48;49]. This allowed for cross-sectional comparison of each gene-carrier group to controls for each set size. We also examined whether groups responded differently in terms of RTs for correct versus incorrect trials. Longitudinal comparisons of each gene carrier group for their set size 5 performance at the second visit (versus their performance at the baseline visit) were compared to that of controls at their 12-month visit (versus their baseline visit).

Finally, to examine the direct relationship between accuracy and RT, we computed separate linear regression models for each set size with RT as the outcome measure. For this analysis, *k* and group (controls, PreHD-A, PreHD-B, HD1 and HD2) were the main predictors. Again, age, gender, education level and study site were covariates. A group versus *k* interaction was included to allow differences in the speed/accuracy relationship between groups to be investigated.

Results

To address our primary objective of examining visual working memory, here we first describe *k* (working memory capacity) in the five groups, including both cross-sectional and longitudinal results. We then present RT findings per set size. Finally, we describe the relationship between RT and accuracy to further characterize the relationship of visual working memory to premanifest and early stage HD. The participant characteristics at baseline are shown in table 1.

Table 1 Participant characteristics at baseline

		Controls	PreHD-A	PreHD-B	HD1	HD2
Number of participants		122	62	58	77	44
Female/male		68 / 54	33 / 29	33 / 25	46 / 31	19 / 25
Age[a]	mean (SD)	46.2(10.1)	41.1 (8.6)	40.6 (9.2)	47.2 (10.3)	51.0 (8.6)
Education level[b]	mean (SD)	4.0 (1.3)	4.1 (1.1)	3.8 (1.3)	3.8 (1.3)	3.3 (1.4)
CAG repeat length	mean (SD)	-	42.1 (1.8)	44.2 (2.5)	43.8 (3.3)	43.5 (2.4)
Expected years to onset[a]	mean (SD)	-	14 (3.1)	9 (1.3)	-	-
Disease duration[a]	mean (SD)		-	-	5 (5.8)	8 (4.5)
Intervisit interval (months)	mean (SD)	11.6 (0.8)	11.5 (0.6)	11.5 (0.9)	11.6 (1.0)	11.6 (0.6)

[a]: Age, expected years to onset and disease duration as at baseline; [b]: Education level as a proxy for Intelligence Quotient, as based on the ISCED education classification system

Working memory capacity (*k*) was significantly lower for the PreHD-B, HD1 and HD2 groups at each visit and for each set size (3, 5 and 7) compared to healthy controls (Table 2 and Figure 1). PreHD-A did not show difference to controls at either visit for any set size. Set size 3 (assessed at the first visit only) demonstrated a ceiling effect in controls and both premanifest groups, but this ceiling effect was not apparent for set size 5 and 7. The 12-month longitudinal effects for set size 5, which was the only set size performed across both visits, indicated that for the HD2 group, *k* decreased by nearly half an item compared to controls over the same period (-0.46 decline in *k*, $p = 0.045$; 95% confidence interval: -0.01 *k* to -0.92 *k*). No other group showed significant decline in performance after 12 months.

Table 2. Adjusted differencesa in working memory capacity and response time for set sizes 3 and 5 at year 1 and set sizes 5 and 7 at year 2 for HD gene-carriers compared to controls

	Working memory capacity (k)			Response time (% increase)[b]						
				Correct			Incorrect			Interaction[(c)]
	Est.	95%CI	*p*	Est.	95% CI	*p*	Est.	95% CI	*p*	*p*
Year 1 – Set Size 3										
PreHD-A	-0.04	*(-0.26 to 0.18)*	0.70	2.6%	*(-2.6 to 8.2)*	0.33	0.8%	*(-8.7 to 11.3)*	0.88	0.66
PreHD-B	-0.26	*(-0.44 to -0.07)*	0.01	7.6%	*(1.6 to 13.9)*	0.01	8.1%	*(-3.8 to 21.4)*	0.19	0.93[(d)]
HD1	-0.78	*(-1.01 to -0.54)*	<0.001	18.9%	*(10.9 to 27.4)*	<0.001	7.3%	*(-2.8 to 18.3)*	0.16	0.004
HD2	-0.95	*(-1.25 to -0.65)*	<0.001	39.4%	*(27.0 to 53.1)*	<0.001	30.9%	*(17.7 to 45.6)*	<0.001	0.09
Year 1 – Set Size 5										
PreHD-A	-0.26	*(-0.61 to 0.09)*	0.14	4.6%	*(-1.2 to 10.8)*	0.12	4.9%	*(-4.3 to 15.0)*	0.31	0.94[(d)]
PreHD-B	-0.71	*(-1.04 to -0.37)*	<0.001	8.3%	*(1.6 to 15.5)*	0.02	2.5%	*(-7.2 to 13.2)*	0.63	0.13
HD1	-1.38	*(-1.71 to -1.04)*	<0.001	14.9%	*(7.2 to 23.3)*	<0.001	1.6%	*(-7.4 to 11.4)*	0.74	<0.001
HD2	-1.45	*(-1.84 to -1.06)*	<0.001	40.1%	*(27.7 to 53.6)*	<0.001	22.2%	*(8.5 to 37.5)*	0.001	<0.001
Year 2 – Set Size 5										
PreHD-A	-0.03	*(-0.31 to 0.26)*	0.87	3.1%	*(-2.2 to 8.6)*	0.26	10.6%	*(3.0 to 18.7)*	0.005	0.005[(d)]
PreHD-B	-0.78	*(-1.19 to -0.36)*	<0.001	4.1%	*(-2.3 to 10.9)*	0.21	1.2%	*(-8.6 to 12.1)*	0.82	0.40
HD1	-1.38	*(-1.72 to -1.03)*	<0.001	21.0%	*(13.4 to 29.2)*	<0.001	10.7%	*(2.9 to 19.2)*	0.007	<0.001
HD2	-1.92	*(-2.40 to -1.43)*	<0.001	37.1%	*(23.6 to 52.0)*	<0.001	19.4%	*(6.3 to 34.1)*	0.003	<0.001
Year 2 – Set Size 7										
PreHD-A	-0.05	*(-0.50 to 0.41)*	0.85	5.5%	*(-0.1 to 11.3)*	0.05	3.6%	*(-3.5 to 11.3)*	0.33	0.40
PreHD-B	-1.10	*(-1.62 to -0.59)*	<0.001	7.3%	*(0.2 to 14.9)*	0.04	-0.9%	*(-9.4 to 8.4)*	0.84	0.002
HD1	-1.38	*(-1.83 to -0.93)*	<0.001	24.2%	*(15.8 to 33.1)*	<0.001	10.5%	*(2.3 to 19.3)*	0.01	<0.001
HD2	-2.01	*(-2.56 to -1.45)*	<0.001	35.1%	*(23.4 to 48.0)*	<0.001	17.4%	*(5.2 to 31.0)*	0.004	<0.001

[a]: All results are adjusted for age, sex, educational level and study site; [b]: For ease of interpretation, the log RT values were back-transformed to the original millisecond scale and these results are reported; [c]: The interaction represents the difference in estimated RTs for Correct and Incorrect responses for each HD subgroup compared with the same difference in Controls; [d]: The difference in estimated RT for Correct and Incorrect responses is larger in the HD subgroup than the difference in Controls. In all other cases the difference in RT for Correct and Incorrect responses is smaller in the HD subgroup than the difference in Controls.

The RTs in the easiest condition, set size 3, were longer in the PreHD-B, HD1 and HD2 than in controls when answering correctly, despite the ceiling effect. For set size 3 incorrect responses, only the HD2 group was significantly slower. At baseline for set size 5 (the moderately difficult trials), correct responses were slower in the PreHD-B, HD1 and HD2 than controls. At the 12-month time point, only HD1 and HD2 were slower at answering correctly than controls. Incorrect responses showed similar sensitivity. Specifically, at baseline, only the HD2 group provided slower incorrect responses compared to controls, and at 12 months, PreHD-A, HD1 and HD2 groups provided slower incorrect responses as compared to controls. In the most challenging condition, set size 7 trials, all four groups (PreHD-A, PreHD-B, HD1 and HD2) were significantly slower than controls when responding correctly, but when responding incorrectly, only HD1 and HD2 were slower.

The relationship between motor performance and working memory capacity (*k*) was examined using only three groups to increase power, including controls, a premanifest group (PreHD-A and PreHD-B combined) and an early HD group (HD1 and HD2 combined). Across all three groups the results showed a significant overall interaction effect between trial accuracy and RT, thereby indicating that response times differed for correct as compared to incorrect responses. This is depicted in Figure 1, which shows that RTs for correct and incorrect responses converge with increasing severity of disease stage as the difficulty of the task increases. In a separate analysis of the relationship between speed and accuracy, we found no evidence for a relationship between speed of response and accuracy for any of the set sizes in either controls or the premanifest gene carrier group. In contrast, we did find that slower response speed was related to lower levels of accuracy in the manifest HD group for all set sizes. Specifically, longer RTs were associated with less accurate responses at baseline for set sizes 3 and 5, and at 12 months for set sizes 5 and 7 (all *p values* < 0.001; see Figure 1).

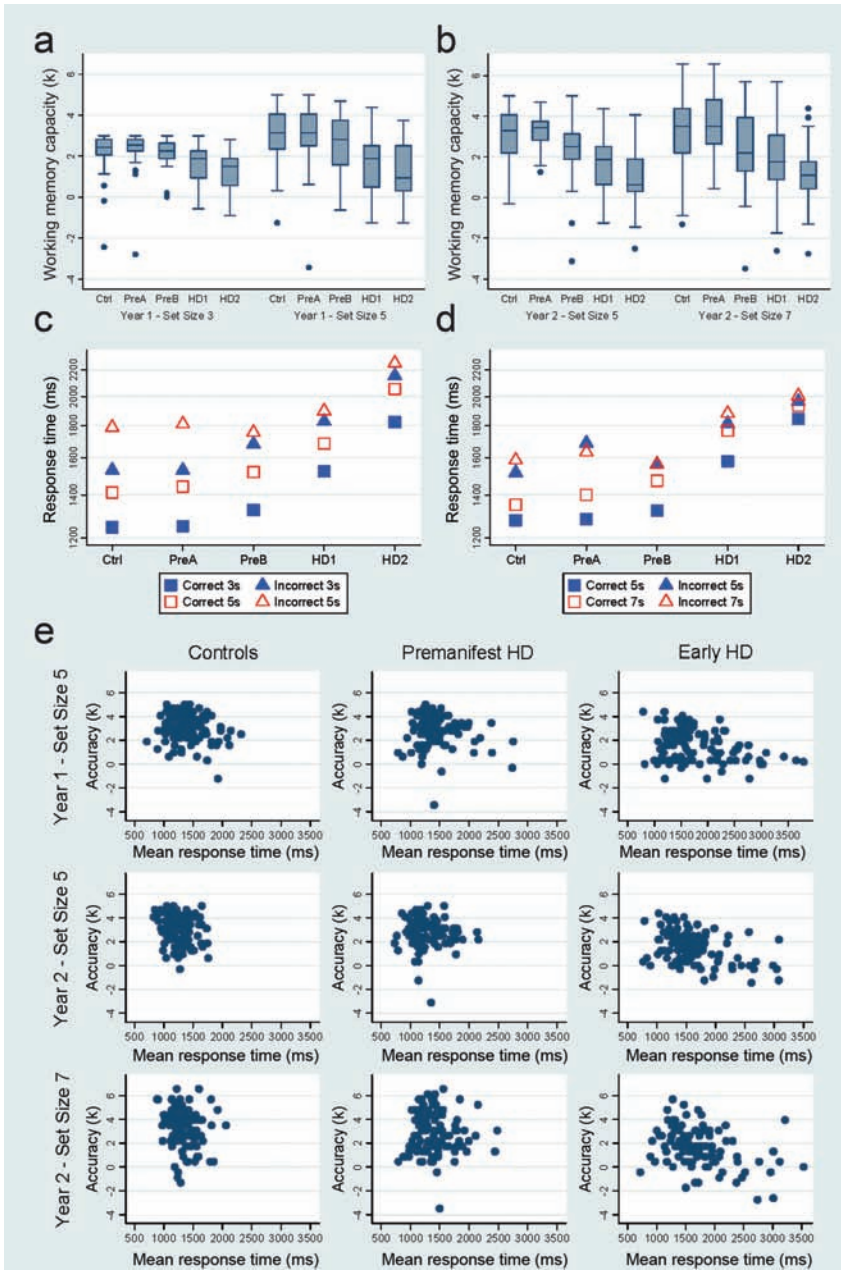

Figure 1. Ctrl = healthy controls, PreA = Premanifest gene carriers far from expected disease onset, PreB = Premanifest gene carriers close to expected disease onset, HD1 = Patients in stage 1 of the disease, H2 = patients in stage 2 of the disease, Year 1 = baseline visit, Year 2 = 12-month visit.
(a) Working memory capacity for set sizes 3 and 5 at Year 1 (b) and set sizes 5 and 7 at Year 2 for HD gene-carriers and healthy controls. (c)Response time for set sizes 3 and 5 at Year 1 (d) and set sizes 5 and 7 at Year 2. (e) Speed vs. accuracy for set sizes 5 and 7 in controls, premanifest gene carriers (pre HD) and patients (set size 3 not shown due to ceiling effect in controls and premanifest gene carriers)

Discussion

This study's main findings were that cross-sectionally, visuospatial working memory capacity is lower in both premanifest HD gene carriers who are within a decade of disease onset and in early HD patients. Secondly, over a period of one year, manifest HD patients show longitudinal decline in working memory capacity. Finally, in manifest HD, despite observing both working memory decline and slower response times, the relationship between motor response speed and accuracy was not a speed-accuracy trade-off, but rather we observed that longer response times corresponded to poorer performance.

From the cross-sectional results we conclude that working memory capacity is impaired in premanifest gene carriers close to expected disease onset (i.e. within 10.8 years of expected diagnosis) and in stage 1 and 2 HD patients as compared to healthy controls. All groups responded very similarly to the moderately difficult trials at both visits. These findings confirm the presence of dysfunction in visual working memory which has previously been found in HD patients in cross-sectional studies[50;51], and extends these findings to premanifest HD. Furthermore, despite the report by Lemiere *et al*. (2004) of a longitudinal decline in general working memory over two and a half years in pre manifest HD[7], we did not find a decline in visual working memory in premanifest HD in our 12-month follow up. Our results add to current literature in that we report, for what we believe is the first time, that decline in visuospatial working memory can be observed over a period of just 12 months in patients with stage 2 HD. We did not find a change over 12-month in premanifest gene carriers or patients at stage 1 of the disease, which could be related to a slower progression of cognitive changes at these stages of the disease. Furthermore, the task used in the current study taxed various levels of working memory capacity and our results demonstrate lower working memory capacity at all complexity levels of the task.

Response time results indicated that premanifest and diagnosed participants were slower than controls at responding to the task correctly. This is particularly relevant as the premanifest gene carriers were restricted to only those who were free of clinically evident motor signs. Given the lack of significant motor signs in this group, we believe that the slowing observed may indicate slowed cognition or information processing rather than evidence of slowed motor processing. Our finding is consistent with previous findings of psychomotor slowing in premanifest groups[15;41]. We also note that the working memory task included a long response time frame (8 seconds), to allow participants, even those with early HD who have proven motor deficits, to respond to the trials within the time frame. The task design therefore eliminated any potential differences in response time being attributed to missing data in the manifest group.

A key strength of the current paper is that in combining assessment of the response speed and accuracy, we can examine how slowing and accuracy are related within a working memory task, whereas in previous studies, psychomotor speed and working memory have been examined in separate tasks. In premanifest gene carriers and controls, we found that response times and working memory capacity were not significantly related to each other. More specifically, we did not find evidence for a speed-accuracy trade-off in any participant group. A speed-accuracy trade-off would have been apparent if faster responders showed generally less accuracy than slower responders[52]. On the contrary, we found evidence that in manifest HD the opposite is true, such that slower responses were associated with less accurate performances and thus lower visual working memory capacity.

Our findings show that declines in visuospatial working memory in early HD are accompanied by both slower speed of responses and lower accuracy. This kind of relationship between speed and accuracy, can be described as a "worse-worse phenomenon", and cannot be explained as an epiphenomenon of HD. An epiphenomenon describes a relationship between two deficits in a disease that occur simultaneously but are in fact not related or caused by each other; however, because they occur together they seem related. The premanifest HD data from this study discount an epiphenomenon as the explanation because although the premanifest gene carriers demonstrated significantly slower response times as well as a poorer working memory capacity, there was no statistical evidence of a relationship between the two. Additionally, because motor slowing can be present at the same time as poor cognitive performance in the absence of a relationship between the two, as is seen in the premanifest gene carriers, the presence of motor slowing does not directly implicate it as a primary cause of poorer cognitive performance. Therefore, the presence of a "worse-worse phenomenon" indicates that poor cognitive performance cannot be explained by slow responses times only.

The visual working memory task applied in this study included a reasonably large number of trials, thereby providing relatively robust estimates of working memory capacity across three difficulty levels. The task can be argued to assess visuospatial rather than verbal working memory because it uses a random selection of colours and location of squares between trial pairs, which makes the use of verbal encoding strategies unlikely. This design also appears to have minimal practice effects.

One limitation is that it is not possible to eliminate deficits in basic attention as a cause for poor task performance. However, the short trial duration was designed to limit the impact of short attention spans on task performance. It is also important to realise that attentional functions are interlinked with working memory, and the role of attention in cognitive processing is complex. In fact, Cowan (2001) argue that working memory tasks, such

as Spot the Change task, assess the scope of attention, a key factor that limits working memory capacity[46]. As HD progresses, there may be a decrease in the ability to adequately attend to and extract relevant information from the task at hand. Therefore, although attention span is not directly assessed by this task, attention processes play a role in the task outcome. This could be reflected in the "worse-worse phenomenon" whereby it may be more difficult for patients with HD to extract the needed information from the stimuli, as well as being slower at integrating the information from the first and second arrays.

In summary, we conclude that visual working memory impairment can be detected in both premanifest gene carriers and early stage HD patients using the Spot the Change task. In the early stages of HD we observed a "worse-worse phenomenon" whereby lower accuracy was associated with slower responses; the opposite of a speed-accuracy trade-off. Importantly, the longitudinal results demonstrated that visual working memory task shows detectable decline across 12 months in stage 2 HD. Our findings, together with other reports in the literature, suggest that working memory tasks are useful markers of cognitive deterioration in HD. Such deterioration may be most sensitively detected in early HD, especially in stage 2, using moderate to higher working memory loads along with measures of working memory capacity and response times for correct trials. This sort of cognitive task may be applicable in short term trials (of 12 or more month duration) of disease modifying or symptomatic treatments for participants in HD stage 2. Future examination of longitudinal effects in the most difficult condition, set size 7, once such data become available from the TRACK-HD study, may reveal added task sensitivity for premanifest gene carriers or stage 1 HD.

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Chapter 4

Early atrophy of pallidum and accumbens nucleus in Huntington's disease

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Abstract

In Huntington's disease (HD) atrophy of the caudate nucleus and putamen has been described many years before clinical manifestation. Volume changes of the pallidum, thalamus, brainstem, accumbens nucleus, hippocampus, and amygdala are less well investigated, or reported with contradicting results. The aim of our study is to provide a more precise view of the specific atrophy of the subcortical grey matter structures in different stages of Huntington's disease, and secondly to investigate how this influences the clinical manifestations. All TRACK-HD subjects underwent standardised T_1-weighted 3T MRI scans encompassing 123 manifest HD (stage 1, n = 77; stage 2, n = 46), 120 premanifest HD (close to onset, n = 58; far from onset, n = 62) and 123 controls. Using FMRIB's FIRST and SIENAX tools the accumbens nucleus, amygdala, brainstem, caudate nucleus, hippocampus, pallidum, putamen, thalamus and whole brain volume were extracted. Results showed that volumes of the caudate nucleus and putamen were reduced in premanifest HD far from predicted onset (> 10.8 years). Atrophy of accumbens nucleus and pallidum was apparent in premanifest HD in the close to onset group (0–10.8 years). All other structures were affected to some degree in the manifest group, although brainstem, thalamus and amygdala were relatively spared. The accumbens nucleus, putamen, pallidum and hippocampus had a strong significant correlation with functional and motor scores. We conclude that volume changes may be a sensitive and reliable measure for early disease detection and in this way serve as a biomarker for Huntington's disease. Besides the caudate nucleus and putamen, the pallidum and the accumbens nucleus show great potential in this respect.

Introduction

Huntington's disease (HD) is an autosomal dominantly inherited, slowly progressive, neurodegenerative disease localised on chromosome four. The pathophysiological mechanism is complex and although impaired energy metabolism and neuronal excitotoxicity are named as major contributors[1;2], it is still not fully understood, but the main result is neuronal dysfunction and loss in the brain. The disease is characterised by clinical symptoms in the motor, cognitive, and psychiatric domains.

Loss of brain cells and their connections, referred to as atrophy, can be visualised with Magnetic Resonance Imaging (MRI) techniques and has been the focus of many studies in search of objective biomarkers for tracking disease progression. The striatum has been the primary region of interest, due to previous findings in pathological studies that demonstrated the most severe loss of neurons in this region[3]. In manifest HD, the evidence for widespread atrophy is extensive, whereby several MRI studies showed striatal[4-12] and cortical atrophy[13;14]. The striatal atrophy occurs in the premanifest stages of HD, with atrophy of the caudate nucleus and putamen present up to a decade or more before clinical manifestation[15-20].

Other subcortical grey matter structures have been examined to a lesser degree. Thalamic[4;5;9;21] and pallidum[9;10;22;23] atrophy is apparent in the manifest stages of HD; however, findings are controversial in the premanifest stage, as not all studies are in agreement[16-19;22;24]. Atrophy of the hippocampus, amygdala, accumbens nucleus, and brainstem has only been reported in the manifest stage in a very limited number of studies[9;22]. Whole brain atrophy has been demonstrated in HD[25;26]. However, the rate of volume reduction of the subcortical structures as compared to whole brain atrophy has not been reported in any of the above mentioned studies and is of importance as this regional atrophy may very well only be a reflection of whole brain atrophy. Correlation studies have been performed for volume reductions and global clinical measures in manifest HD[8;9;22]. However, reports on the relationship of atrophy with clinical measures of all these structures in different disease stages in one large cohort has not been previously reported.

With the possibilities of new therapies for HD, there has been increasing interest in the development of robust, reproducible biomarkers to assess the efficacy of potential treatments. The TRACK-HD study is an international multi-centre study investigating candidate biomarkers for HD and encompasses a large, well defined, sample of HD gene carriers[26]. Application of a fully automated MRI-analysis tool to this study sample provides the opportunity to study brain atrophy in detail within a very large HD cohort. The aim of this study is to provide a more precise overview of the specific atrophy of the subcortical

grey matter structures in different disease stages, whilst taking into account whole brain atrophy and provide better targets for biomarker research. Secondly, we aim to examine the relationship of all subcortical grey matter structure volumes to clinical measures for HD.

Methods

Subjects

The TRACK-HD study recruited 366 participants from four centres (the National Hospital for Neurology and Neurosurgery in London, the Department of Medical Genetics at University of British Columbia in Vancouver, the Department of Genetics and Cytogenetics at the Hôpital de la Salpetrière-Université Pierre and Marie Curie in Paris and the Department of Neurology at Leiden University Medical Center in Leiden) totalling 123 early manifest HD, 120 premanifest HD and 123 control subjects. Inclusion criteria for the early manifest HD group consisted of genetic confirmation of an expanded CAG repeat of ≥ 40, presence of motor disturbances on the Unified Huntington's Disease Rating Scale (UHDRS), defined as a diagnostic motor confidence score of 4 and a total motor score (TMS) of > 5, and a Total Functional Capacity (TFC) of ≥ 7. Inclusion criteria for the premanifest group consisted of genetic confirmation of an expanded CAG repeat of ≥ 40, absence of motor disturbances on the UHDRS, defined as a TMS of ≤ 5. Furthermore, premanifest gene carriers required a burden of pathology score > 250, based on CAG length and age[27], to ensure a premanifest HD sample close to onset. Spouses or partners of premanifest and affected subjects or gene-negative siblings were recruited as healthy control subjects. The control group was age-and gender-matched to the combined premanifest and manifest HD group. Further subdivision of the premanifest group was performed on the basis of predicted years to diagnosis into preHD-A (10.8 or more years to disease onset) and preHD-B (closer than 10.8 years to disease onset) based on Langbehn's *et al.* (2004) survival analysis formula[28;29]. The early manifest HD subjects were also divided into two groups based on disease stage by means of TFC score, the HD1 group (TFC score: 11-13) and the HD2 group (TFC score: 7-10)[30]. The study was approved by the local ethical committees and written informed consent was obtained from each subject. For full details on study design see Tabrizi *et al.* (2009)[26].

Clinical measures

From the total TRACK-HD assessment battery, the UHDRS motor scale and TFC were used for this study. The UHDRS TMS is a representation of the severity of motor disturbances, scores potentially range from 0-124, with higher scores indicating more severe motor abnormalities. The TFC is a scale used for assessment of global impairments within daily activities, ranging from 0-13, with lower scores indicating impaired global functioning.

MRI acquisition

All participants underwent 3Tesla MRI scanning. T_1-weighted image volumes were acquired using a 3D MPRAGE acquisition sequence on a 3.0T Siemens or a 3.0T Phillips whole body scanner with the following imaging parameters: TR = 2200ms (Siemens) / 7.7ms (Philips), TE=2.2ms (Siemens) / 3.5ms (Philips), FA=10° (Siemens) / 8° (Philips), FOV= 28cm (Siemens) / 24cm (Philips), matrix size 256x256 (Siemens) / 224x224 (Philips), 208 (Siemens) / 164 (Philips), sagittal slices to cover the entire brain with a slice thickness of 1.0 mm with no gap between slices.

Post-processing

All T_1-weighed scans were analysed using software provided by FMRIB's Software Library (FSL)[31]. Eight subcortical regions were assessed using FMRIB's integrated registration and segmentation tool (FIRST)[32-34], namely the accumbens nucleus, amygdala, brainstem, caudate nucleus, hippocampus, pallidum, putamen, and the thalamus. Total brain tissue volume was estimated with SIENAX[35;35;36;36]. For details on MRI post-processing please see the Supplementary Material. The whole brain volume was used to calculate ratios of atrophy of the subcortical structures to whole brain atrophy. The obtained ratio was divided by the control group value in order to obtain an easily interpretable ratio whereby 1.00 is the control (normal) value ratio. The following formula was used:
[volume structure / (whole brain volume - volume structure)] / control group value
A value below 1.00 demonstrates that the structure displays more atrophy than the overall whole brain atrophy. A value greater than 1.00 demonstrates that the overall whole brain atrophy is greater than the atrophy of the structure at hand.

Statistics

Analysis of variance for group comparison was performed for volumes and ratios of the eight different subcortical structures, whilst correcting for intracranial volume, study site, age and gender. (Note that each site used a different MRI Scanner model, and thus potential scanner effects can not be differentiated from site effects.) Comparisons of each structure were made for each disease group as compared to the control group, and as compared to the previous disease stage group, e.g. the preHD-B group was, besides comparison to the control group, also compared to the preHD-A group. We calculated partial correlations between the various subcortical structures and total motor score and total functional capacity from the UHDRS battery. These correlations used volumes corrected for intracranial volume and were further controlled for age, site, and gender by "partialing" these covariates out during the correlation calculations. The UHDRS TMS and TFC analyses were performed on the basis of a two-way group division, namely the premanifest gene carriers and the manifest HD group in order to keep sufficient power to perform this analysis. The significance level was set at p value < 0.05.

Results

Characteristics

The clinical characteristics of the five study groups are shown (Table 1). The progressive nature of HD inherently leads to a higher age of the manifest HD groups. Except for the HD2 group, which displayed lower levels of education, there were no significant differences in education level between the disease groups and the controls. For full details on group characteristics we refer to the recent baseline TRACK-HD paper[26].

Table 1: Group characteristics

	Control	PreHD-A	PreHD-B	HD1	HD2
N	123	62	58	75	46
Leiden	30	16	14	76	14
London	30	14	16	19	11
Paris	30	14	16	25	4
Vancouver	33	18	12	15	17
Age (SD) (range; years)	46.1 (10.2) (23.0–65.7)	41.1 (8.6) (18.6–59.4)	40.6 (9.2) (22.3–64.1)	46.9 (10.2) (22.8–64.1)	51.4 (8.6) (33.3–63.3)
Women (%)	68 (55%)	33 (53%)	33 (57%)	45 (60%)	21 (46%)
Education (SD) levels 1-6	4.0 (1.3)	4.1 (1.1)	3.8 (1.3)	3.8 (1.3)	3.2 (1.4)
Disease-burden score (SD)	n.a.	259.1 (30.1)	333.1 (30.0)	364.1 (75.0)	397.6 (67.5)

Descriptive statistics of the five different groups. Data are mean (SD, range) or number (%).

Volumes

Examples of a typical output from the segmentation by the FIRST and SIENAX tools are depicted (Figure 1), for the FIRST examples only the left hemisphere segmentations are shown, however both sides were segmented for the analysis.

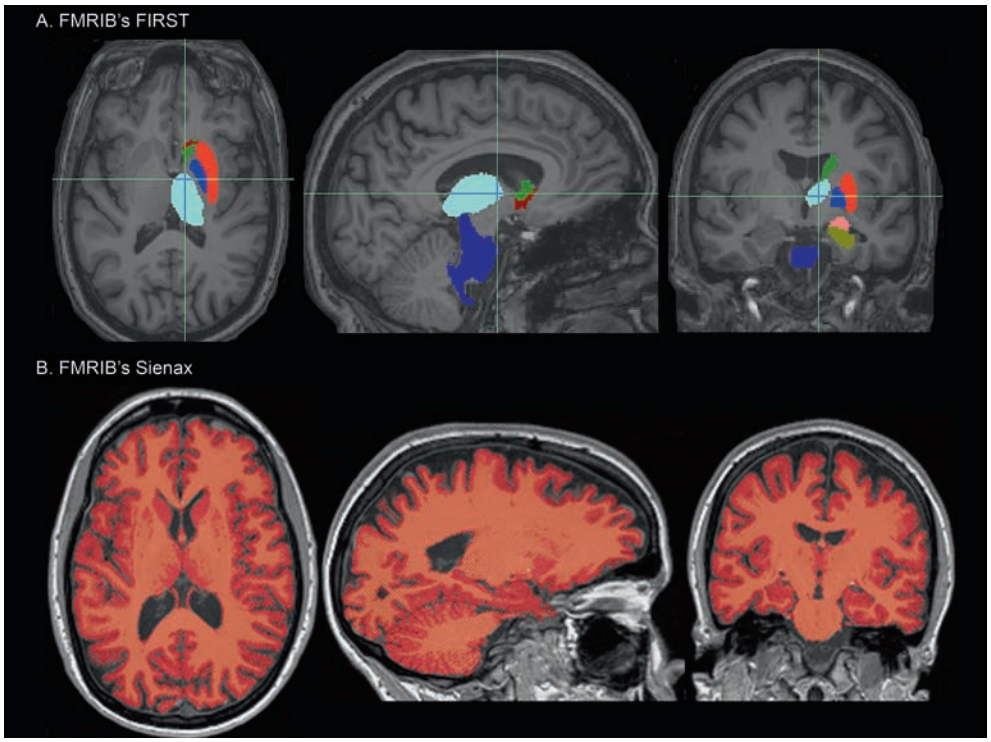

Figure 1: FSL-FIRST and SIENAX. A. Example segementations by FSL FIRST. Dark blue = brainstem, blue = pallidum, light blue = thalamus, green = caudate nucleus, dark red = accumbens nucleus, red = putamen, copper = hippocampus, pink = amygdala. B. Example output from the FSL SIENAX tool. Red depicts the final sienax segmentation results.

Volume comparisons for eight subcortical structures in five groups are shown (Table 2). Furthermore, figure 2 shows the amount of volume reduction in terms of percentage of the control value. The putamen and the caudate nucleus show a similar pattern of a smaller volume in the preHD-A group and in every subsequent disease stage group with a continuous decline in volume. The accumbens nucleus and pallidum show a decreased volume in the preHD-B group and from every following disease stage group as compared to the controls as well as to the previous disease stage group except for the accumbens nucleus between HD1 and HD2.

Table 2: Volumetric analysis of eight structures

	Control		PreHD-A		PreHD-B		HD1		HD2	
	Mean volume	SD	Mean Volume	SD	Mean volume	SD	Mean Volume	SD	Mean volume	SD
Accumbens	1.202	0.251	1.170	0.258	1.038**††	0.245	0.873**††	0.239	0.752**	0.239
Amygdala	3.345	0.825	3.331	0.939	3.346	0.869	3.246	0.757	2.914	0.851
Brainstem	24.043	2.672	23.828	3.001	23.011*	2.947	22.204*	2.592	21.098**††	3.083
Caudate	6.915	0.989	6.325**	1.067	5.722**††	0.923	5.057**††	0.756	4.830**	0.816
Hippocampus	8.417	1.271	8.391	1.319	8.115*†	1.290	7.668**	1.233	6.617**††	1.351
Pallidum	3.593	0.505	3.526	0.562	3.099**††	0.510	2.718**††	0.615	2.238**††	0.679
Putamen	10.277	1.383	9.610**	1.442	8.644**††	1.220	7.548**††	0.987	6.935**††	1.203
Thalamus	16.112	1.707	16.152	1.718	15.620**	1.656	14.525**†	1.524	13.681**	1.448

Means are the observed unadjusted measured values (mm^3). Significance is after correction for ICV, site, gender and age.

* significant difference from controls $p < 0.05$

* * significant difference from controls $p < 0.001$

† significant difference from previous group $p < 0.05$

†† significant difference from previous group $p < 0.01$

The brainstem and thalamus show signs of reduced volume in preHD-B, HD1 and HD2 as compared to the control group.

The amygdala showed no reduced volume as compared to the control group. The hippocampus showed volume reduction in stages preHD-B, HD1 and HD2 as compared to controls. In terms of percentages of volume reduction as compared to the control group value it is apparent that the pallidum and accumbens nucleus show marked atrophy with only 62% and 60% of the normal volume remaining in the HD2 group, respectively. These structures are closely followed by the putamen and the caudate nucleus with 67% and 70% remaining volume, respectively. Percentages for all structures in the four disease stages are also available in the supplementary material.

Ratios

Comparisons for volume ratio of eight structures as compared to whole brain atrophy are shown (Table 3). A smaller ratio indicates that the volume of this specific structure declines faster than the overall brain atrophy. The putamen atrophy ratio showed a continuous smaller value across subsequent disease stage groups. The caudate nucleus atrophy ratio showed a similar pattern, except within the manifest stage this ratio did not show a significant difference across HD1 and HD2. The accumbens nucleus and the pallidum atrophy ratio showed a decrease over disease stages from the preHD-B group onwards.

The ratio of whole brain atrophy to amygdala atrophy is comparable or slightly higher to the control group ratio. The atrophy ratio for the brainstem and thalamus shows no or slightly higher ratios as compared to the control group, stating that atrophy of these structures is comparable or slower to whole brain atrophy. Finally, the hippocampus atrophy ratio shows a drop in value in HD2 as compared to control and HD1.

Clinical measures

The UHDRS total motor score in the premanifest stage is most strongly correlated to the atrophy of the caudate nucleus (R=-0.272, p=0.003) and putamen (R=-0.240, p=0.009), but also to the atrophy of accumbens nucleus, pallidum and thalamus. The TFC demonstrates a strong ceiling effect in the premanifest stage and no significant correlations are to be found. In the manifest stage of the disease the UHDRS total motor score is most strongly related to the putamen (R=-0.530, p <0.0001), pallidum (R=-0.390, p <0.0001) and accumbens nucleus (R<-0.380, p<0.0001), but also to the hippocampus, thalamus, and brainstem.

Table 3: Ratio analysis of volumes for eight structures

	PreHD-A		PreHD-B		HD1		HD2	
	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD
Accumbens Nucleus	1.00	0.18	0.91**†	0.18	0.82**†	0.18	0.73**†	0.18
Amygdala	0.97	0.26	1.03	0.23	1.06*	0.23	1.00*	0.29
Brainstem	0.99	0.07	0.99	0.08	1.04*†	0.10	1.00†	0.11
Caudate Nucleus	0.92**	0.13	0.87**†	0.11	0.83**†	0.13	0.81**	0.14
Hippocampus	1.00	0.13	1.01	0.13	1.03	0.16	0.91**††	0.18
Pallidum	0.97	0.12	0.91**††	0.12	0.85**†	0.21	0.70**††	0.21
Putamen	0.93**	0.11	0.87**††	0.08	0.82**††	0.08	0.77**†	0.09
Thalamus	1.00	0.73	1.01	0.73	1.01	0.86	0.97	0.08

Ratios are the observed unadjusted measured values. Significance is after correction for ICV, site, gender and age

* = $p<0.05$ from control group

** = $p<0.001$ from control group

† = $p<0.05$ from previous group

†† = $p<0.001$ from previous group

Table 4: Volume Correlations with Motor Score and Functional Capacity

	UDHRS Total Motor Score				Total Functional Capacity			
	PreHD		HD		PreHD		HD	
	Partial R	*p*	Partial R	*p*	Partial R	*p*	Partial R	*p*
Accumbens Nucleus	-0.24	0.01	-0.38	<0.0001	-0.025	0.79	0.21	0.03
Amygdala	-0.121	0.21	-0.11	0.23	-0.14	0.13	0.10	0.29
Brainstem	-0.12	0.23	-0.22	0.02	0.00	>0.99	0.20	0.03
Caudate Nucleus	-0.272	0.003	-0.14	0.12	-0.15	0.10	0.02	0.82
Hippocampus	0.03	0.78	-0.31	0.0008	0.03	0.73	0.24	0.01
Pallidum	-0.20	0.03	-0.39	<0.0001	0.03	0.75	0.23	0.01
Putamen	-0.24	0.009	-0.53	<0.0001	0.00	0.99	0.27	0.004
Thalamus	-0.23	0.015	-0.29	0.002	0.05	0.59	0.13	0.17

Statistic is partial correlation of volumes corrected for intracranial volume with statistical (partial) correction for site, gender and age.
UHDRS = Unified Hunitington's Disease Rating Scale, preHD = premanifest Huntington's disease, HD = manifest Huntington's disease.

The TFC is most strongly correlated to atrophy of the putamen (R=0.270, *p*=0.004), hippocampus (R=0.240, *p*=0.01) and pallidum (R=0.230, *p*=0.01) and a weak correlation to accumbens nucleus and brainstem.

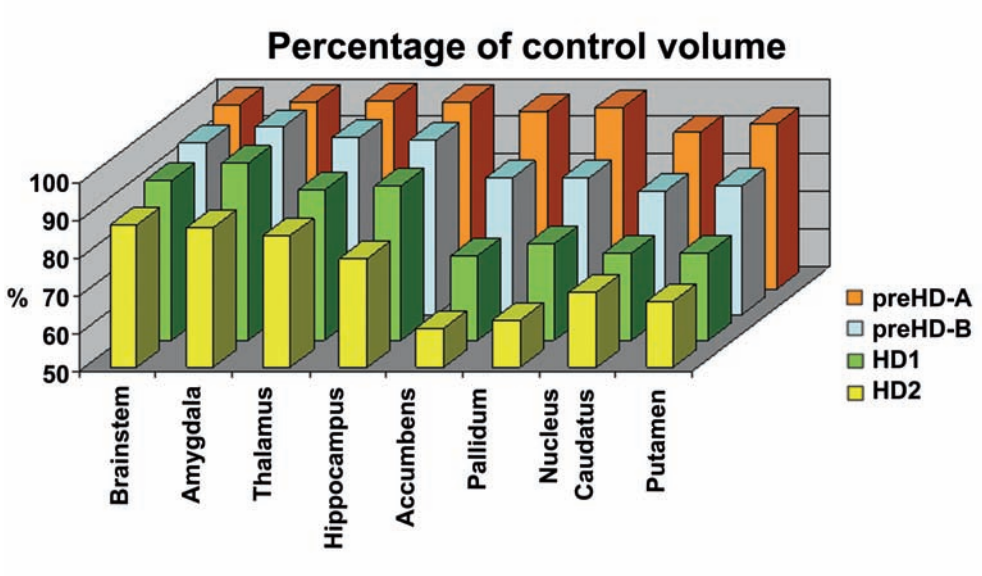

Figure 2: Percentage of volume for eight structures as compared to the control value. PreHD-A = premanifest gene carriers far from expected disease onset, preHD-B = premanifest gene carriers close to disease onset, HD1 = manifest HD stage 1, HD2 = manifest HD stage 2.

Discussion

The aim of this study was to provide a comprehensive overview of the atrophy of subcortical structures, with respect to disease stage while taking into account whole brain atrophy. The main findings show that all subcortical grey matter structures at some point have a reduced absolute volume. However, the accumbens nucleus, the caudate nucleus, the pallidum and the putamen display a true progressive decline in volume reduction which is disproportionate to overall whole brain atrophy and this is already visible in the premanifest stages of HD. Conversely, the amygdala, brainstem and thalamus do show reduced volumes, but this merely reflects the overall whole brain atrophy rate. The hippocampus is exceptional in the fact that only in the manifest stage a true rapid decline in this structure is observed. Furthermore, the volume reductions of the accumbens nucleus, putamen, pallidum and hippocampus have a severe impact on clinical measures in manifest HD.

The atrophy of the caudate nucleus and putamen in the premanifest stages confirms earlier studies. The fact that this happens a decade or more before estimated time to onset is in accordance with the findings of Aylward *et al.* (1996)[37], the findings of the PREDICT-HD study[20;20] and with the results reported in TRACK-HD baseline paper[26].

Atrophy of the pallidum and thalamus in the premanifest stages of HD is controversial. Campodonico *et al.* (1998) and Harris *et al.* (1999) report no significant reduction in volume of the pallidum[18;24], while others do report this finding[16;17;19]. With regard to the thalamus in the premanifest stage, reports of reduced volume are available in favor[16;24] and in dispute[19;38] of thalamic involvement. Our findings, using 3T MRI and in the largest cohort reported thus far, confirms that atrophy in the pallidum is apparent in the premanifest stage closer to predicted disease onset, but not in the further from predicted onset stage. Although thalamic atrophy is also apparent in the premanifest close to onset stage, this merely reflects the whole brain atrophy.

The brainstem, thalamus, and amygdala follow the normal atrophy rate of the whole brain in the manifest stages of the disease. In fact, in HD stage 1 the brainstem and the amygdala show signs of being relatively spared. The hippocampus shows a different pattern of atrophy as it starts to show signs of atrophy in preHD-B stage, but really becomes significant in terms of volume loss in HD stage 2. To some degree the above described findings have been reported by Rosas *et al.* (2003) and Douaud *et al.* (2006) [9;22]. The added value of our study lies not only in the large sample size facilitating the further specification of the premanifest and manifest groups, but particularly in the fact that the atrophy described takes into account whole brain atrophy. It should be noted that some caution is appropriate when interpreting the ratio to whole brain atrophy, as this method does not necessarily imply that the volume reduction of a certain structure has to be greater than the whole brain atrophy to be of significance in terms of clinical symptoms.

The clinical impact of atrophy of subcortical grey matter is difficult to assess as many brain structures are involved in complex functionality. We have demonstrated that of the subcortical grey matter structures, specifically volume reductions of the accumbens nucleus, putamen and pallidum have the greatest influence on predicting the motor disturbances in manifest HD, and perhaps surprisingly, the impact of the caudate nucleus is minimal. The role of the pallidum in motor function is underestimated and surpasses the caudate nucleus and the thalamus. However, the putamen is the most important structure for this clinical symptom. Our study shows that decreased global functioning of manifest HD was mainly related to volume loss of the hippocampus, putamen and pallidum. Cognitive and motor deficiencies have been shown in a number of separate studies, all examining only part of the deep grey matter structures, to relate to volume reductions of the basal ganglia, thalamus and frontal lobe[4;6;11;18;19;24;39;40]. Within one small cohort

examination of clinical measures with the TFC was carried out by Rosas *et al.* (2003) who provided evidence for an association between each separate structure and the TFC in the manifest stage[22], which, apart from some differences between studies, is largely confirmed by our study. All these studies show that there is an association between volumes and clinical measures; however, we hope by examining all structures within one large, well defined cohort we can provide evidence for which structure has the most contribution (or at least the strongest correlation) to global functioning and motor disturbances.

TRACK-HD is a longitudinal observational biomarker study aimed at providing essential methodology for the assessment of therapeutic interventions. In respect to this goal we can add that the accumbens nucleus and pallidum show similar biomarker potential in addition to the well recognised structures putamen and caudate nucleus. The hippocampus is also of importance as it has a high correlation with clinical symptoms. The important role of the caudate nucleus in the premanifest stage of the disease seems to diminish after manifestation, as was also suggested by the TRACK-HD baseline paper[26]. It is possibly fair to say that at different disease stages different structures play a role. A biomarker would ideally show sensitivity across all disease stages and would reflect the underlying pathologic processes. From this cross-sectional analysis it is not possible to draw any definitive conclusions, however, the putamen possibly shows the greatest potential. Longitudinal evaluation has to be performed to confirm the putamen's true potential. Furthermore we would like to stress how taking whole brain atrophy into account aids our interpretation of regional atrophy rates and in this way their potential as biomarkers can be assessed more effectively.

A limitation of our study could be the use of a relatively new software package from FSL used for segmentation, which hasn't specifically been validated in HD. Visual inspection, however, did not reveal any significant mismatches. The method applied gives structure segmentation on an individual basis and can therefore be used to compare groups. In contrast to this limitation several clear reasons exist in favor of using FIRST; first of all, compared to manual segmentation the automated segmentation uses voxel intensity in contrast to the sometimes difficult visual contrast differences, reducing a rater dependent bias. Secondly this automated technique is suitable for implantation on large datasets, whereas manual segmentation is labour intensive and prone to human error.
In conclusion, we have demonstrated that atrophy of the pallidum and the accumbens nucleus exists in the premanifest stages of the disease and confirmed the well known atrophy of the caudate nucleus and putamen. Furthermore, we have shown the important correlation of the accumbens nucleus, putamen, pallidum and hippocampus with clinical symptoms. The importance of the remaining subcortical structures should be regarded when taking into account that they show similar amounts of atrophy as the brain as a whole. These findings have implications for biomarker research, as several subcortical structures now show great potential for use as a disease progression measure.

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Supplementary Material

Table 1: Percentage of the volume as compared to the control group value for eight separate structures.

	PreHD-A	PreHD-B	HD1	HD2
Accumbens Nucleus	97.4 %	86.4 %	72.6 %	60.3 %
Amygdala	99.6 %	100.0 %	97.0 %	87.1 %
Brainstem	99.1 %	95.7 %	92.4 %	87.7 %
Caudate Nucleus	91.5 %	82.8 %	73.1 %	69.8 %
Hippocampus	99.7 %	96.4 %	91.1 %	78.6 %
Pallidum	98.1 %	86.2 %	75.6 %	62.3 %
Putamen	93.5 %	84.1 %	73.4 %	67.5 %
Thalamus	100.3 %	97.0 %	90.2 %	84.9 %

Values represent percentage of the volume as compared to the control group value for eight separate subcortical structures. PreHD-A = premanifest gene carriers far from expected disease onset, preHD-B = premanifest gene carriers close to disease onset, HD1 = manifest HD stage 1, HD2 = manifest HD stage 2.

Methods: MRI Postprocessing

All T_1-weighed scans were analysed using software provided by FMRIB's Software Library (FSL)[1]. Eight subcortical regions were assessed using FMRIB's Integrated Registration and Segmentation Tool (FIRST)[2-4], namely the accumbens nucleus, amygdala, brainstem, caudate nucleus, hippocampus, pallidum, putamen, and the thalamus. Using FIRST, the T_1-weighted images were first registered to MNI (Montreal Neurological Institute) 152 standard space, using linear registration with 12 degrees of freedom[5-6]. FIRST draws upon a vast library of manually segmented images, which were transformed into deformable surface meshes. These meshes were applied to our dataset to be used as subcortical masks to locate the specific structures. Subsequently, segmentation was carried out using the voxel intensities and mesh models. Finally a boundary correction was applied to prevent overlap with adjacent structures. After registration and segmentation the absolute volume was calculated having taken into account the obtained information from previous steps. A visual inspection of all registrations was performed and of the final segmentations 20% were selected at random and visually inspected for accuracy.Total brain tissue volume was estimated with SIENAX[7-8].This program extracts brain and skull images from the single whole-head input data[9].The brain images were then affine-registered to MNI152 space, using the skull images to determine the registration scaling. Next, tissue-type segmentation with partial volume estimation was carried out[10].

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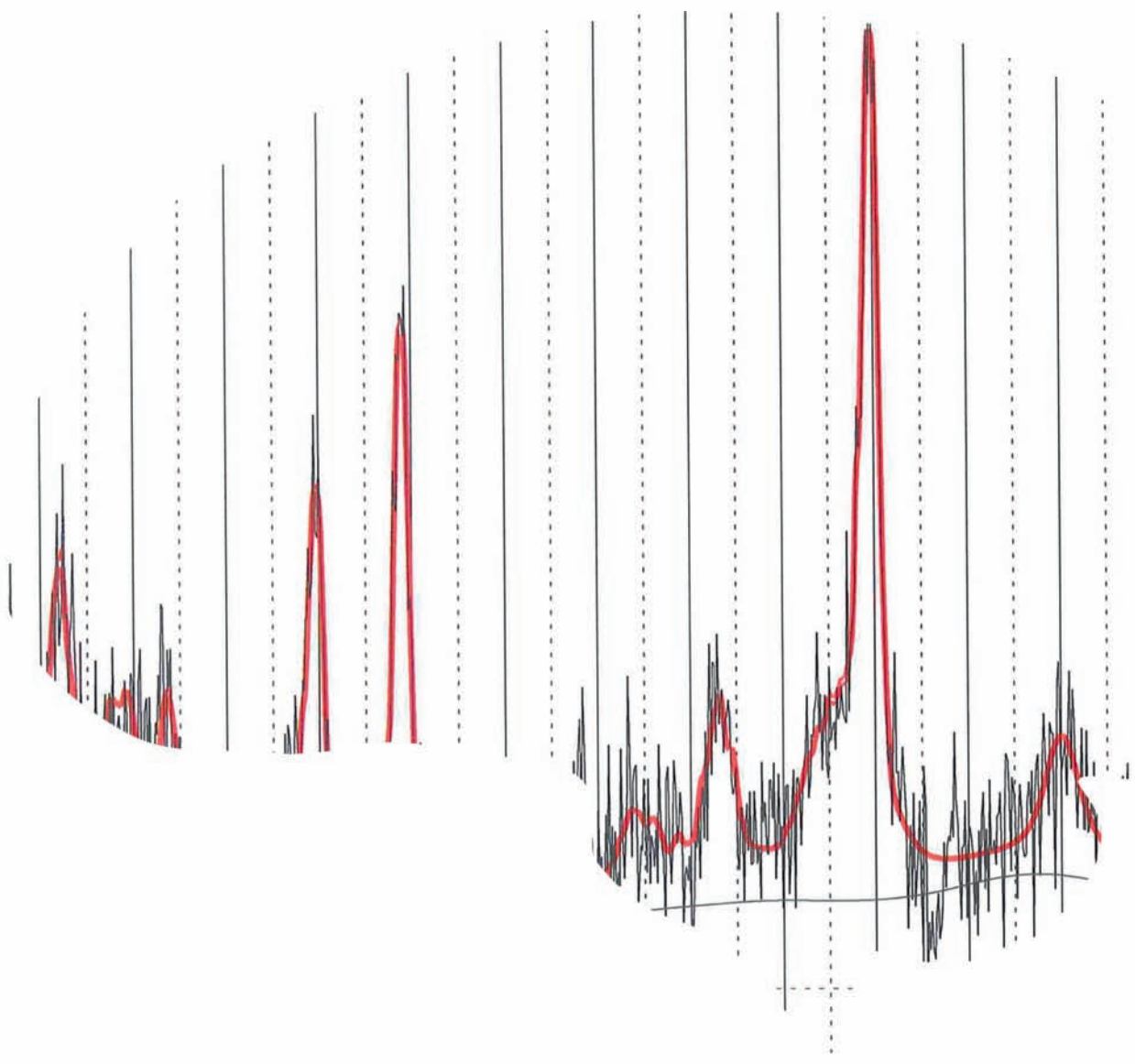

Chapter 5

Exploratory 7-Tesla Magnetic Resonance Spectroscopy in Huntington's disease provides in vivo evidence for impaired energy metabolism

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Abstract

Background

Huntington's Disease (HD) is a genetic disorder affecting the brain. Atrophy of deep grey matter structures has been reported and it is likely that underlying pathologic processes occur before or in concurrence with volumetric changes. Measurement of metabolite concentrations in these brain structures has the potential to provide insight into pathological processes. We aim to gain understanding of metabolite changes with respect to the disease stage and pathophysiological changes.

Methods

We studied five brain regions using magnetic resonance spectroscopy (MRS) using a 7-Tesla MRI scanner. Localised proton spectra were acquired to obtain five metabolite concentrations. MRS was performed in the caudate nucleus, putamen, thalamus, hypothalamus and frontal lobe in 44 control subjects, premanifest gene carriers and manifest HD.

Results

In the caudate nucleus HD patients display lower NAA (p=0.009) and lower creatine concentration (p=0.001) as compared to controls. In the putamen, manifest HD patients show lower NAA (p=0.024), lower creatine concentration (p=0.027) and lower glutamate (p=0.013). Although absolute values of NAA, creatine and glutamate were lower, no significant differences to controls were found in the premanifest gene carriers.

Conclusion

The lower concentrations of NAA and creatine in the caudate nucleus and putamen of early manifest HD suggest deficits in neuronal integrity and energy metabolism. The changes in glutamate could support the excitotoxicity theory. These findings not only give insight in neuropathological changes in HD, but also indicate that MRS can possibly be applied in future clinical trials to evaluate medication targeted at specific metabolic processes.

Introduction

Huntington's Disease (HD) is a neurodegenerative autosomal dominant disorder. The causative gene mutation is located on the short arm of chromosome 4 and consists of an expanded Cytosine-Adenine-Guanine (CAG) repeat within the *Htt*-gene. This expansion results in the synthesis of an abnormal huntingtin protein that causes neuronal damage, brain atrophy, ultimately leading to functional disturbances of motor, cognition and behavior.

HD research has revealed widespread changes throughout the brain[1]. Controversy remains as to which structures are affected at different disease stages. Atrophy of the striatum is regarded as the hallmark of the pathologic findings in HD[2]. MRI studies demonstrate that the caudate nucleus and putamen begin to show atrophy up to a decade before clinical manifestations occur[3]. Structures such as the thalamus, hypothalamus, the frontal lobe, white matter and cortical grey matter have all been implicated to some degree at this pre-manifest stage, although findings differ[1;4-6].

The pathophysiological mechanism leading to neuronal damage remains unclear. Currently the two most accepted hypotheses describe impaired energy metabolism and the excitotoxicity of neurons[7;8]. Both hypotheses can potentially be explored by means of non-invasive in vivo measurements of metabolites such as creatine and glutamate, using localised magnetic resonance spectroscopy (MRS). However, previous studies measuring the changes in the metabolite levels related to the damaging processes in HD have reported conflicting results. In one study a lower level of N-acetylaspartate (NAA) was confirmed post-mortem in the putamen and cortex in manifest HD[9]. In vivo altered levels of NAA, creatine, choline and glutamate have been reported in both premanifest and manifest HD in several brain structures, such as the striatum and thalamus using MRS[10-16]. However, in contrast, other studies using localised MRS did not detect changes in these metabolite levels in either manifest or premanifest HD[17-19].

One of the factors that might explain discrepancies between previous studies is the relatively poor spatial and/or spectral resolution of localised spectra. Improvement in MRS methodology in terms of spectral resolution in combination with small voxel size and total scanning time can be achieved using a high field MRI scanner[20;21]. This allows metabolite quantification in small, well defined anatomical structures, such as the caudate nucleus and putamen.

The major aims of the present study were to assess metabolite differences between manifest HD patients or premanifest HD gene carriers and controls, and to assess the association between these metabolite differences and clinical measures of disease severity in order to obtain a greater understanding of the pathophysiological changes in HD with

respect to the disease stage. The hypotheses were as follows; as the neuronal integrity and the energy metabolism would be compromised, we expected the NAA and creatine to be lower in premanifest and manifest HD, especially in the striatum. Possible changes in glutamate could be expected based on the excitotoxicity theory, with higher levels in both premanifest and manifest HD.

Materials and Methods

Subjects

Participants were recruited from the outpatient neurology clinic of the Leiden University Medical Center. Inclusion criteria for the early manifest HD consisted of genetic confirmation with a CAG repeat ≥ 39, and the presence of motor disturbances as measured by the Unified Huntington's Disease Rating Scale '99 (UHDRS) defined as a total motor score (TMS) > 5 and a Total Functional Capacity (TFC) ≥ 7. All participants received a diagnostic confidence rating of 4, representing "motor abnormalities that are unequivocal signs of HD (>99% confidence)". Inclusion criteria for the premanifest HD participants consisted of genetic confirmation with a CAG repeat ≥ 39, and the absence of motor disturbances on the UHDRS defined as a TMS ≤ 5. Healthy gene negative family members or partner/spouses were recruited as control subjects in an effort to reduce influences of environmental factors. Exclusion criteria for all participants consisted of significant (neurological) comorbidity, a major psychiatric diagnosis, history of severe head injury or incompatibility for MRI. In total 44 subjects were recruited to undergo MRS in one or more regions of interest (ROI). Time constraints and HD related issues (such as patient motion) accounted for not all participants completing the MRS protocol in all five of the ROI's. The study was approved by the local Medical Ethical Committee of the Leiden University Medical Center. All participants provided written informed consent.

Clinical measures

Clinical evaluation for all subjects consisted of the UHDRS motor scale (score 0-124) and TFC, a global scale of impairment in daily life activities, score 0 -13. Furthermore, a short cognitive battery was administered, consisting of the Mini Mental State Exam (MMSE), Stroop test word reading card (Stoop-II), the Symbol Digit Modality Test (SDMT), Trail making Test part B (TMT-B), the Wechsler Memory Scale (MQ) and premorbid IQ estimation using the Dutch Adult Reading Test (DART)[22]. Behavioural disturbances were evaluated with the Beck Depression Inventory 2nd version (BDI-II)[23] and the Problem Behaviour Assessment short version (PBA-s). Predicted years to onset were calculated from current age and CAG repeat length using the formula by Langbehn *et al.* (2004)[24].

MRI/MRS acquisition:

MRI and MRS were performed on a Philips 7-Tesla Achieva whole body scanner (Philips Healthcare, Best, The Netherlands) with a NOVA Medical quadrature transmit coil and

16 channel receive coil array. For accurate planning, a high resolution, three-dimensional T_1-weighted GRE scan was acquired (TR/TE = 11/5.4 ms, voxel size 0.44 x 0.44 x 0.84 mm, total scan time 1:46). Localised proton spectra were acquired using a stimulated echo acquisition mode (STEAM) sequence. The voxel was placed within the region of interest with the maximum volume containing only tissue from the intended structure, minimising the contribution from surrounding tissue, and also partial volume effects. Regions of interest consisted of the caudate nucleus, putamen, thalamus, hypothalamus and the prefrontal white and cortical grey matter. For the prefrontal voxel, positioning included approximately 50% white and 50% cortical grey matter. Only for the hypothalamus voxel was some cerebral spinal fluid (CSF) necessarily present since the voxel was placed bilaterally. For each voxel in each ROI a water reference signal was acquired for adequate quantification. The following scan parameters were used for the STEAM spectra: TR/TE/TM = 2000/19/25 ms, BW 4 kHz, 2048 complex data points and 128 signal averages, giving a data acquisition time of approximately 5 minutes per spectrum. The chemical shift artifact was minimal (<1 mm), which is shown in figure 1. Water suppression was performed using a frequency-selective RF pulse and gradient spoiling, six saturation bands were additionally applied to suppress signal from surrounding tissue. All scans included a reference scan without water suppression for quantification. When an MRS scan failed (for example due to motion) it was standard protocol to try the same ROI again: however, this did, in some cases, result in insufficient time for the full protocol to be completed since the time for each patient in the magnet was strictly limited to one hour.

MRS post-processing

MRS data were analysed with LCModel[25;26], using the unsuppressed water as an internal reference to calculate the concentrations of metabolites. Concentrations of choline, creatine, glutamine+glutamate (Glx), total NAA (N-acetylaspartateglutamate + NAA), myo-inositol and lactate were calculated for analysis.

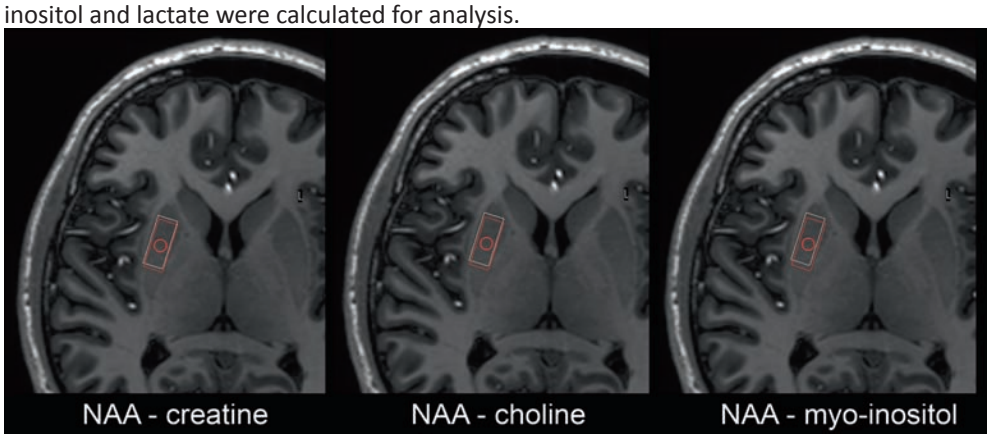

Figure 1. Example of chemical shifts at 7-Tesla using STEAM provided for an actual planning. Red box = NAA, white box = metabolite as stated below each figure. Only a minimal chemical shift exists. The chemical shift of water is almost zero, as the separate water file is planned according to the NAA-voxel. NAA= N-acetylaspartate

Stringent quality control was enforced to exclude data with high estimated errors. A signal to noise ratio of ≤ 3 and very high values of residual errors in the LCmodel fitting were applied as initial spectral exclusion criteria. Furthermore, the LCModel fit for metabolites were required to have a Cramér-Rao Lower Bound (CRLB) of 20% or less. This was required for all metabolites within a single spectrum, with the exception of lactate. For lactate CRLB above 100% were excluded from further analysis. If lactate was not quantified with CRLB <100% in at least 50% of analysed spectra, the analysis of lactate was deemed not detectable; this method was first presented by Tkac *et al.* (2009)[20]. In total 30 spectra were included in the analysis for the hypothalamus , 36 of the thalamus, 28 of the caudate nucleus, 27 of the putamen and 32 subjects of the prefrontal region.

Statistics

Statistical analyses were performed using SPSS for Windows (SPSS version 17.0, SPSS inc, Chicago, IL). All clinical variables were assessed between groups with a one-way analysis of variance (ANOVA), with post-hoc testing. To compare all metabolites in the five brain regions between the manifest and premanifest HD group versus the control group, an ANOVA with planned comparisons was performed. The nature of this study is exploratory, and therefore in order to prevent inflation of type II errors and avoid important results going unreported, no correction for multiple testing was performed, as is accepted practice in such types of study[27]. To examine the relationship between metabolic concentrations and clinical measures in gene carriers (manifest and premanifest HD), partial Pearson correlation analysis corrected for age was performed.

Results

Demographics

No differences in age, education level or IQ were present between the manifest HD and control group, or between the premanifest HD and control group. No significant difference exists in CAG repeat length between the premanifest and the manifest HD group. For all other clinical assessments variables we refer to the results in table 1.

Voxel planning

Typical examples of localised proton spectra for the five examined regions, along with metabolite identification, are shown in figure 2. The voxel location is shown on the left side, for clarity the voxel is only shown in one orientation. Actual planning of the voxel required oblique planning in all three dimensions. Figure 3 provides a typical example spectrum and fit from the LCmodel.

Voxel sizes were individually set, based upon the different sizes of structures in different individuals, although the voxel size was never below 1.0 ml. With these sizes it was

possible in all cases to include only the intended (grey matter) structure, without contamination of other (white matter) structures, the exception being the prefrontal region. The voxel size ranged from 1.0 ml in the caudate nucleus for a manifest HD participant to a maximum of 3.4 ml for the putamen of a participant in the control group.

Metabolite analysis

The concentrations of the six metabolites (NAA, creatine, choline, glutamate, lactate and myo-inositol) in the five brain regions are shown in table 2 for the three groups. In the caudate nucleus the manifest HD group demonstrated significantly lower NAA ($F = 8.12$, $p = 0.009$) and lower creatine concentration ($F = 13.60$, $p = 0.001$) compared to control subjects. In the putamen, manifest HD showed significantly lower NAA ($F = 5.81$, $p = 0.024$), lower creatine concentration ($F = 5.58$, $p = 0.027$) and glutamate/glutamine ($F = 7.21$, $p = 0.013$) compared to controls.

Table 1: Demographic variables and scores on clinical assessments for the three groups.

	Control n: 18 (m:9 f:9)		*Premanifest* n: 14 (m:6 f:8)		*Manifest* n:12 (m:5 f:7)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age	47.7	7.4	42.9	11.0	48.6	7.0
Years of education	15.9	3.2	14.3	3.3	15.3	2.7
IQ	106.7	7.8	102.2	12.5	102.3	9.5
CAG	20.3 [c]	2.9	43.1	3.4	43.7	2.3
Disease burden	n.a.	n.a.	306.4	87.0	385.4 [e]	72.5
Predicted YTO	n.a.	n.a.	10.4	8.2	n.a.	n.a.
UHDRS TMS	2.0	2.4	2.6	1.3	20.9 [a]	14.1
UHDRS TFC	12.9	0.2	12.6	0.6	11.3 [a]	1.9
PBA-s	5.9	1.4	5.0	1.3	13.2 [a]	3.8
BDI-II	3.9	4.6	5.2	5.5	6.4	6.1
MMSE	29.5	0.7	28.5	1.7	28.3 [b]	1.6
SDMT	59.2	8.9	48.5	10.8	37.3 [d]	6.7
TMT-B	46.7	15.1	67.8	27.8	101.4 [a]	51.2
Stroop II	104.0	15.4	95.1	19.4	79.3 [b]	19.9
MQ	127.3	11.5	115.5	23.2	99.7 [b]	13.2

SD = Standard Deviation. CAG = CAG repeat length of larger allele. Predicted YTO = predicted years to onset. UHDRS TMS = Unified Huntington's Disease Rating Scale Total Motor Score, PBA-s = Problem Behaviour Assessment short version, TFC = Total Functional Capacity, BDI-II = Beck Depression Inventory 2nd version, MMSE = Mini Mental State Exam, TMT = Trail Making Test part B, SDMT = Symbol Digit Modality Test, MQ = Memory Quotient, Stroop II = Stroop word reading card, n.a.=not applicable. a = significant differences between early HD compared to both premanifest and controls. b = significant differences between early HD and controls. c=significant differences between control and both premanifest and manifest HD. d=significant differences between all three groups. e=significant difference between premanifest and manifest HD

No significant differences were found in the hypothalamus, thalamus or prefrontal region. Comparison of the premanifest gene carrier group and the control group did not show any significant differences in any of the regions. However, the absolute values of NAA, creatine and glutamate were lower in the caudate nucleus and putamen in the premanifest gene carrier group, but did not reach significance.

Table 2: Metabolite concentrations from five brain regions in HD

	Control			Premanifest			Manifest		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
Hypothalamus	11			10			9		
Creatine		10.7	2.1		9.4	2.0		9.3	1.7
Choline		3.7	0.7		3.4	0.6		3.3	0.8
NAA		12.1	1.9		11.9	1.5		11.3	2.1
Glx		12.5	3.0		12.0	4.0		11.0	4.8
Myo-inositol		13.4	4.8		12.7	3.1		13.3	2.8
Lactate		1.5	1.0		1.1	1.1		1.0	1.4
Thalamus	14			13			9		
Creatine		12.7	3.5		12.8	3.6		12.1	1.6
Choline		3.1	0.8		3.0	0.7		3.1	0.4
NAA		17.8	6.0		15.8	2.6		15.9	1.6
Glu/Gln		14.8	5.1		15.1	3.4		12.8	3.4
Myo-inositol		8.5	4.1		7.3	2.6		9.5	4.4
Lactate		1.1	1.0		0.5	0.7		1.8	0.9
Caudate nucleus	12			11			5		
Creatine		12.5	1.7		11.7	2.2		**8.9***	1.2
Choline		3.1	0.7		2.8	0.5		2.6	0.6
NAA		12.1	2.3		10.8	2.0		**9.0****	1.1
Glx		15.4	5.2		13.3	5.7		10.8	1.6
Myo-inositol		6.2	1.4		7.1	2.0		7.9	2.0
Lactate		1.2	0.9		1.5	0.9		0.5	0.8
Putamen	13			9			5		
Creatine		13.6	2.3		12.1	2.2		**10.9***	0.9
Choline		3.4	0.8		3.1	0.5		3.0	0.4
NAA		14.8	1.6		14.4	1.7		**12.8***	0.9
Glx		16.3	3.4		14.2	3.4		**11.4***	3.8
Myo-inositol		7.3	2.1		6.5	3.1		10.5	4.0
Lactate		0.8	0.6		1.0	1.3		0.3	0.4
Prefrontal	13			9			10		
Creatine		9.1	2.4		9.2	1.6		9.9	2.1
Choline		2.3	0.5		2.4	0.4		2.3	0.5
NAA		11.7	2.4		11.6	1.5		11.9	3.0
Glx		11.3	2.8		9.8	2.2		13.3	4.0
Myo-inositol		9.5	2.4		8.8	1.9		11.9	4.9
Lactate		1.2	1.1		1.5	1.5		0.8	0.9

Concentrations of metabolites in the three groups. Since the water concentration and the T_1 and T_2 relaxation times of the individual metabolites in the specific voxels of interest are unknown at the field strength of 7 Tesla, data are expressed as relative values to the water peak in arbitrary units (AU). NAA = N-acetylaspartate, Glx = glutamate + glutamine, *p<0,05, ** p<0.005

Relationship to clinical measures

The association of metabolite levels with clinical measures is shown in table 3. TFC was associated with creatine levels in caudate nucleus and putamen and with NAA in the putamen, showing decreased metabolite levels corresponding to poorer clinical scores. The NAA concentration in the putamen was associated with higher scores on the UHDRS TMS, indicating lower NAA to correspond to more motor disturbances. Lower glutamate/glutamine related to poorer scores on the cognitive tests SDMT and TMT. Finally, a higher PBA score, indicating more behavioural disturbances, corresponded to lower creatine in the caudate nucleus.

Table 3: Relationship of the clinical measures with metabolite concentration in the caudate nucleus and putamen

		Caudate nucleus		Putamen		
		Creatine	**NAA**	**Creatine**	**NAA**	**Glx**
Disease burden	**R**	-0,266	-0,473	0.168	0.284	-0.400
	p	0.339	0.075	0.584	0.347	0.157
UHDRS TMS	**R**	-0.280	-0.228	-0.324	**-0.415**	-0.255
	p	0.157	0.253	0.106	**0.035***	0.200
PBA-s	**R**	**-0.545**	-0.207	-0.063	0.064	0.052
	p	**0.003***	0.300	0.758	0.754	0.797
TFC	**R**	**0.409**	0.106	**0.440**	**0.556**	0.095
	p	**0.034***	0.598	**0.025***	**0.003***	0.636
BDI-II	**R**	-0.256	-0.022	0.005	0.206	0.152
	p	0.197	0.914	0.983	0.323	0.457
MMSE	**R**	-0.196	-0.106	-0.070	-0.108	-0.223
	p	0.328	0.598	0.735	0.601	0.265
TMT-B	**R**	0.310	0.270	0.185	0.049	**0.438**
	p	0.115	0.173	0.366	0.813	**0.022***
SDMT	**R**	0.171	0.133	0.239	0.125	**0.555**
	p	0.404	0.518	0.250	0.552	**0.003***
MQ	**R**	0.151	0.078	-0.049	-0.013	0.326
	p	0.451	0.700	0.811	0.951	0.097
Stroop II	**R**	-0.196	-0.106	-0.070	-0.108	-0.223
	p	0.328	0.598	0.735	0.601	0.265

Correlation analysis of metabolite concentration with clinical measures. UHDRS TMS = Unified Huntington's Disease Rating Scale Total Motor Score, PBA-s = Problem Behaviour Assessment short version, TFC = Total Functional Capacity, BDI-II = Beck Depression Inventory 2nd version, MMSE = Mini Mental State Exam, TMT = Trail Making Test part B, SDMT = Symbol Digit Modality Test, MQ = Memory Quotient, Stroop II = Stroop word reading card, R = partial r correlation coefficient.*$p<0.05$

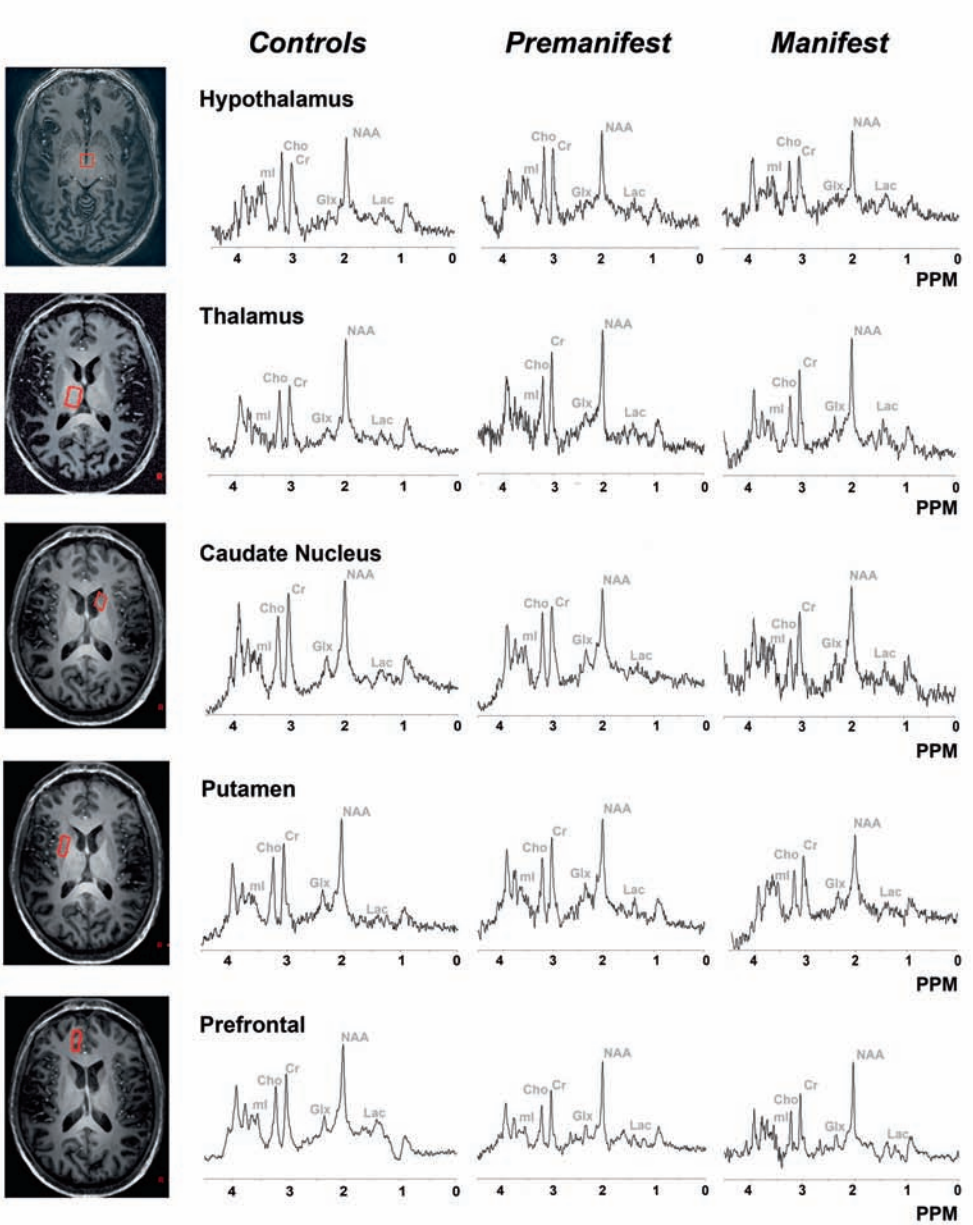

Figure 2: Localised proton MR spectra from different regions of the brain On the left side the voxel is displayed in the transverse direction, a typical spectrum of that structure is shown on the right side for the three groups. Five different regions are displayed: hypothalamus, thalamus, caudate nucleus, putamen, prefrontal region. Cho=choline, Cr=creatine, NAA=N-acetylaspratate, mI=myo-inositol, Glx=glutmate+glutamine, Lac=lactate, PPM=parts per million. A Gaussian filter of 4 Hz was applied.

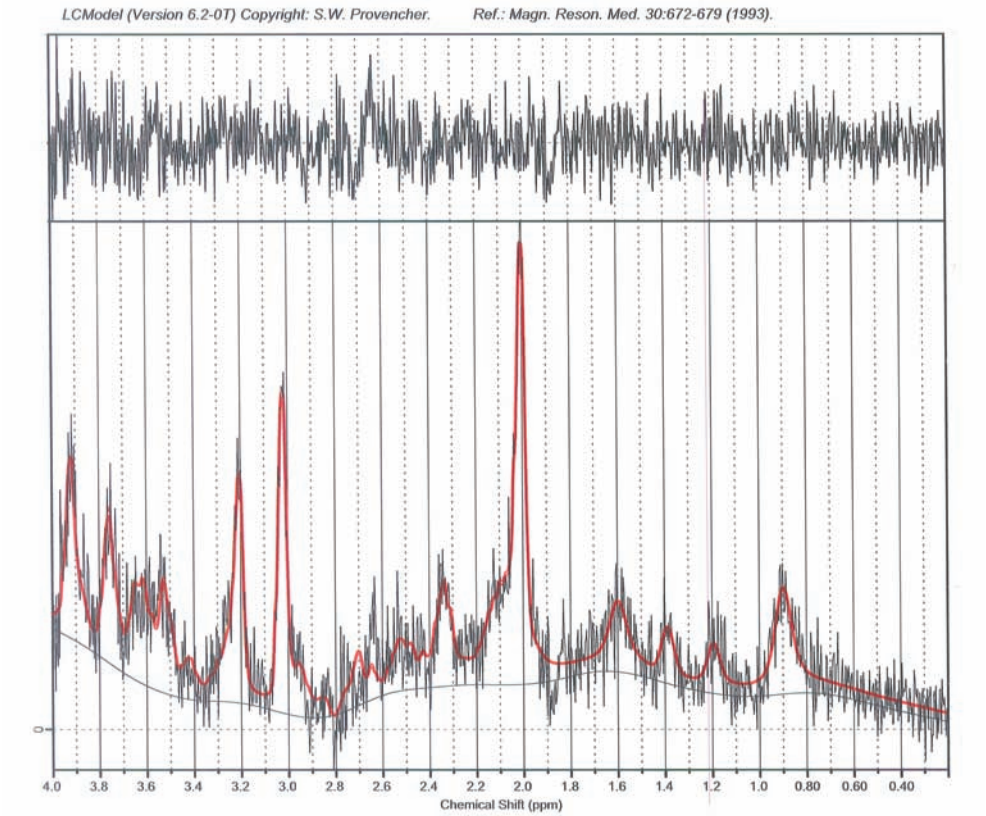

Figure 3: LCmodel output example. The black line is the raw spectrum, the red line is the fit by LCmodel. PPM = parts per million

Discussion

The major findings of this study are lower concentrations of creatine and NAA in the caudate nucleus and the putamen, and a reduction of glutamate in the putamen in manifest HD. A relationship between differences in these metabolic levels and clinical measures of disease severity, especially global functioning, was demonstrated. No statistically significant differences in any metabolite concentration were observed when the premanifest group was compared to controls, although the absolute lower values could indicate a subtle decline.

As creatine is considered an important marker for brain energy metabolism[28], the finding of lower creatine levels suggests impaired energy metabolism in manifest HD. In the healthy population, the concentration of creatine in the brain is considered to be fairly stable, however, specific pathology has been shown to influence creatine concentrations[28]. The finding of lower creatine in putamen and caudate nucleus is supported by findings of Sanchez *et al.* (1999), who observed a reduction of both creatine and NAA in the

striatum[10]. Reynolds *et al.* (2005) went on to propose creatine as a possible biomarker, when they demonstrated lower levels in the caudate nucleus in premanifest HD[18]. Contrary to our findings, Reynolds *et al.* (2005) did not report altered creatine levels in the putamen. Results from the TRACK-HD study reported by Sturrock *et al.* (2010) show similar lower values of creatine in the putamen as our study[29]. Jenkins *et al.* (1998) found additional evidence for impaired energy metabolism as displayed by elevated lactate levels in HD within the occipital cortex[11;12]. However, our study did not show elevated lactate in any of the examined structures. This finding can possibly be explained by the fact that Jenkins *et al.* (1993) used a more severely affected HD population with a lower mean TFC score of 7.3[11]. Moreover, the region analysed by Jenkins *et al.* (1993, 1998)[11;12] did not consist of the basal ganglia, but of the occipital cortex, which is a very metabolically active area of the cortex and is also severely atrophied in HD[30]. Although, these studies are not completely comparable on the basis of methodology and results, they do all point towards the importance of assessment of metabolic changes and specifically the energy metabolism markers such as creatine.

NAA is a marker for the integrity of neurons and axons[28] and lower NAA levels suggest a decrease in neuronal integrity. As atrophy of numerous brain structures is apparent in manifest HD, it is likely that neurons are affected and decrease in NAA may occur. This hypothesis was previously confirmed within the striatum[10;11;17]. Again the recent report from Sturrock *et al.* (2010) is similar to our findings of lower NAA in the putamen[29]. Our study confirms the finding of decreased NAA in the striatum (caudate nucleus plus putamen), and more importantly, our data show that neuronal damage may be present in both the caudate nucleus and putamen separately. The ability to acquire spectra from relatively small voxels is an important advantage of higher magnetic fields in localised MR spectroscopy.

Glutamate is of interest in light of the excitotoxicity theory which states that an overstimulation of neurons causes cell damage and eventually cell death. In neurons this can occur either due to increased levels of glutamate (and/or its precursor glutamine) or due to an increase in sensitivity of the glutamate receptors, both resulting in the same effect[7;8]. Taylor *et al.* (1994,1996) described increased levels of glutamate, supporting this theory[14;15]. However, the glutamate levels in these studies were expressed as a ratio to creatine, and could also be influenced by changes of the creatine level. Our study demonstrates reduced glutamate levels in the putamen. An explanation may be that the number of viable neurons is decreased to an extent where glutamate is lowered along with the neuron count. Also, the sensitivity of glutamate receptors could be altered, resulting in altered levels of glutamate. However, without further investigation these propositions are highly speculative.

In the premanifest HD group no changes in any of the examined metabolites levels could be demonstrated. This was an unexpected finding, as previous studies have reported structural abnormalities, e.g. atrophy, at this stage[1;31;32] and some reports exist of lowered levels of metabolites in premanifest HD gene carriers[10;18]. Sanchez-Pernaute *et al.* (1999) did report lower NAA and lower creatine in the striatum in 4 premanifest gene carriers; however the article already stated that there were soft motor signs present in two out of four in this group[10]. Reynolds *et al.* (2005) reported on lower values of creatine and NAA, yet he concluded that there was no pathognomonic profile in metabolite changes, but stated that there was great heterogeneity in this respect[18]. Nonetheless, we did hypothesize that premanifest HD would show altered levels of metabolites as atrophy (which is already present more than a decade before disease onset[3;30-32] is logically the result of underlying processes. We must therefore conclude that either we cannot (yet) measure these changes or the processes involved are more complex than simple linear correlation between metabolite levels and disease severity. For instance the excitotoxicity theory can possibly be a process measured by increased (damaging effects) or decreased (loss of healthy neurons) levels of glutamate depending on the individual disease stage. When performing group analysis, these measurements can become diluted. Individual assessment of longitudinal changes could shed more light on these changes. An explanation as to why no significant differences in our premanifest population were found, may be that our premanifest group was too heterogeneous in terms of proximity to disease onset. When taking into consideration the 'disease burden score', a proven correlate of striatal damage and thereby proximity to onset[33], a range of 121.5 to 450.5 exists within our premanifest HD sample. This large range suggests a great deal of variability in striatal damage and therefore of possible metabolite differences. Even so, despite this large range in disease burden score, a decline in absolute values of NAA and creatine was observed in the premanifest group, although this did not reach statistical significance. A second explanation could be our very stringent inclusion criteria. Only premanifest participants were included when there was no evidence of motor symptoms, as quantified by an UHDRS motor score of 5 or less, whereas other studies may have allowed participation of premanifest gene carriers with a higher degree of clinical abnormalities.

Our data show that metabolite levels are associated with clinical measures of disease severity. This finding highlights the feasibility of MRS in clinical trials, whereby metabolite levels could be target outcome measures. Endeavours using creatine as a treatment for HD with MRS as a monitoring tool have demonstrated the feasibility of this method[34].

This study shows the clinical application of high field 7-Tesla MRI. The spectral resolution in combination with small voxel size and total scanning time is a clear improvement in MRS methodology[21;35]. This allows the examination of metabolites in small, well defined anatomical structures, such as the caudate nucleus and putamen for HD, and can be used

in the examination of many other disorders where spectral and spatial resolution is of great importance.

A limitation of our study was the fact that the premanifest group was not uniformly distributed according to expected disease onset, which may have lead to a relatively large spread of the metabolite concentrations. A more homogeneous group close to onset could possibly reveal more significant results already in the premanifest stage of the disease. Also the small number of participants included in the manifest groups could be seen as a limitation. However, despite the small amount of participants the results were still significant. Furthermore, the hypothalamus region (bilaterally) included some CSF in the voxel, especially in the manifest HD group, as a result of atrophy, which could account for less reliable measurements. This was not a problem for any of the other regions. Finally, the frontal region consisted of approximately 50% of both grey and white matter, which could lead to false negative findings if grey or white matter would be unequally affected.

In conclusion, in manifest HD, lower NAA and creatine were found in the caudate nucleus and putamen, supporting the theory of impaired energy metabolism as part of the pathophysiology of Huntington's disease. Glutamate levels were lowered in the putamen, however to which extent this finding supports the excitotoxicity theory remains unclear. The relationship with clinical measures of function makes MRS a potential disease monitor and could also possibly be used to evaluate therapeutics targeted at metabolic processes in HD.

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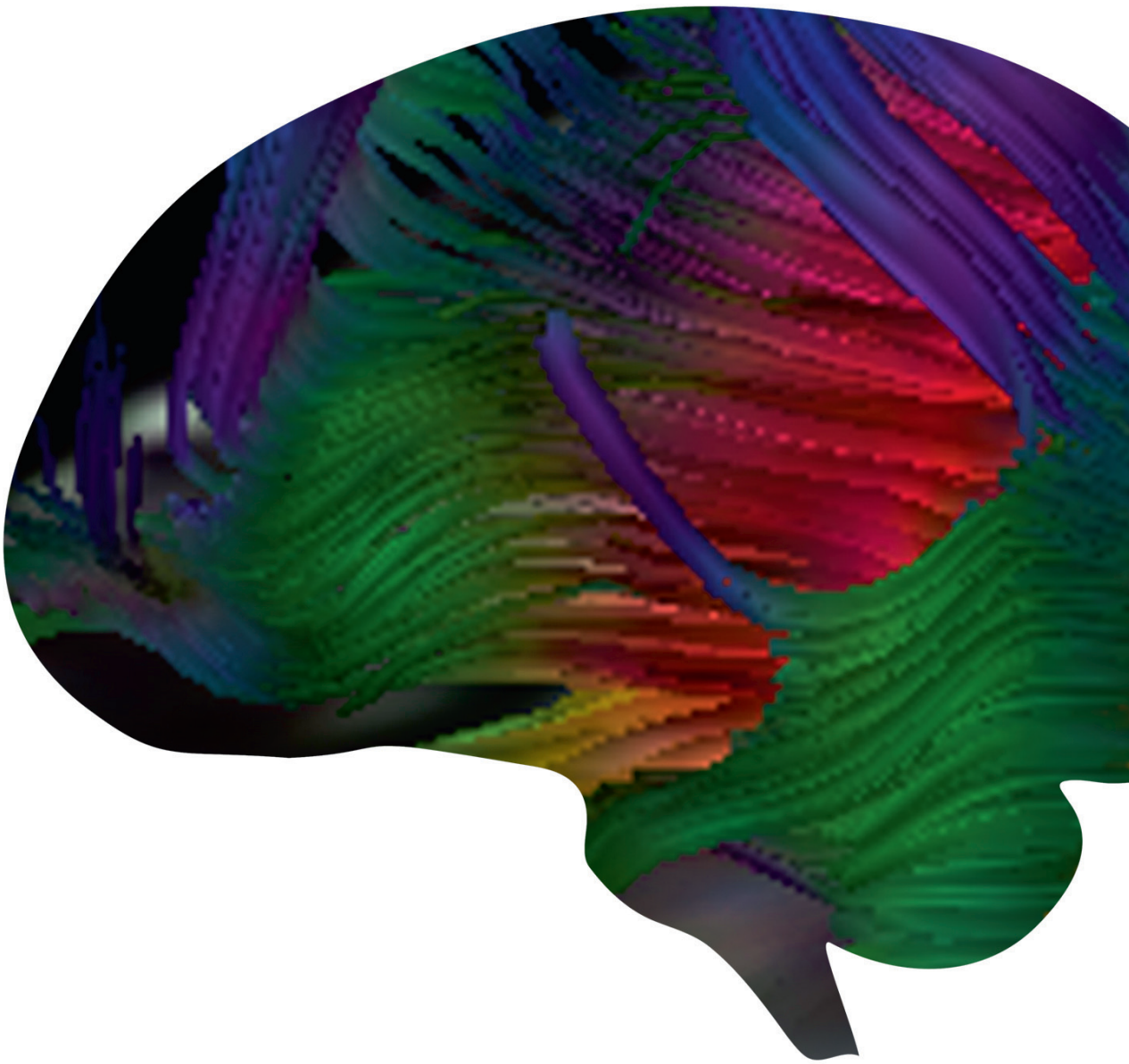
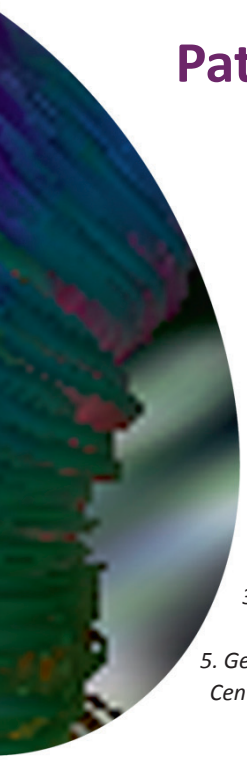


Chapter 6



Early Changes in White Matter Pathways of the Sensorimotor Cortex in Premanifest Huntington's Disease

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Abstract

Objectives

To investigate the function-structure relationship of white matter within different stages of Huntington's disease using diffusion tensor imaging (DTI).

Experimental design

From the TRACK-HD study, an early stage HD group and a pre-manifest gene carrier group (PMGC) were age-matched to two healthy control groups; all underwent 3T MRI scanning of the brain. Region of interest (ROI) segmentation of the corpus callosum, caudate nucleus, thalamus, prefrontal cortex and sensorimotor cortex was applied, and the apparent fiber pathways of these regions were analysed. Functional measures of motor, oculomotor, cognition, and behavior were correlated to DTI measures.

Principle observations

In PMGC versus controls, higher apparent diffusion coefficient (ADC) was seen in white matter pathways of the sensorimotor cortex ($p<0.01$) and in the ROI of corpus callosum ($p<0.017$). In early HD, fiber tract analysis showed higher ADC in pathways of the corpus callosum, thalamus, sensorimotor and pre-frontal region ($p<0.01$). ROI analysis showed higher diffusivity in the corpus callosum and caudate nucleus ($p<0.017$). Motor, oculomotor, cognition, and probability of onset within 2 and 5 years, correlated well with ADC measures of the corpus callosum ($p<0.01$ – $p<0.005$), sensorimotor ($p<0.01$ – $p<0.005$) and prefrontal region ($p<0.01$).

Conclusions

Disturbances in the white matter connections of the sensorimotor cortex can be demonstrated not only in manifest HD but also in pre-manifest gene carriers. Connectivity measures are well related to clinical functioning. DTI measures can be regarded as a potential biomarker for HD, due to their ability to objectify changes in brain structures and their role within brain networks.

Introduction

Huntington's disease (HD) is a neurodegenerative genetic disorder characterised by a progressive deterioration of motor control, cognitive functioning, and mood and behavioral functioning. The presence of an abnormal expansion of CAG repeats in the *HTT* gene, on chromosome four is responsible for the disease. The effect of the HD gene is seen in the brain as progressive cerebral atrophy of the basal ganglia and cortex[1-4]. It has been shown that the onset of atrophy may already be present in gene carriers up to 10 years prior to disease manifestation[4;5]. On the contrary, less is known about white matter changes in HD. Some reports demonstrate global atrophy of the white matter in manifest HD[6;7], whereas others show regional differences only[8;9]. A global volume reduction of white matter was seen only in one study of premanifest gene carriers[10]. The specific impact of HD on specific white matter pathways, and the clinical relevance of these changes, remains unclear.

The development and clinical application of magnetic resonance diffusion tensor imaging (DTI) has increased knowledge of grey and white matter structure in a variety of neurodegenerative diseases[11]. In patients with HD, a few studies have applied DTI to characterise changes in the macrostructure and microstructure of the basal ganglia. Lower fractional anisotropy (FA) values were found for premanifest gene carriers in the putamen, caudate nucleus[12] and thalamus[3]. On the contrary, Rosas *et al.* (2006) found higher FA in the putamen and pallidum[14]. In healthy subjects, FA values of grey matter structures are generally below 0.15. In white matter, values tend to be much higher ranging from 0.2 up to 1[15]. In general, the higher the FA value the more directional the organisation of the tissue is regarded to be - as seen in white matter fiber tracts. For this reason FA is generally accepted as an indication of tissue integrity.

In white matter, lower FA and increased apparent diffusion coefficient (ADC) values have been found in the internal capsule and corpus callosum in premanifest and manifest HD compared with healthy control subjects[13;14]. In several of neurodegenerative disorders, such as Alzheimer's and Parkinson's Disease, ADC values have been found to higher, indicating that degeneration negatively affects the brain tissue structure[16-18]. Higher ADC values indicate that the microstructure of the tissue allows a faster movement of water molecules. These higher values were also found in manifest HD[19-22]. To find white matter differences that are related to the earliest changes in HD, specific white matter fiber tracts that are related to HD symptomatology should preferably be investigated. A reduction in cognitive, motor, oculomotor performance is present up to a decade before clinical manifestation of HD[5;23;24], therefore, it can be hypothesized that the fibers associated with these function may also be affected in the premanifest phase. The direct nature of this relationship is unknown, and therefore, the aim of the present study is, first, to investigate

early FA and ADC changes in white matter fiber bundles running to and from brain areas known to be affected by HD (e.g. caudate nucleus) or those related to the clinical characteristics of HD (e.g sensorimotor cortex or prefrontal cortex). Second, to investigate to which extent changes in brain tissue structure and integrity in white matter fibers are related to clinical functioning.

Method

Participants

As part of the Track-HD study 90 participants were included at the Leiden University Medical Centre study site (for details see Tabrizi *et al.*, 2009[4]). Diffusion tensor magnetic resonance imaging was added to the standard MRI protocol. DTI was not performed because of claustrophobia in 10 participants, and another nine were excluded from analysis due to movement artifacts. Of the remaining 71 subjects, 16 subjects had early HD, 27 were premanifest gene carriers and 28 were healthy control subjects. Inclusion criteria for premanifest HD gene carriers were a CAG repeat ≥ 40 with a total motor score on the Unified Huntington's Disease Rating Scale 1999 (UHDRS) ≤ 5. Inclusion criteria for the early manifest HD patients were a CAG repeat ≥ 40, with a UHDRS motor score > 5 and a total functional capacity (TFC) score ≥ 7. Healthy gene negative family members or partners were recruited as control subjects. Because the early HD group is inherently older than the premanifest group, the control group was split into two separate groups of each 14 subjects to achieve age-matching. The younger healthy control subjects were age matched to the premanifest gene carriers (control group A). The older healthy controls subjects were age-matched to the manifest group (control group B). None of the participants suffered from a neurological disorder, a major psychiatric diagnosis, or had a history of severe head injury. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre. All participants gave informed consent.

DTI acquisition

MRI acquisition was performed on a 3 Tesla whole body scanner (Philips Achieva, Healthcare, Best, the Netherlands) with an eight channel SENSE head coil. T_1-weighted image volumes were acquired using a 3D MPRAGE acquisition sequence with the following imaging parameters: TR = 7.7 ms, TE = 3.5 ms, FOV = 24 cm, matrix size 224x224, number of slices = 164, slice thickness = 1.00 mm, slice gap = 0. A volumetric T_2-weighted image (VISTA) was acquired with the same parameters for field of view, acquisition matrix, and slice thickness as the T_1-weighted images, with TE = 250 ms and TR = 2500 ms. A single-shot echo-planar DTI sequence was applied with 32 measurement directions and the following scan parameters: TR = 10004 ms, TE = 56 ms, FOV = 220 x 220 x 128 with an acquisition matrix of 112 x 110, 2.00 mm slice thickness, transversal slice orientation, slice gap = 0, flip angle = 90°, single reconstruction voxel dimensions were 1.96 x 1.96 x

2.00 mm, number of slices = 64, B factor = 1000, halfscan factor = 0.61. Parallel imaging (SENSE) was used with a reduction factor of 2, NSA = 1 and fat suppression was applied. DTI acquisition time was 6.55 minutes.

Regions of interest segmentation

A priori, the caudate nuclei, thalami, and the corpus callosum were determined as regions of interest (ROI). A semiautomatic segmentation and analysis procedure was used as part of the software program FibreTrak (release 2.5.3, Philips Medical Systems, Best, the Netherlands). The caudate nucleus and thalamus were segmented separately on each side, but considered as one ROI during data analysis. On the DTI scans, special caution was taken to prevent inclusion of any non grey matter voxels, as both the caudate nucleus and thalamus are laterally bordered by the internal capsule. All analyses and segmentations were performed blinded to group status. For more detailed information on segmentations see the supplementary material.

Fiber tract analysis

Fiber analysis of fibers running through the following five structures was performed: the corpus callosum (Figure 1A), sensorimotor cortex (Figure 1B), caudate nucleus (Figure 1C), superior prefrontal cortex (Figure 1D) and thalamus (Figure 1E). To analyse fibers running through the corpus callosum, caudate nucleus and thalamus, the previously segmented ROIs were used. Additional segmentations for ROIs of the superior prefrontal cortex and sensorimotor cortex were performed. The superior prefrontal region was segmented on the basis of Brodmann areas 9 and 10. The sensorimotor cortex on Brodmann areas 1, 2, 3 and 4[12]. To calculate the average ADC and FA of the five white matter pathways FibreTrak was used (release 2.5.3, Philips Medical Systems, Best, The Netherlands). The software applies fiber assignment by continuous tracking[25]. The following standard parameters were implemented: minimum FA value 0.10, maximum angle change 27º, minimum fiber length 10 mm. For more detailed information on the application of the fiber tracking software see the supplementary material.

Clinical Assessments

From the extensive assessment battery in the TRACK-HD study, specific tasks were chosen that gave a representation of functioning in each symptom domain. Furthermore, these were tasks that had been proved to provide sensitive outcome measures for group comparisons, even in the premanifest stages[4]: index finger speeded tapping and sustained tongue force measures (motor), anti-saccade latency and error rate (oculomotor), symbol digit modalities test, the Stroop word reading test, trail making task part B, and a visual working memory task – the spot the change task (cognition); Beck's depression inventory 2nd version (BDI-II) and the frontal systems behavior inventory which yields three subscores of disinhibition, executive functioning and apathy (psychiatry). CAG repeat length and

probability of onset within 5 years and Burden of pathology ((CAG – 35.5) x age))[26] were also added to the analysis. For the complete set of clinical assessments and variables see Tabrizi *et al.* (2009)[4].

Statistics

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS for Windows; version 17.0.2, SPSS inc, Chicago, IL). Distributions and assumptions were checked. Independent student's t-tests and Chi-square tests were applied where appropriate in the analysis of group differences based on descriptive data; age, CAG, Dutch adult reading test (IQ), TFC and UHDRS. To test for differences in FA and ADC in the three ROIs and the five white matter fiber bundles between premanifest or manifest gene carriers and their corresponding control groups, analyses were performed using independent student's t-tests. Correction for multiple comparisons was applied to each analysis. Each analysis of ADC in the basal ganglia ROIs, the FA in the basal ganglia ROIs, the FA of the fiber pathways, and the ADC of the fiber pathways was regarded as a separate analyses. Therefore a Bonferroni correction was applied to each analysis. For the ADC and FA ROI analysis this lead to 0.05/3 = 0.017, and for the ADC and FA fiber pathways analysis this lead this to 0.05/5 = 0.01. The number of voxels in each seed ROI was compared between groups. Partial Pearson correlations analysis was performed to explore and test for possible associations between measures of white matter fiber pathway integrity and clinical measures. Age and gender were added into this analysis as covariates. The number of voxels in a ROI were also correlated to the clinical variables in order to examine the possibility that grey matter volume explains any possible correlations. The ADC and FA values of the white matter fiber pathways were correlated to each other to examine other explanations for possible results.

Results

Premanifest

No differences in age, gender, IQ, TFC, and UHDRS motor score between the premanifest gene carriers and their controls were present (Table I). The ADC and FA values of the ROIs and fiber pathways are shown in Table I. Premanifest gene carriers showed increased ADC values in the corpus callosum compared with controls. A significant increase in ADC of the white matter fibers of the sensorimotor cortex was also found between premanifest gene carriers and controls. No differences in ADC were found in the regions of the caudate nucleus and thalamus. No difference in FA was found in any of the three regions between the two groups. Also, no FA or ADC differences were found in any of the fiber pathways between the two groups. The number of voxels in the caudate nucleus ROI of premanifest group is significantly lower than the number in the control group ROI.

Early HD

The demographic data of the early HD and control group B showed no differences in age, gender or IQ. TFC and UHDRS differed significantly (Table II). In the corpus callosum and the caudate nucleus, the ADC values were larger in early HD when compared with controls. Between the two groups, no difference in FA was found in any of the ROIs. ADC values were significantly increased in fibers from all regions, except for the caudate nucleus fibers. FA was decreased in fibers running to and from the prefrontal cortex in early HD patients. No difference in FA was found in fibers passing through the corpus callosum, caudate nucleus, thalamus and sensorimotor cortex. The number of voxels of the caudate nucleus as well as in the corpus callosum was significantly lower in the patient group than in the control group (Table II).

Clinical correlations

The correlation analysis between the ADC of the fiber bundles with genetics, probability of onset within five years, burden of pathology, motor, oculomotor, cognition and behavior in all participants are shown in Table III.

Tapping was moderately associated with the diffusivity (ADC) of bundles through the corpus callosum, thalamus, sensorimotor cortex and prefrontal cortex. For the tongue force, similar but weaker associations were found. Latency of anti-saccades and the percentage of antisaccade errors in the oculomotor task were found to correlate with increased diffusivity of the corpus callosum, sensorimotor cortex and prefrontal cortex fibers. All cognitive measures were moderately to strongly associated with the ADC of fibers through the corpus callosum, sensorimotor cortex and to a lesser extent the prefrontal cortex. In premanifest gene carriers, probability of expected onset within 5 years and burden of pathology showed a high positive correlation with the diffusivity of the corpus callosum fibers and with those of the sensorimotor cortex, whereby a loss of integrity related to a higher probability of onset within five years and higher burden of pathology. No association in any cognitive domain with fibers of the thalamus was found. CAG repeat length and behavioral measures did not show association with the ADC of any of the fiber bundles studied. No measures showed association with the fibers of the caudate nucleus. FA of the corpus callosum and sensorimotor cortex fibers negatively correlated with the latency ($r = -0.52$, $p < 0.001$) and percentage of errors ($r = -.038$, $p = 0.001$) in the anti-saccade oculomotor task. FA did not demonstrate any significant correlation with genetics, motor, cognition, and behavior.

The results from a correlational analysis between number of voxels in a seed region and clinical variables showed five significant moderate correlations. Only one of these also showed a significant correlation in the analysis of ADC values with clinical variables. This was the correlation between the caudate nucleus and TMT task performance ($r = -0.36$,

$p < 0.01$). Directly correlating the ADC and FA values of the white matter pathways with each other revealed a number of significant correlations which is shown in table IV in the supplementary material. The direct correlation of ADC and FA of the same fiber bundle shows that the ADC and FA of the sensorimotor cortex and prefrontal white matter pathways are correlated.

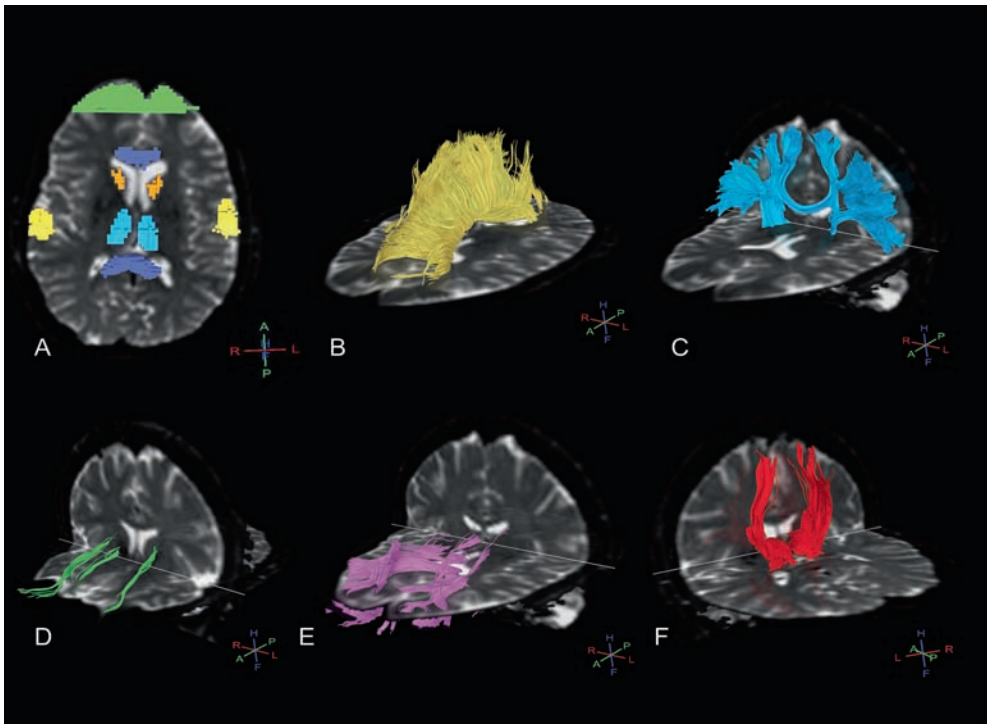

Figure 1. Typical example of ROIs (A) and subsequent white matter fiber pathways in a premanifest gene carrier of the corpus callosum (B), sensorimotor cortex (C), caudate nucleus (D), prefrontal cortex (E) and thalamus (F). 3D crosses depict orientation, whereby H = head, F = feet, A = anterior, P = posterior, R = right, L = left.

Table 1. Group demographics, ADC and FA values, and number of seed voxels per region of interest for the premanifest gene carriers and their controls.

	Control group A n: 14, male: 7		Premanifest gene carriers n: 27, male: 16		
	Mean (SD)	range	Mean (SD)	range	
Age (yrs)	46 (7,5)	35 - 58	43 (8,3)	26 - 61	
CAG	- (-)	-	43 (2,5)	39 – 50	
IQ	105,6 (11,3)	88 - 130	101,3 (11,3)	76 - 118	
TFC	13 (0)	13 - 13	12,6 (0,8)	10 - 13	
UHDRS	1,9 (1,8)	0 - 6	2,4 (1,4)	0 - 4	
ADC ROI	# voxels ± SD	mean ± SD	# voxels ± SD	mean ± SD	*p*
Corpus Callosum	1884 ± 378	0.78 ± 0.028	1767 ± 319	0.81 ± 0.033*	**0.014**
Caudate Nucleus	**58 ± 13**	0.73 ± 0.071	**47 ± 11*** **	0.76 ± 0.053	0.160
Thalamus	2700 ± 843	0.73 ±0.023	2601 ± 657	0.73 ± 0.023	0.994
FA ROI					
Corpus Callosum	1884 ± 378	0.76 ± 0.019	1767 ± 319	0.75 ± 0.021	0.461
Caudate Nucleus	**58 ± 13**	0.17 ± 0.015	**47 ± 11*** **	0.18 ± 0.023	0.058
Thalamus	2700 ± 843	0.34 ± 0.025	2601 ± 657	0.33 ± 0.022	0.368
ADC Fiber pathway	# voxels ± SD	mean ± SD	# voxels ± SD	mean ± SD	
Corpus Callosum	1884 ± 378	0.89 ± 0.049	1767 ± 319	0.91 ± 0.054	0.206
Caudate Nucleus	**58 ± 13**	0.98 ± 0.116	**47 ± 11*** **	0.94 ± 0.075	0.166
Thalamus	2700 ± 843	0.87 ± 0.055	2601 ± 657	0.89 ± 0.071	0.476
Motor cortex	595 ± 86	**0.78 ± 0.017**	580 ± 71	**0.80 ± 0.029****	**0.009**
Prefrontal cortex	1136 ± 183	0.88 ± 0.044	1117 ± 322	0.89 ± 0.037	0.631
FA Fiber pathway					
Corpus Callosum	1884 ± 378	0.49 ± 0.015	1767 ± 319	0.49 ± 0.016	0.785
Caudate Nucleus	**58 ± 13**	0.36 ± 0.017	**47 ± 11*** **	0.35 ± 0.031	0.683
Thalamus	2700 ± 843	0.42 ± 0.018	2601 ± 657	0.41 ± 0.021	0.515
Motor cortex	595 ± 86	0.40 ± 0.017	580 ± 71	0.39 ± 0.020	0.190
Prefrontal cortex	1136 ± 183	0.41 ± 0.018	1117 ± 322	0.40 ± 0.021	0.120

SD: standard deviation, CAG: CAG repeat length, IQ: estimate of premorbid intelligence quotient. TFC: Total Functional Capacity score.UHDRS: Unified Huntington's Disease Rating Scale total motor score. ROI: Region of interest analysis. ADC: Apparent Diffusion Coefficient in $\mu m^2/ms$, FA: Fractional Anisotropy (no unit), Fiber pathway: fiber pathways analysis between the listed region and the rest of the brain. *p <0.017, **p<0.01 (adjusted for multiple comparisons). # voxels: mean number of voxels in the listed seed region. ***Number of voxels differs significantly from healthy controls

Table 2. Group demographics, ADC and FA values, and number of seed voxels per region of interest for the early HD patients and their controls.

	Control group B n: 14, male: 7		Early HD n: 16, male: 12		
	Mean (SD)	range	Mean (SD)	range	
Age (yrs)	51.4 (7.9)	42 - 65	48.2 (10)	31 - 63	
CAG	- (-)	-	43 (1.6)	41 - 46	
IQ	102.6 (6.6)	88 - 115	99.6 (12)	72 - 118	
TFC	12.9 (0.3)	12 - 13	10.6[§] (2)	7 - 13	
UHDRS	3.1 (2.8)	0 - 7	18.3[§] (10.4)	6 - 45	
ADC ROI	# voxels ± SD	mean ± SD	# voxels ± SD	mean ± SD	*p*
Corpus Callosum	**1934 ± 312**	**0.80 ± 0.031**	**1445 ± 371*** **	**0.85 ± 0.040***	**0.000**
Caudate Nucleus	**57 ± 12**	**0.74 ± 0.048**	**40 ± 7*** **	**0.83 ± 0.069***	**0.001**
Thalamus	2480 ± 531	0.74 ± 0.032	3102 ± 1062	0.74 ± 0.034	0.863
FA ROI					
Corpus Callosum	**1934 ± 312**	0.75 ± 0.012	**1445 ± 371*** **	0.73 ± 0.033	0.046
Caudate Nucleus	**57 ± 12**	0.17 ± 0.018	**40 ± 7*** **	0.19 ± 0.019	0.072
Thalamus	2480 ± 531	0.33 ± 0.021	3102 ± 1062	0.32 ± 0.034	0.95
ADC Fiber pathway	# voxels ± SD	mean ± SD	# voxels ± SD	mean ± SD	
Corpus Callosum	**1934 ± 312**	**0.89 ± 0.051**	**1445 ± 371*** **	**0.95 ± 0.067****	**0.008**
Caudate Nucleus	**57 ± 12**	0.88 ± 0.107	**40 ± 7*** **	0.97 ± 0.079	0.023
Thalamus	2480 ± 531	**0.85 ± 0.080**	3102 ± 1062	**0.93 ± 0.063****	**0.007**
Motor cortex	589 ± 117	**0.79 ± 0.023**	593 ± 110	**0.82 ± 0.027****	**0.001**
Prefrontal cortex	1118 ± 239	**0.88 ± 0.023**	1202 ± 274	**0.95 ± 0.056****	**0.000**
FA Fiber pathway					
Corpus Callosum	**1934 ± 312**	0.48 ± 0.013	**1445 ± 371*** **	0.47 ± 0.017	0.027
Caudate Nucleus	**57 ± 12**	0.35 ± 0.027	**40 ± 7*** **	0.35 ± 0.021	0.592
Thalamus	2480 ± 531	0.41 ± 0.016	3102 ± 1062	0.41 ± 0.024	0.644
Motor cortex	589 ± 117	0.38 ± 0.019	593 ± 110	0.37 ± 0.024	0.059
Prefrontal cortex	1118 ± 239	**0.39 ± 0.016**	1202 ± 274	**0.38 ± 0.014****	**0.006**

SD: standard deviation, CAG: CAG repeat length, IQ: estimate of premorbid intelligence quotient. TFC: Total Functional Capacity score. UHDRS: Unified Huntington's Disease Rating Scale total motor score. [§]Significant difference from control group, $p < 0.01$. ROI: Region of interest analysis. ADC: Apparent Diffusion Coefficient in µm2/ms, FA: Fractional Anisotropy (no unit), Fiber pathway: fiber pathways analysis between the listed region and the rest of the brain. *p <0.017, **p<0.01 (adjusted for multiple comparisons). # voxels: mean number of voxels in the listed seed region. ***Number of voxels differs significantly from healthy controls.

Table 3. Standardised correlation coefficients matrix for ADC of white matter fiber bundles and clinical measures in all participants.

	Motor		Cognitive				Behavior			
	Tapping	Tongue	SDMT	SWR	TMT B	SPOT	BDI-II	FrSBe Dis-inhib	FrSBe Exec dysf	FrSBe apathy
Corpus Callosum	0.38**	0.27	**-0.32***	**-0.39****	**0.36****	**-0.39****	0.11	0.01	0.09	0.10
Caudate Nucleus	0.13	0.23	-0.08	-0.04	0.13	-0.19	-0.05	0.05	-0.02	-0.01
Thalamus	**0.41****	**0.33***	-0.27	-0.28	0.26	-0.30	0.02	0.03	0.09	0.07
Motor cortex	**0.46****	**0.32***	**-0.46****	**-0.52****	**0.55****	**-0.33***	0.15	-0.02	0.11	0.17
Prefrontal cortex	**0.33***	0.28	-0.28	**-0.34***	0.25	-0.28	0.15	-0.02	-0.06	0.04

	Genetics	Probability of onset	Burden of Pathology	Oculomotor	
	CAG Gene carrier only	within 5 years (preHD only)	(CAG-35.5) x age	Latency of anti-saccades	Error % of anti-saccades
Corpus Callosum	0.36	**0.61****	**0.51****	**-0.32***	**-0.39****
Caudate Nucleus	0.09	0.42	0.27	-0.08	-0.04
Thalamus	0.10	0.06	0.19	-0.27	-0.28
Motor cortex	0.32	0.58**	0.52**	-0.46**	-0.52**
Prefrontal cortex	0.09	**0.41***	0.37	-0.28	-0.34*

Tapping = Average speeded tapping intertap variability for left and right index finger, Tongue = sustained tongue force measure, SDMT = Symbol Digit Modalities test, SWR = Stroop word reading task, TMT B = Trail making test part B, SPOT = Visual array comparison task for visual short-term memory capacity, BDI-II = Becks Depression Inventory 2nd version, FrSBe = Frontal Systems Behaviour rating scale Self Report, disinhibtion, executive dysfunction and apathy subscores, CAG = CAG repeat length (in gene carriers only), Expected years to onset correlations are only for premanifest gene carriers. All correlations are controlled for the effects of age and gender. *p<0.01 **p<0.005

Discussion

The main finding of this study is that in premanifest gene carriers, the white matter pathway of the sensorimotor cortex is impaired. Furthermore, our data show that in the early manifest phase of the disease impairment is more widespread and present in the white matter pathways of the sensorimotor cortex, corpus callosum, thalamus, and prefrontal cortex. Finally, a relationship is seen between the changes in white matter pathways and functionality in the domains of motor, oculomotor and cognition. Moreover this study confirms findings of regional differences in the corpus callosum of premanifest and the caudate nuclei and corpus callosum of early HD[14;27].

In the premanifest phase of the disease, our study demonstrated a reduction of integrity of only the sensorimotor cortex fibers pathway, therefore this suggests that this pathway may be one of the first to be affected by HD. This is supported by the positive relationship between a higher probability of onset within 5 years, and a higher burden of pathology, with the loss of integrity of the sensorimotor cortex fibers. This premanifest cohort is 'free' of motor symptoms, and therefore these findings in a truly 'premotor' premanifest group further support the idea that these white matter changes are among the first to occur. This study is the first to demonstrate this change across the whole pathway; however, other studies examining this area do provide support for this finding. The findings of the voxel–by–voxel white matter analysis of Reading *et al.* (2005)[12] found differences in a cluster of voxels coinciding with the primary motor cortex (Brodmann area 4). Atrophy of the sensorimotor cortex was demonstrated as the only cortical region to be affected in premanifest gene carriers far from predicted onset[4]. Furthermore differences have been reported in functions that utilise this area in premanifest gene carriers, such as measures of motor function[5;28]. The other important finding of our study in premanifest gene carriers is the differences in diffusivity in the corpus callosum, thereby replicating previous findings by Rosas *et al.* (2006,2009)[14;27]. However we add that these differences are not seen in the white matter projections specifically going to and from the corpus callosum. Therefore, when looking beyond the main structure it can be suggested that the loss of integrity of the main structure is apparent, but not spread over its entire network, as is the case in manifest HD.

In the early manifest phase of HD, our data show that the diffusivity of the fiber pathways of the thalamus, corpus callosum, sensorimotor, and prefrontal cortex was higher in HD than in healthy controls. This suggests a disintegration of these structures. Of the five white matter pathways examined in HD all were found to be affected except the pathways of the caudate nuclei. The absence of affected white matter from the caudate nucleus should be interpreted with some caution. It may suggest that the magnitude of integrity loss may not be the same for a structure as for its fibers at a given stage of the disease. However the variance of these measurements is larger than that of the other fibers

pathways. The remaining results, such as that of reduced integrity of the prefrontal cortex fibers, find support in the regional differences demonstrated by Rosas *et al.* (2006)[14]. Our results show changes in ADC and almost no changes in FA. This suggests that of these two closely related measures, ADC is more sensitive in demonstrating changes across large pathways in HD. The results of the regional analysis showed higher diffusivity in the caudate nucleus and corpus callosum in HD than in healthy controls. This was not the case in the thalamus, whereby similar diffusion properties were seen in both HD and controls. Our findings concur with previous cross-sectional and longitudinal findings of a widespread effect of HD on white matter[14;20;22;29]. The finding that a region was not affected, but its fibers were, demonstrates the need to embrace the full potential of DTI measures for HD, as this shows that despite a structure not showing integrity differences, the fibers that are needed for this structure to communicate with the brain may well be affected. In the case of the thalamus this is especially relevant to the clinical expression of the disease as this structure is vital to a large number of functional processes.

The previously discussed outcomes demonstrate that almost all fiber pathways that we examined are affected in early manifest HD and that one specific pathway is also affected in premanifest gene carriers. In exploring the clinical implications of these findings we see strong relationships between both motor, oculomotor and cognitive measures, and diffusivity measures of the pathways of the corpus callosum, thalamus, sensorimotor and prefrontal cortex. Although one cognitive measure does correlate to the prefrontal cortex fiber the cognitive measures are most strongly related to the sensorimotor cortex fibers. This finding can be explained with two complementary hypotheses. First, as seen in the premanifest group, the fibers of the sensorimotor cortex are the first to show decreased integrity. This suggests that these are the most severely affected fibers in the earliest (premanifest) stages of HD; therefore, it is not surprising that the clinical measures of decline relate to these fibers. Second, HD effects both cognition and motor function, and all cognitive tests require a motor response. A consequent finding in cognitive HD literature is that cognitive tests sensitive to psychomotor speed are the most sensitive tasks. A great deal of voluntary motor functioning is initiated in the sensorimotor cortex. Because of this we were not entirely surprised that cognitive tests relate strongly to the fibers of the sensorimotor cortex. The tasks chosen for analysis were those that provide meaningful outcomes for all study groups, and did not show ceiling or floor effects. Therefore, we can conclude that fibers associated with higher order cognitive and motor coordination are affected in a manner that is congruent to clinical manifestations and that for this reason, DTI measures can be applied to characterise the structure-function relationship of white matter. This conclusion is supported in other studies of white and grey matter and clinical measures[6,30]. In another study of eye movements and fiber tracking, similar results were found as ours, especially finding a relationship between eye movements and fiber FA[31]. We did not show a relationship between CAG repeat length or behavioral processes and changes in white matter integrity. The CAG repeat length is

not dependent on disease progression or white matter pathways and this stable quality may, in part, explain this finding. A possible explanation for the absence of a relationship between behavioral measures and diffusivity may be the complex pathophysiological and psychological process underlying these behavioral changes that may not be primarily dependent on white matter. Alternatively, the use of medication may be a factor in the level of symptoms reported (full details of medication use are outlined in Tabrizi *et al.* (2009)[4] leading to an underestimation of neuropsychiatric problem behavior. However, a recent study has shown that behavioral changes in premanifest HD remain stable[32], thereby reinforcing the idea that degenerative processes are not at the root of these neuropsychiatric differences. Therefore we conclude that behavioral changes may not be directly reflected by white matter changes.

This study examined and demonstrated differences in white matter pathways of five HD relevant regions, this restriction is a limitation of the study. Differences were found in the number of seed voxels in the caudate nucleus in premanifest gene carriers and in the caudate nucleus and corpus callosum in manifest HD. On the contrary, differences were not seen in the number of voxels in the thalamus, sensorimotor region, or prefrontal regions in the patient group. These reductions do seem to reflect expected atrophy, but do not follow the pattern of differences found in ADC. Therefore these differences do not seem to explain the differences in diffusivity. The results from a correlation analysis between number of voxels in a seed region and clinical variables reveal only five significant moderate correlations. This is in contrast to the analysis of relationship between average ADC and the clinical variables whereby 25 moderate to high correlations were significant. Furthermore only one of these five was the same as one of the 25 clinical correlations. These results suggest the volume of the seed region does not explain the observed original correlations. With this possibility excluded, it can be stated with more certainty that the relationships observed reflect the underlying changes in structure of the white matter pathways. Furthermore, direct correlation of the ADC and FA of the white matter pathways also supports this conclusion. It can be seen that only significant relationships between the ADC and FA of the same pathways were apparent for pathways that showed (nearly) significant group differences. Overall, the results call for further examination of white matter pathways. The findings warrant confirmation with longitudinal follow-up.

In conclusion, we demonstrated that the sensorimotor cortex fibers are affected already in the premanifest phase of HD and therefore may be a good target for following progression of the disease. Our data show that impairment is seen in corpus callosum, nuclei and fiber projections of HD relevant brain regions in both premanifest and early manifest HD. These impairments relate to proven correlates of clinical dysfunction. Overall, the findings of this study confirm the feasibility and use of DTI measures in HD research, whereby we show that ADC is a good measure in characterising the impaired function-structure relationship present in HD.

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Supplementary Material

Supplementary Methods

Segmentation

Based on the anatomical co-registration images, the ROIs were segmented in native space for each individual. This was done by two experienced radiographers, blinded to patient group. Two types of ROIs were defined. Basal ganglia ROIs for analysis, and seed-ROIs as starting point for fiber tracking. The basal ganglia ROIs were segmented as follows. For the caudate nucleus all voxels clearly bounded by the lateral ventricle and the anterior limb of the internal capsule were selected. The putamen segmentation included all voxels within the structure bounded on the medial side by the globus pallidus and the anterior limb of the internal capsule, and on the lateral side by the capsula extrema. The thalamus segmentation was performed by allocating all voxels to the ROI that medially bordered the third ventricle, were superiorly bordered by the lateral ventricle, and were laterally bordered by the internal capsule. The seed ROIs were cortically based, and because these areas were not as clearly anatomically bounded as the other structures, Brodmann areas were used to define the segmentation. Reading *et al.* (2005) defined areas of the cortex in their analysis of presymptomatic HD by means of these Brodmann areas[1]. The superior prefrontal region was segmented on areas 9 (frontal portion) and 10. Brodmann area 10 is medially bound by the superior rostral suclus, and dorsally by the inferior frontal suclus and the frontomarginal sulcus. Area 9 bordered inferiorally by area 10 and dorsally by the superior frontal sulcus. After all ROIs were drawn, the radiographers switched scans and carefully checked the segmentations for potential miss-segmentation. After all scans had been segmented a random 20% were selected for verification by a neurologist specialised in neuro-imaging. Any segmentation voxels that were felt to be outside of the targeted anatomy were discussed until a consensus was formed and these few voxels were changed accordingly.

Analysis

We applied the software supplied by the manufacture by transferring the raw DTI data and the anatomic co-registration images to an off-line manufacturer provided console. In doing so the data were loaded into the Philips Research Image-processing Development Environment (PRIDE) (Philips Medical Systems). Then the diffusion tensor and derived measures, including ADC and FA, were calculated using the Philips PRIDE Fiber Tracking tool (version 2.5.3). This allows fiber tracking based on the Fiber Assignment by Continuous Tracking (FACT) algorithm[2]. The algorithm values for restricting fiber tracking were kept at default and were: minimum FA value of 0.10, maximum angle change: 27°, and a minimum fiber length of 10mm. This same method and software has been applied in a number of disorders of both brain and muscle, such as developmental CNS anomalies[3], corticospinal tract stroke[4], lateral patellar dislocation[5] and Lissencephaly[6].

Supplementary Results

Table 4. Cross tabulation of correlation coefficients of the FA and ADC of the white matter fiber pathways with each other.

		Corpus Callosum		Caudate Nucleus		Thalamus		Motor cortex		Prefrontal cortex	
		ADC	FA	ADC	FA	ADC	FA	ADC	FA	ADC	FA
Corpus Callosum	**ADC**	-	-.123	**.482**	-.262	**.705**	.069	**.644**	**-.312**	**.572**	**-.432**
	FA		-	.002	.136	-.065	**.472**	**-.439**	**.581**	-.272	**.516**
Caudate Nucleus	**ADC**			-	.028	**.594**	.153	.185	.091	.206	-.077
	FA				-	-.116	**.404**	-.052	.190	**-.352**	.046
Thalamus	**ADC**					-	.034	**.476**	-.145	**.353**	-.183
	FA						-	-.021	.290	-.050	.126
Motor cortex	**ADC**							-	**-.604**	**.548**	**-.530**
	FA								-	**-.492**	**.473**
Prefrontal cortex	**ADC**									**-**	**-.483**
	FA										-

ADC: Apparent Diffusion Coefficient, FA: Fractional Anisotropy. **Bold** = significant correlation

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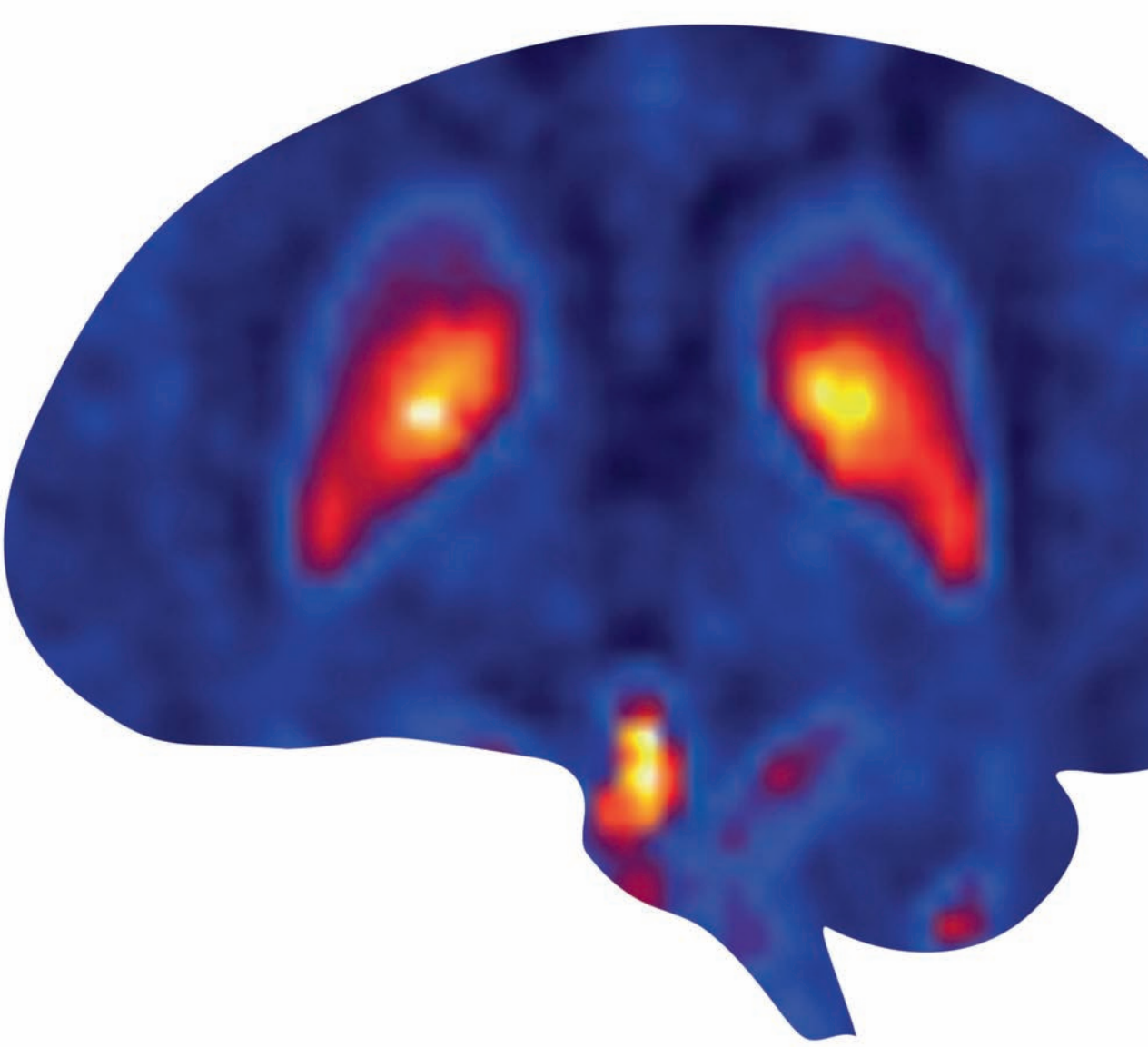

Chapter 7

Elevated brain iron is independent from atrophy in Huntington's Disease

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Abstract

Increased iron in subcortical structures in patients with Huntington's Disease (HD) has been suggested as a causal factor of neuronal degeneration. The present study examines iron accumulation, measured using magnetic resonance imaging (MRI), in premanifest gene carriers and in early HD patients as compared to healthy controls. In total 27 early HD patients, 22 premanifest gene carriers and 25 healthy controls, from the Leiden site of the TRACK-HD study, underwent 3T MRI including high resolution 3D T_1- and T_2-weighted and asymmetric spin echo (ASE) sequences. Magnetic Field Correlation (MFC) maps of iron levels were constructed to assess magnetic field inhomogeneities and compared between groups in the caudate nucleus, putamen, globus pallidus, hippocampus, amygdala, accumbens nucleus, and thalamus. Subsequently the relationship of MFC value to volumetric data and disease state was examined. Higher MFC values were found in the caudate nucleus ($p<0.05$) and putamen ($p<0.005$) of early HD compared to controls and premanifest gene carriers. No differences in MFC were found between premanifest gene carriers and controls. MFC in the caudate nucleus and putamen is a predictor of disease state in HD. No correlation was found between the MFC value and volume of these subcortical structures. We conclude that Huntington's disease patients in the early stages of the disease, but not premanifest gene carriers, have higher iron concentrations in the caudate nucleus and putamen. We have demonstrated that the iron content of these structures relates to disease state in gene carriers, independently of the measured volume of these structures.

Introduction

Huntington's Disease (HD) is an autosomal dominant neurodegenerative disorder characterised by brain atrophy and clinical deterioration in the domains of motor function, cognition and behaviour. HD is caused by an expanded CAG repeat in the *HTT* gene on the short arm of chromosome 4. Genetic testing can be performed in those at risk, prior to disease onset, to ascertain that they carry the gene and will develop the disease in the future. Histological reports describe profound cellular structure deterioration of the putamen and caudate nucleus[1-3], as well as iron accumulation[4]. Autopsy brain tissue extractions confirmed these findings in end-stage patients by demonstrating increased absolute iron levels in these structures[5].

MRI has been shown to be a valuable tool for estimating iron levels in vivo[6;7]. Several MRI techniques have been used to assess the distribution of iron in the brain in normal aging[8-10] and in neurodegenerative diseases[11-14]. These include T_2 and T_2* mapping, as well as susceptibility weighted imaging (SWI). Each of these techniques is simple to perform, but have significant potential limitations. A confounding factor of both T_2- and T_2*-based imaging is that measurements are also affected by changes in the water content, associated for example with destructive brain processes[15], and can mask the changes in iron content[6;9;14;16;17]. Ex vivo histological staining of putamen samples from HD patients demonstrated elevated putamen iron levels[18] but there was little correlation to T_2-weighted imaging of these samples. To overcome these limitations T_2 can be measured at two different field strengths to remove the field independent contribution to T_2 changes. Because the T_2 value of water is relatively field strength independent[19], this method is a more sensitive measurement of iron content. However, scanning subjects on two different MRI systems is clinically impractical. MRI approaches based on changes in T_2* relaxation time have also been widely applied[16]. In addition to the sensitivity of the measurement to water content, a specific limitation of T_2* imaging techniques is that measurements are affected by local background sources of magnetic field inhomogeneities that cause signal loss unrelated to the internal iron content of the tissue[6]. SWI is a technique that provides an additional measure for detecting iron related changes by combining magnitude and phase data into a single image[20]. Because changes in the magnetic field also lead to changes in the MRI signal phase, it has been suggested as a more sensitive method for detecting neurodegenerative disease related iron changes[17]. However SWI is not a quantitative method and the limitations mentioned above for T_2* techniques are also true for SWI. Hence, the limitations posed by the above described methods demonstrates the need for further development and application of existing and new techniques[12].

The recently developed quantitative technique of magnetic field correlation (MFC) imaging has the potential to solve the limitations of previously applied MRI techniques in HD.

MFC is sensitive to spatially inhomogeneous magnetic fields, such as those generated by iron-rich regions. An advantage is that this technique is insensitive to changes in water concentration. For this reason MFC has previously been used to study increased iron concentrations in the basal ganglia in patients with aceruloplasminemia[21], patients with traumatic brain injury[22] and in patients with multiple sclerosis[23]. The relationship between the measured MFC values and magnetic field inhomogeneities is more direct compared to other relaxometry measurements, allowing for a clearer physical interpretation of MFC measurements[21;24]. Furthermore, MFC imaging can be implemented practically in a single short scan at one field strength.

Previous MRI studies have suggested that increased iron in the striatum could be a causal factor of the symptoms of HD[11;19]. Given that both prior to, and after, disease onset, brain changes in the form of volumetric reductions have been systematically reported[25-28], it is possible that iron levels change both in the premanifest and in the manifest stages of the disease. However, this hypothesis has only been examined previously in one group of premanifest gene carriers of HD[29]. It has been suggested that increases in iron could promote neurotoxicity through the induction of oxidative reactions[12;30]. A question still debated is whether these iron changes are causal or secondary factors of disease processes. With this current study we aim to determine at which disease stage iron accumulations increase in HD.

It remains unclear to what extent iron changes are independent of volume decreases in both the premanifest and manifest phases of the disease, or whether iron levels alter as a direct result of volumetric change[25;28;31]. Volume decreases may concentrate the iron that is already present, or alternatively more iron may accumulate as the disease progresses. It is important for our understanding of HD, as well as for future research, to differentiate between these pathophysiological mechanisms. For this reason, the second aim of this study is to examine and relate the potential iron changes to the amount of atrophy present in the related subcortical grey matters structures.

The current reports of iron in HD examined only a selection of the subcortical grey matter structures. However, recently atrophy has been demonstrated in seven major subcortical structures in the progressive stages of HD[27]. In this current study we investigate in both premanifest and early HD the extent to which elevated iron may be present. We hypothesize that iron concentrations will be higher in these subcortical grey matter structures in HD patients. Furthermore, we expect that iron may play a role in HD that is not explained by volumetric differences.

Material and Methods

Participants

Participants were recruited from the Leiden University Medical Centre (LUMC) study site of the longitudinal TRACK-HD study[28], 27 manifest gene carriers in the early disease stages one or two (early HD)[32], 22 premanifest gene carriers (prior to disease onset) and 25 healthy controls underwent 3T MRI scanning including an asymmetric spin echo sequence and functional assessment.

Inclusion criteria for the early HD group were a positive genetic test for the HTT gene with 40 or more CAG repeats, the presence of motor disturbances quantified by more than five points on the Unified Huntington's Disease Rating Scale – motor score (UHDRS-TMS), and a minimum Total Functional Capacity (TFC) score of seven points[32]. Inclusion criteria for premanifest gene carriers consisted of 40 or more CAG repeats, and the absence of motor disturbances with five or less points on the UHDRS-TMS. A burden of pathology score greater than 250[33] was required. Age- and gender-matched gene-negative relatives of HD gene carriers were included as healthy controls. Level of education in accordance to the International Standard Classification of Education (ISCED) was recorded. The study was approved by the Medical Ethical Committee and all participants gave informed consent.

MRI protocol

An MRI protocol including high-resolution 3D T_1-weighted and asymmetric spin echo (ASE) sequences was applied using a 3 Tesla whole body scanner (Achieva, Philips Healthcare, Best, The Netherlands) with an eight channel receive array head coil. Participants were positioned carefully with the application of strapping or cushioning where needed, to reduce the potential occurrence of involuntary head movement.

A 9.5 minute isotropic 1 mm^3 3D T_1-weighted scan was acquired with the following parameters: repetition time (TR)/ echo time (TE) = 7.7 ms/3.5 ms, field-of-view (FOV) = 24x24x16.4 cm^3. A T_2-weighted image (turbo spin echo) was acquired with the same volumetric spatial resolutions as the T_1-weighted images, with TE = 250 ms and TR = 2500 ms, also with a duration of 9.5 minutes. The T_2-weighted image for this study was only used to exclude any possible comorbidity. An 8 minute ASE sequence was implemented with the following scan parameters: TR/TE/flip angle = 1005 ms/38 ms/90°. The FOV was 22x19x7cm^3 with a voxel size of 1.9x1.7x2mm^3 for 18 slices with an interslice gap of 2 mm, positioned through the basal ganglia and an acquisition bandwidth of 290 Hz. The position of the refocusing radiofrequency pulse was shifted from its original position towards the excitation radiofrequency pulse by several time shifts = 0, 2.3, 6.9, 11.5 and 13.8 ms, thus varying the sensitivity of the sequence to magnetic field variations.

Post-processing of MRI images

Magnetic field correlation

Prior to post-processing all images were screened for artifacts and clinical abnormalities by a clinical neuroradiologist from the radiology department of the LUMC. Furthermore structural images were subjected to external quality control by a contract research organisation (IXICO Ltd, London, UK). ASE images were fitted to a theoretical model relating the signal decay to the homogeneity of the magnetic field[24]. The resulting MFC maps display the amount of magnetic field inhomogeneities present for each voxel. Higher MFC values correspond to a more inhomogeneous magnetic field. A correction was applied to the MFC maps to account for the contributions of macroscopic magnetic field inhomogeneities[21] using the non shifted image and the first asymmetric echo image of 2.3 ms. These macroscopic field inhomogeneities arising from, for example, the cavernous sinus and the skull are unrelated to iron content. The contributions from such areas were corrected to retain the underlying microscopic variations, such as those caused by iron, in the magnetic field in the remaining brain tissue.

Image Segmentation

Segmentation of the accumbens nucleus, amygdala, caudate nucleus, hippocampus, globus pallidus, putamen and thalamus was performed on the T_1-weighted scans using the FIRST tool[34] from FMRIB's Software Library (FSL, Oxford). Based on these segmentations, the absolute volume of each structure was calculated with FSLstats (FSL, Oxford), where the resulting value represents the total bi-lateral volume of a single structure. The volumetric analysis procedure, including a correction for intracranial volume, was identical to that described previously[27] making use of the FSL tools (FSL oxford). The segmentation masks were registered to the MFC maps using the T_1-weighted scans and the quality of the registration was visually inspected. Average MFC values were calculated for each segmented bilateral structure.

Statistics

To examine differences in MFC and volume between the groups, one-way Multivariate Analyses of Covariance (ANCOVA) were performed with the MFC value, or volumetric value (corrected for intracranial volume), of each subcortical structure as the outcome variable, while controlling for age and gender. The *p* value after post-hoc Bonferroni correction was considered significant at $p<0.05$.

To determine the independent potential for MFC value as a marker of disease state in HD, again only gene carriers were examined (premanifest+early HD, n=49). Logistic regression analysis was performed with disease state (premanifest vs. early HD) as the variable to be predicted. Age, gender, CAG repeat length, MFC value and volume of the relevant subcortical structures were included as predictor variables[19;35]. These were entered in one block (ENTER method) during analysis. All statistical analyses were performed with the

SPSS 17.0 package (SPSS Inc., Chicago, USA).

Subsequently, two analyses were performed to investigate the potential correlation between MFC values in any affected regions and volume changes in all gene carriers (premanifest + early HD). First, to investigate the direct relationship between MFC value and volume of the individual subcortical structures, Pearson's partial correlation analysis was performed, whilst controlling for the same covariates as in the logistic regression, namely age, gender and CAG repeat length. Secondly, to test for the potential influence of the interaction term (MFC value*volume) between MFC value and volume on disease state (premanifest or early HD), a general linear model univariate analysis of variance was performed.

To understand the potential relationship between iron levels and clinical measures, Pearson's partial correlation analysis was performed between iron in regions showing aberrant levels and clinical measures while controlling for age, gender and CAG repeat length. This was performed in the gene carrier group for the Unified Huntington's Disease Rating Scale – Total Motor Score (UHDRS), the Unified Huntington's Disease Rating Scale - Total functional Capacity measure (TFC), the number of correct answers on the Symbol Digit Modalities Test (SDMT), the number of correct answer on the Stroop word reading test (SWR) and the Beck's Depression Inventory 2nd Edition (BDI-II). For the premanifest gene carriers only an additional analysis was performed to understand any relationship between iron levels and the number of years to predicted disease onset, calculated according to the method by Langbehn *et al*. (2004)[35]. Pearson correlation was applied without covariates, as it is not appropriate to include covariates that are used in the prediction of expected disease onset. The *p* value was Bonferroni corrected for multiple comparisons.

Results

MFC value

No significant group differences were found for age, gender or education levels between controls, premanifest gene carriers, and early HD (table 1). The early HD group demonstrated lower scores on the TFC and higher total scores on the UHDRS ($p<0.001$) compared to the healthy control group and premanifest gene carriers.

Typical examples of the brain structure segmentations (figure 1a) and MFC maps in a healthy control (figure 1b), a premanifest gene carrier (figure 1c) and an early manifest patient (figure 1d) are shown in figure 1. This figure demonstrates the spatial distribution of the magnetic field inhomogeneities, with the highest values visible in the caudate nucleus, putamen, and globus pallidus. Higher MFC values were observed in the early HD group: the MFC group averages can be found in table 2 and are displayed in figure

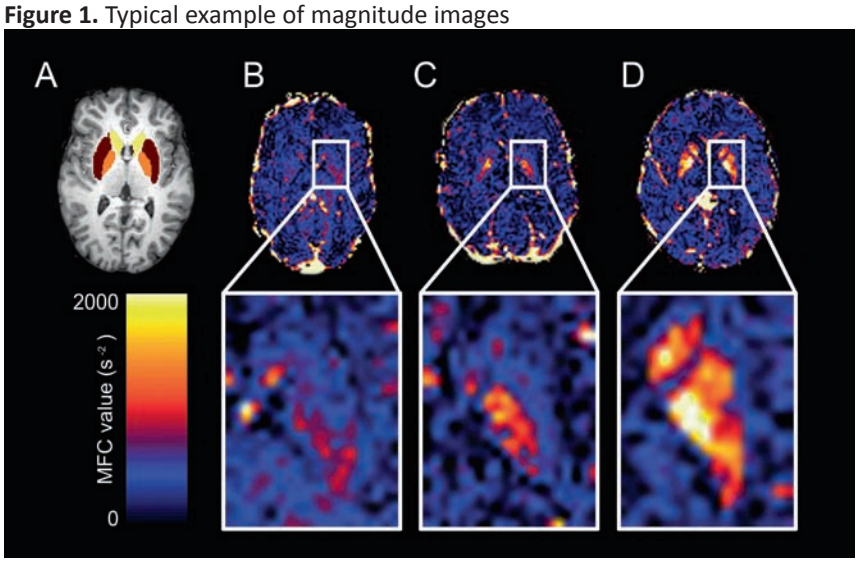
2. The MFC values in the caudate nucleus and putamen of HD patients were found to be significantly higher than in healthy controls (p = 0.03 and p = 0.003, respectively). No significant differences were found between premanifest gene carriers and controls. No other nuclei showed differences either between early HD patients and controls, or between premanifest gene carriers and controls.

Table 1. Demographic information of controls, premanifest gene carriers, and early Huntington's disease

		Healthy Controls	Premanifest Gene Carriers	Early Manifest Patients
N		25	22	27
Gender	Female/Male	13/12	13/9	19/8
Age	Mean yrs ± SD	50.3 ± 8.3	45.6 ± 8.5	50.0 ± 9.9
	Range (min – max)	36 - 66	27 - 62	30 - 64
Education level	Mean ISCED level ± SD	3.4 ± 1.1	3.8 ± 1.1	3.2 ± 1.3
UHDRS	Mean total score ± SD	2.1 ± 1.7	2.6 ± 1.4	25.2 ± 15.4*
TFC	Mean ± SD; Range: 0-13	12.9 ± 0.2	12.5 ± 0.8	9.8 ± 2.8*

N = number of participants, SD = Standard Deviation, ISCED = International Standard Classification of Education (range 0-6), UHDRS = Unified Huntington's Disease Rating Scale – Total Motor Score, TFC = Total Functional Capacity, * Significantly different from both healthy controls and premanifest HD at p<0.001.

Figure 1. Typical example of magnitude images



A) Magnitude image showing segmentation of caudate nucleus (yellow), putamen (brown) and globus pallidus (orange). The corresponding MFC maps for a healthy control (B), premanifest gene carrier (C) and early Huntington's disease patient (D). High MFC values are found in the subcortical grey matter structures, known to correspond with high iron concentrations. Highest values are found in the early HD patient. The high MFC values found laterally near the tissue-skull interface are caused by macroscopic magnetic field inhomogeneities from the skull tissue interface that could not be properly corrected. The distance to the areas of interest is so large that no interference with measurements in the deep gray matter structures is expected.

Figure 2. Groups differences in MFC

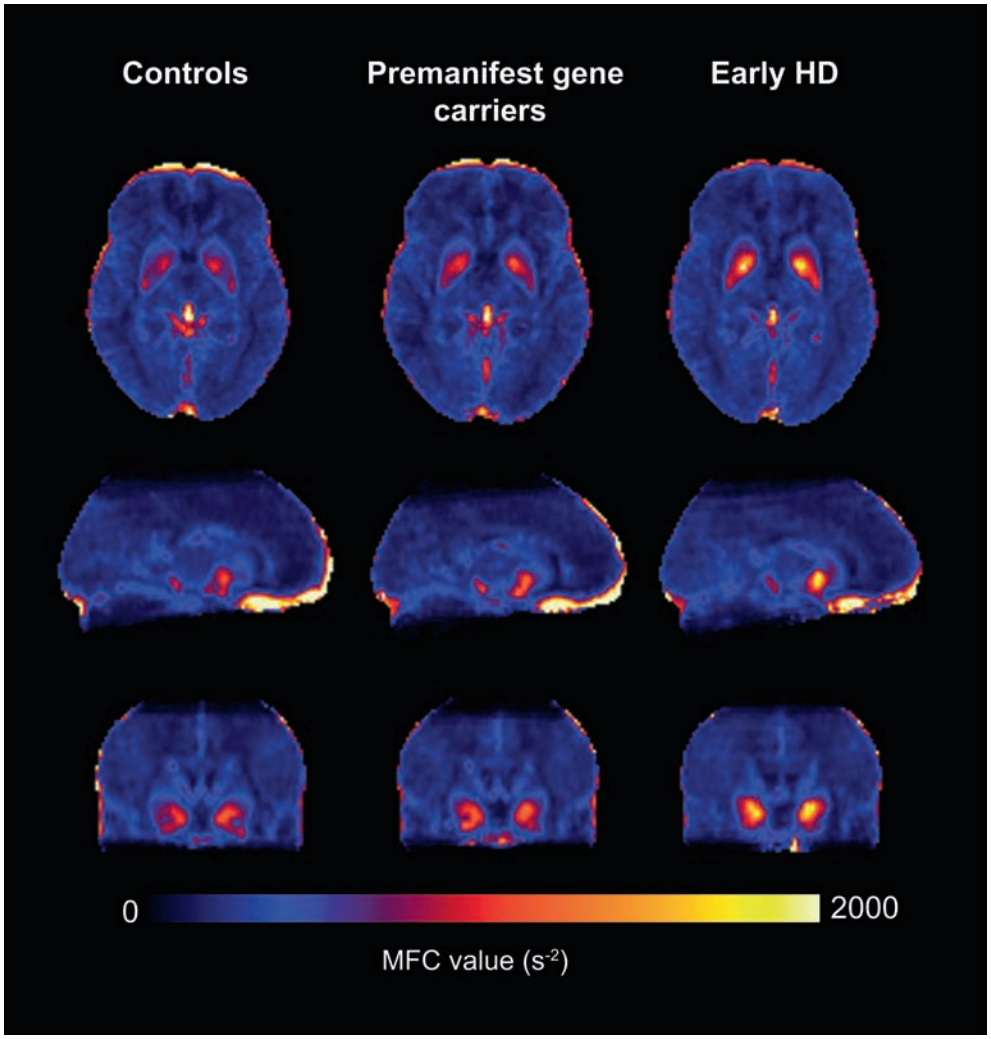


Figure 2: MFC group averages for controls, premanifest and manifest HD. All images were registered to standard space and averaged. The resulting average is a visual display of the values shown in table 2, however more localised information is present in this visual display. High MFC values are found in the subcortical grey matter structures, known to correspond with high iron concentrations.

Volume

The volumes of the caudate nucleus and putamen were smaller in early HD patients compared to premanifest gene carriers and controls, and also smaller in premanifest gene carriers as compared to controls (all $p<0.0001$). Smaller volumes of the accumbens nucleus ($p<0.0001$), globus pallidus ($p<0.0001$) and thalamus ($p=0.001$) were also found in early HD as compared to healthy controls only (table 2).

Table 2. Average MFC values and subcortical grey matter volumes per subcortical nucleus per study group

	Mean MFC(s^{-2})	SD	Volume (ml)	SD
Accumbens nucleus				
Control group	385.6	101.2	1.10	.25
Premanifest	343.2	93.7	**.98***	.19
Early HD	450.3	219.1	**.76**§**	.17
Amygdala				
Control group	446.6	122.0	2.54	.44
Premanifest	419.0	108.6	2.46	.45
Early HD	412.2	111.9	2.29	.42
Caudate nucleus				
Control group	398.6	55.0	6.96	.91
Premanifest	372.4	76.8	**5.86****	.72
Early HD	478.1*§§	153.1	**4.72**§§**	.76
Hippocampus				
Control group	452.0	102.8	7.91	.82
Premanifest	400.0	59.8	7.80	.80
Early HD	409.6	102.2	7.21	.86
Globus pallidus				
Control group	643.7	120.8	3.54	.42
Premanifest	715.3	215.2	3.27	.47
Early HD	807.4	368.9	**2.67**§§**	.44
Putamen				
Control group	481.8	120.6	4.83	.71
Premanifest	491.8	154.0	**4.14****	.50
Early HD	**632.1**§**	190.6	**3.28**§§**	.50
Thalamus				
Control group	388.4	60.3	15.23	1.45
Premanifest	377.8	92.9	14.80	1.39
Early HD	373.6	82.1	**13.67****	1.39

MFC values represent average value in s^{-2}. Volumetric analysis performed with correction for intercranial volume, volumes reported represent absolute total volume of each left and right nucleus.

* Significantly different from healthy controls (p<0.05)

** Significantly different from healthy controls (p<0.005)

§ Significantly different from premanifest gene carriers (p<0.05)

§§ Significantly different from premanifest gene carriers (p<0.005)

Predicting disease state

Logistic regression was performed to assess the predictive quality of subcortical MFC values of the caudate nucleus and putamen to disease state in all gene carriers (premanifest or early HD) as shown in table 3. The MFC values and volumes of the caudate nucleus, and putamen were significant predictors of disease state. In contrast, age, gender and CAG repeat length were not significant contributors to the prediction of disease state. Figure 3 shows the relationship between volume and MFC value for the caudate nucleus and putamen. In assessing further the independence of these two significant predictors the Pearson's partial correlation showed that the MFC value and volume were not related

to one another in the caudate nucleus (r=-0.105, *p*=0.486) or putamen (r=-0.132, *p*=0.382) in gene carriers (premanifest + early HD). The univariate analysis of variance assessing any potential interaction effect of MFC and volume on disease state also showed no interaction (caudate nucleus: *p*=0.916; putamen: *p*=0.992).

Table 3. Logistic regression model for predicting disease state (premanifest vs. early manifest) in all gene carrying participants

	Total model			Level of statistical significance (p value)	
	χ^2	DF	p	MFC value	Volume
Caudate nucleus	33.3	5	<0.0005	0.02	0.01
Putamen	40	5	<0.0005	0.04	0.01

Table shows only those predictors that significantly contributed to the model. MFC values represent average value per subcortical nucleus. DF = degrees of freedom. Volumes included are total volume of left and right nucleus per subcortical structure.

Relationship to clinical measures

The relationship between clinical measures and iron levels in the caudate nucleus and putamen was examined for both gene carriers groups. No relationship was found between UHDRS total motor score, TFC, SDMT, SWR or BDI-II and iron levels in the putamen or caudate nucleus. For the premanifest group only correlation no significant relationship between predicted years to onset and iron in the caudate nucleus or putamen was shown. Individual iron levels versus a measure of disease load, namely burden of disease pathology[33], are displayed for individual gene carriers in figure 4.

Figure 3: Individual MFC values set out against volumetric data for (A) caudate nucleus and (B) putamen.

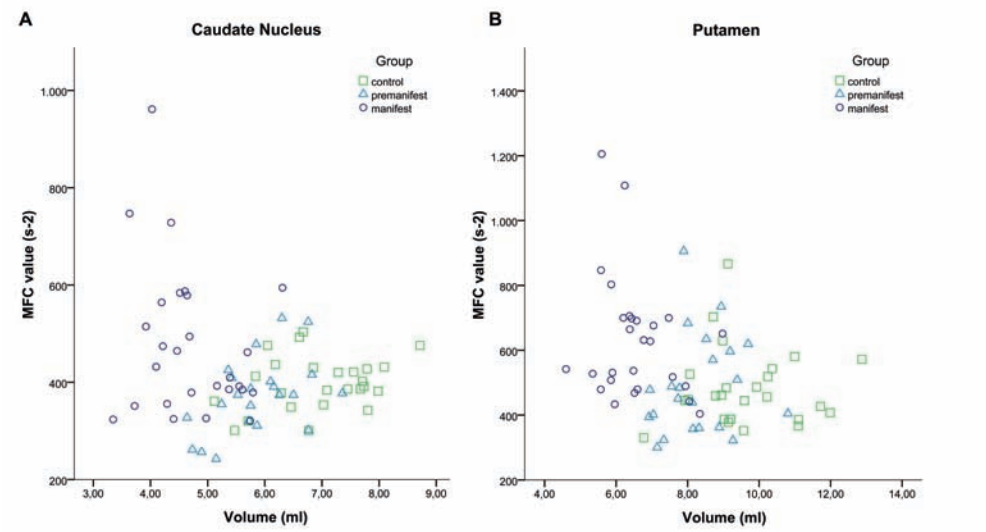
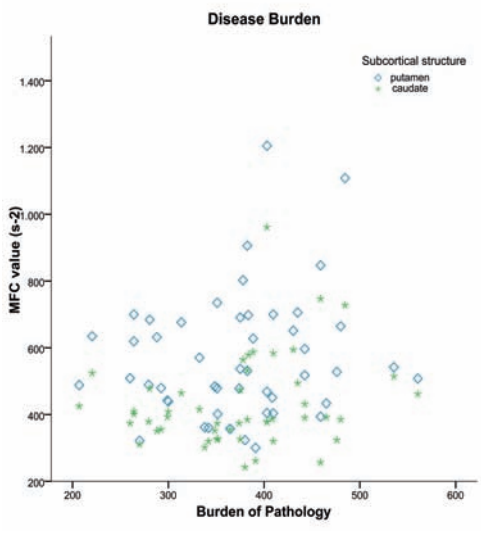


Figure 4: Burden of disease pathology scores ((CAG - 35.5) * age) versus MFC values for all gene carriers (premanifest + manifest) in the caudate nucleus and putamen.



Discussion

This study's main findings show increased levels of iron, as demonstrated by higher MFC values, in caudate nucleus and putamen in patients with early HD. MFC values were not significantly different to controls in premanifest gene carriers. For clarity and consistency with other studies, we will refer to changes in the measured iron-dependent quantity, namely MFC values, as changes in iron[11;16;36]. The iron levels in the putamen and caudate nucleus are not related to the volume of these structures. Furthermore, iron levels appear to predict the disease state (premanifest or early manifest) of HD gene carriers.

In early HD the caudate nucleus and putamen show significantly higher MFC values. This is in line with in vivo[11;19] and ex vivo[5;18] findings. The relative MFC values in the healthy controls correspond to ex-vivo-determined levels of iron, whereby the globus pallidus showed highest values for all participant groups, which is in agreement with previous studies of both aging and neurodegeneration[36-38].

No significant differences in iron were observed between premanifest gene carriers and healthy controls. The only other report in premanifest gene carriers found elevated iron in the globus pallidus[29]. However, this report was based on a group with less stringent inclusion criteria of individuals, namely without overt motor signs as opposed to a UHDRS cut-off point of 5. The correlation which Jurgens *et al*. (2010) found between the number of hypointense pixels and total motor score, demonstrates that all of the participants with higher iron levels would have been classified as having early manifest HD under our criteria[29]. It is certainly possible that the iron levels are not high enough for MFC, or any other existing imaging techniques, to pick up small changes in premanifest gene carriers, or that abnormal iron depositions do not occur until later in the disease process. The volumetric decreases of the caudate nucleus and putamen in premanifest gene carriers found in this present study are in line with previous reports[25-27].

This study has demonstrated that the individual iron content of the caudate nucleus and putamen independently predicts disease state in gene carriers (premanifest versus early manifest), also when taking into consideration the predictive value of other commonly related factors, such as volume, CAG repeat length or age. Furthermore the iron content in the putamen and caudate nucleus is not directly related to the volume of these structures, and there is no interaction effect present that explains disease state. Therefore, our results indicate that volumetric decrease and iron increase are two independent processes in HD, with iron accumulation not occurring as a direct result of volume decrease (Figure 4). This is an important finding as it reiterates the need for increasing our understanding of the pathophysiological processes related to iron in HD. When considering this conclusion it is important to exclude the possibility of epiphenomenonological results, especially in early

HD. We addressed this by accounting for those factors most commonly found to explain the variance between premanifest and manifest gene carriers[33;35]. We propose that iron levels may be regarded as a marker of disease state, as iron does not differentiate those prior to disease onset from controls, but does distinguish between premanifest gene carriers and those in the earliest stages of HD. Reproduction and longitudinal evaluation of potential iron accumulation may demonstrate the capacity to predict symptom progression or, as previously suggested, the age of disease onset[39].

Iron content in early HD was not found to be higher than in controls in the amygdala, hippocampus, nucleus accumbens or thalamus. This both replicates and also adds to previous findings in which no differences were found in the hippocampus and thalamus[19]. We have measured a pattern of elevated iron in early HD that could be toxic or an accelerated process of normal aging. However, oxidative stress related mechanisms, as a result of higher free radicals cannot be excluded[40]. It may be that iron depositions primarily affect nuclei that consist of the same types of cells, such as the medium spiny neurons common to the putamen and caudate nucleus, or as demonstrated in HD patients and mice, the microglia[4]. Many hypotheses on the role of iron in neurodegeneration have been formulated but the exact mechanisms remains unclear[12;41;42].

Globus pallidus iron depositions showed an increase in the early HD group as compared to controls: however, this was not statistically significant. This may be related to the sequence parameters, and not due to an intrinsic absence of higher iron levels, especially as another study in manifest HD[19] found higher iron in the globus pallidus. The chosen ASE parameters could be suboptimal for measurements in areas of very high iron concentration as a result of the pronounced signal reduction. As a result a higher standard deviation can be expected in the MFC maps within the globus pallidus, which was indeed the case. Future research at ultra high field strength (7 Tesla and above) could increase the sensitivity of the MFC method.

We believe that the technique used in our study is a more direct measure of iron than conventional T_2-, T_2^*- and SWI based methods that have been used extensively to study the distribution of iron in the brain[8;9;14], due primarily to the robustness with respect to any altered water concentrations. In the study by Bartzokis *et al*. (2007) T_2 relaxation values were obtained by scanning patients at two different field strengths and significant differences were found in iron concentration of the putamen, globus pallidus, and caudate nucleus in manifest HD patients[19]. This is comparable to the results of our study. Vymazal *et al*. (2007) measured T_2 relaxation values at a single field strength and found changes in the globus pallidus and white matter only in HD patients[16]. The discrepancy between these and our results can potentially be explained by the sensitivity of T_2 measurements to water content, which is thought to change during disease progression due to, for example,

breakdown of the structural integrity of myelin[9;15;19]. Although we cannot definitively exclude all potential sources of magnetic field variation[43], we believe that based on ex-vivo studies[4;14] the changes observed in our and previous studies are related primarily to increased iron. An advantage of MFC measurements is that the macroscopic component of the field inhomogeneities can be separated from the microscopic components, which is a confounding factor for T_2^* based techniques.

A possible limitation could be the influence of other sources of magnetic field variation, such as the cavernous sinus or skull. However all possible steps were taken to prevent this influence. After correction a remainder of macroscopic field variations is still present located laterally near skull-tissue interface and around large vessels. However, upon visual inspection, the macroscopic contribution of these regions around the basal ganglia are adequately corrected.

In conclusion, we have demonstrated that patients with early HD have higher magnetic field inhomogeneities in the caudate nucleus and putamen. This is not found in premanifest gene carriers. The iron content of the caudate nucleus, putamen seems to independently predicts disease state in HD gene carriers. Futhermore, we have demonstrated that increased iron accumulation is an independent disease process not related to structural atrophy.

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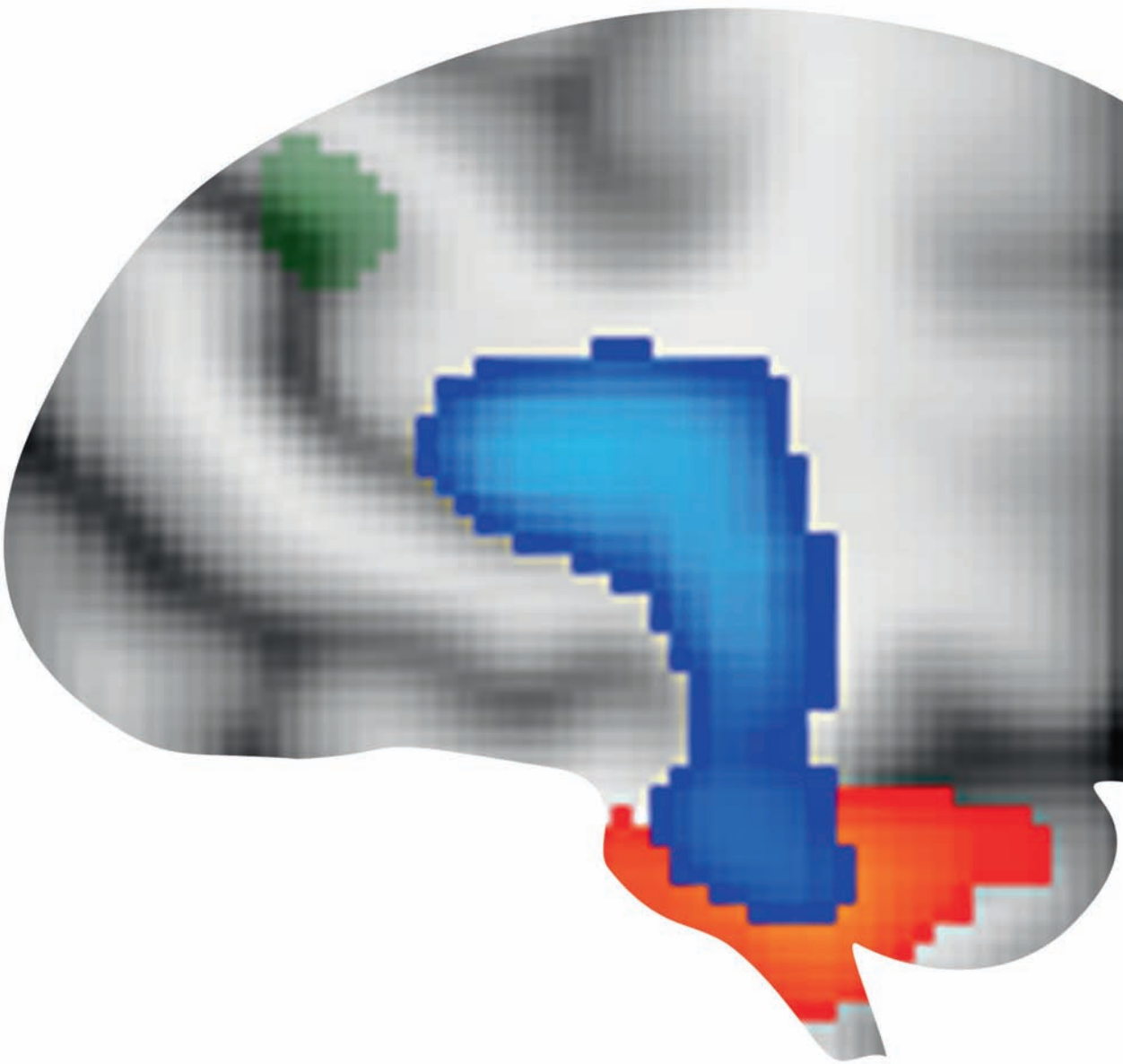

Chapter 8

Reduced functional brain connectivity prior to disease onset in Huntington's Disease

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submitted

Abstract

Background

Huntington's disease (HD) is characterised by both regional and generalised neuronal cell loss in the brain. Investigating functional brain connectivity patterns in rest in HD has the potential to broaden the understanding of brain functionality in relation to disease progression. This study aims to establish whether brain connectivity during rest is different in premanifest and manifest HD as compared to controls.

Methods

At the Leiden University Medical Centre study site of the TRACK-HD study, 20 early HD patients (disease stage 1 and 2), 28 premanifest gene carriers and 28 healthy controls underwent 3T MRI scanning. Standard and high-resolution T_1-weighted images and a resting state fMRI scan were acquired. Using FSL, group differences in resting state connectivity were examined for eight networks of interest using a dual regression method. With a voxelwise correction for localised atrophy, group differences in functional connectivity were examined.

Results

Brain connectivity of the left middle frontal and pre-central gyrus, and right post central gyrus with the medial visual network was reduced in premanifest and manifest HD as compared to controls ($0.05>p>0.0001$). In manifest HD connectivity of numerous widespread brain regions with the default mode network and the executive control network were reduced ($0.05>p>0.0001$).

Discussion

Brain regions that show reduced intrinsic functional connectivity are present in premanifest gene carriers and to a much larger extent in manifest HD patients. These differences are present even when the potential influence of atrophy is taken into account. Resting state fMRI can potentially be used for early disease detection in the premanifest phase of HD and for monitoring of disease modifying compounds.

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterised by progressive motor-, behavioural- and cognitive-dysfunction. The expansion of the HTT gene on chromosome 4 is eventually responsible for neuronal loss and dysfunction throughout the brain[1;2]. Previous studies have demonstrated that atrophy of both the deep grey matter structures and of the cortex are apparent in patients with HD, and also to a lesser degree in HD gene carriers prior to disease onset[3-6]. These premanifest gene carriers, who do not show symptoms of the disease but are certain of eventual disease onset, have also been found to show reduced integrity of white matter[7-9]. In patients with HD, both extensive white matter integrity loss and atrophy of white matter has been shown[9-11].

Clinical assessments in multiple functional domains have extensively objectified the impairments reported by patients and their companions[12-15]. Also in premanifest gene carriers numerous tests of functioning have shown diminished performance[16-18]. In an effort to bridge the gap between the observed clinical deteriorations and structural brain deficits, a number of studies have applied clinical assessments whilst observation of brain activity was performed using functional magnetic resonance imaging (fMRI).

Four task-based fMRI studies in manifest HD demonstrated a fairly homogenous profile, with reductions in brain activation in numerous cortical and sub-cortical brain regions[19-22]. However the results from the limited number of task-based fMRI studies in premanifest HD report a more heterogeneous pattern. Increased activation in several brain regions was found in premanifest gene carriers far from expected disease onset, and reduced activation was reported in premanifest gene carriers close to expected disease onset[23;24]. These task-based fMRI studies all challenged the brain during the MRI scanning yielding assessment and performance dependent results. An alternative approach is to examine the brain connectivity patterns without taxation.

Brain function depends on large-scale brain interactions[25]. Functional brain connectivity patterns can be examined at rest with fMRI and this approach is recognised as an important step towards understanding functional brain networks[26]. Hence, recent reports have incorporated resting state (RS) fMRI to examine the brain during both normal aging and disease[27-30]. Currently the earliest detectable brain changes in HD are atrophy of subcortical grey matter structures[17;31]. Given that cell loss presents as the result of a pathologic cascade it is plausible to expect functional brain changes prior to cell loss. In carriers of the APOE-4 gene, alterations in intrinsic functional connectivity have been observed even in the absence of changes in brain structure[32]. Such interactions have not been studied in premanifest HD. Functional brain changes may also occur in HD, either prior to, or as a result of brain atrophy. RS fMRI has the potential to give insight into

potential functional changes. RS fMRI could be implemented for early disease detection and could evaluate the effect of future neuroprotective or therapeutic compounds. This study aims to establish whether functional brain connectivity at rest is altered in both premanifest HD gene carriers and early manifest patients.

Methods

Participants

At the Leiden University Medical Centre study site of the TRACK-HD study, subjects participating in the longitudinal TRACK-HD study underwent MRI scanning including fMRI during the baseline visit. Of the 90 participants included, 11 did not undergo the additional fMRI scan due to time constraints. Furthermore after quality control of the fMRI data, both visually and by means of the scan analysis reports generated during post-processing of the MRI data, three manifest HD participants were excluded from the analysis because of excessive motion (>4mm). In total 20 stage 1 and 2 HD patients, 28 premanifest gene carriers and 28 healthy controls were included in the fMRI analysis (table 1).

Inclusion criteria for HD patients included a positive genetic test for the *HTT* gene with 40 or more CAG repeats; the presence of motor disturbances defined as more than five points on the Unified Huntington's Disease Rating Scale – total motor score (UHDRS-TMS), and a Total Functional Capacity score (TFC) greater than or equal to seven points, thereby only including patients in the earliest two disease stages[33]. Inclusion criteria for premanifest gene carriers consisted of a positive genetic test with 40 or more CAG repeats, and the absence of motor disturbances with five or less points on the UHDRS-TMS. Finally, a burden of pathology score ((CAG repeat length -35.5) x age) greater than 250[34] was required. Age- and gender-matched gene-negative relatives of HD gene carriers and unaffected spouses were included as healthy controls. Exclusion criteria for all participants included previous significant head injury, any other neurological or major psychiatric disorder, or unwillingness to undergo MRI scanning. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre. All participants gave written informed consent. For full details of study parameters see Tabrizi *et al*. (2009)[31].

During further medical history taking, handedness was recorded by means of the Edinburgh Inventory 2nd version (Oldfield, 1970). For early HD patients, the rater's estimate of disease onset was determined, based on the rater's observations, reports by the patients and information from companions or relatives. With this information the current disease duration was calculated. For premanifest gene carriers the estimated number of years until disease onset was calculated based on their current age and CAG repeat length, by means of the formula developed by Langbehn *et al*. (2004)[35].

MRI protocol

MRI acquisition was performed on a 3 Tesla whole body scanner (Philips Achieva, Healthcare, Best, The Netherlands) with an eight channel receive array head coil. An anatomical T_1-weighted scan was acquired using an ultrafast gradient echo 3D acquisition sequence with the following imaging parameters: repetition time (TR) = 7.7 ms, echo time (TE) = 3.5 ms, field-of-view = 24 x 24 x 16.4 cm, matrix size 224 x 224, with a duration of 9 minutes. For post-processing registration purposes, a high resolution T_1-weighted scan, with the following parameters was collected; repetition time (TR) = 2200 ms, echo time (TE) = 30 ms, field-of-view = 220 x220x168 mm, flip angle = 80°, matrix size= 112x109mm, with a duration of 46 seconds. A RS fMRI scan with the following parameters was also obtained: repetition time (TR) = 2200 ms, echo time (TE) = 30 ms, field-of-view = 220 x 220 x 10.4 cm, resolution = 1.96 x 1.96 x 2, no slice gap, flip angle = 80°, matrix size 80 x 79, with a duration of 7.5 minutes. To reduce unnecessary sensory input that could influence the results, participants were not allowed to listen to music during the RS fMRI scan, and to ensure a wakeful disposition participants were asked to keep their eyes open with normal background light.

Pre-processing for RS fMRI analysis

Pre-processing of the RS fMRI data using the standard procedure was carried out using FSL 4.1.8[36]. The following steps were preformed: head motion correction[37], brain extraction[38], spatial smoothing using a Gaussian kernel of 5 mm full width at half maximum (FWHM). All volumes were normalised based on mean intensity and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, FWHM = 100s). The middle (reference scan) of each individuals RS fMRI time series was affine registered to MNI152 standard space (Montreal Neurological Institute, Montreal, QC, Cananda): initially, it was registered to the high resolution T_1-weighted scan. This high resolution T_1-weighted scan was subsequently registered to the anatomical T_1-weighted scan. Finally, the anatomical scan was registered to MNI152 standard space. By first registering the functional data to the high resolution scan and then to the anatomical T_1-weighted scan allows for better registration of the data. These three registration matrices were combined to obtain a matrix for transforming fMRI data from native space to standard space, using interpolation to 2x2x2 mm voxels. Visual quality control was performed to ensure correct registration.

Statistical analyses

Statistical analysis of group demographic was compared using SPSS (version 17, SPSS, USA). Where appropriate either Analysis of Variance or Chi-squared tests were applied. Resting state connectivity was examined using a dual regression method[32;39;40]. In doing so the similarity of the haemodynamic response patterns (fMRI signal) for each brain voxel was compared to the fMRI signal in eight pre-defined, well established, networks

of interest (NOIs)[41]. These networks encompass over 80% of the entire brain volume. The NOIs represent spatial template maps corresponding to medial visual (NOI1), lateral visual (NOI2), auditory (NOI3), sensorimotor (NOI4), the default mode (NOI5), executive control (NOI6), visual-spatial memory (NOI7), and working memory (NOI8) networks.

First, a spatial regression was applied: The eight NOIs and a CSF mask were used as spatial regressors in a general linear model (GLM) to obtain the nine corresponding dynamic patterns of fMRI signal fluctuations in each network from each individual's RS fMRI scan. Next, these nine time series, together with six motion correction parameters derived during preprocessing (three translations and three rotations) were used as temporal regressors in a second (temporal) GLM. For each voxel, the z-score corresponding to each of these 15 temporal regressors was obtained. A GLM was applied, resulting in spatial z-score maps for each individual's RS fMRI scan, for each NOI. This dual-regression method thereby generated eight z-scores maps reflecting the connectivity strength of each voxel in the brain to each of the eight NOIs. A voxel with a high z-score demonstrated a highly similar pattern of fMRI fluctuation to the voxels in the NOI.

These z-score maps were constructed to compare the groups. The group statistical analysis was performed to determine which brain regions showed statistically significant differences in connectivity to any of the NOIs between groups by applying three independent sample t-tests. This was performed with a voxel-wise correction for localised grey matter concentration to rule-out any potentially confounding impact of local structural loss on brain connectivity, as described by Oakes *et al*. (2007)[42]. In short, per individual the anatomical T_1-weighted scans were processed to provide voxel-based intensity maps of grey matter concentration, which were included as a voxel-wise covariate in the mixed effects model group analysis. Non-parametric permutation based statistical inference was used with 5000 repeated permutations per NOI for the comparisons; controls vs premanifest, controls vs manifest HD and premanifest vs manifest HD. Correction for multiple comparisons was applied using threshold free cluster enhancement based correction whereby all results under the threshold of $p<0.05$ were considered statistically significant[43]. This provided spatial information per NOI of brain regions demonstrating different connectivity patterns between the study groups.

Results

The groups did not differ in terms of age, gender, handedness and education level. Early HD patients had significantly higher UHDRS motor scores, CAG repeat lengths and lower TFC scores than premanifest gene carriers and/or healthy controls.

Table 1: Group characteristics of the study groups

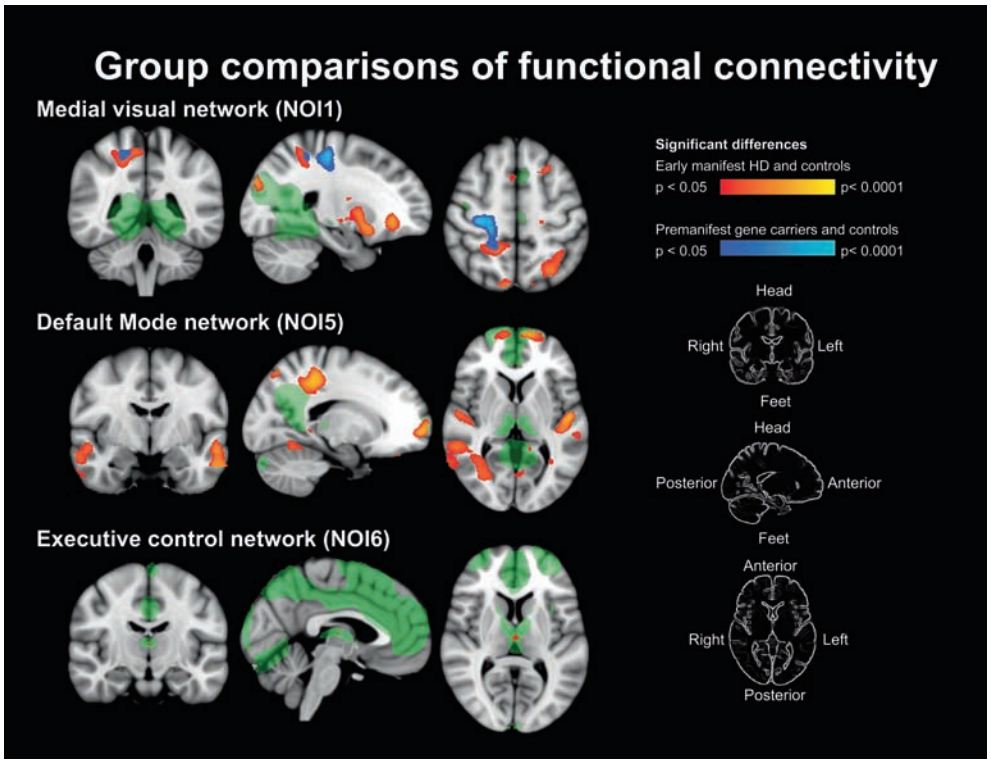
	Healthy controls	Premanifest gene carriers	Early HD patients
N	28	28	20
Gender M/F	13/15	11/17	5/15
Age (years) Mean (SD)	48.5 (8.5)	43.21 (8.2)	46.5 (10.6)
CAG repeat length Mean (SD)	n/a	42.5 (2.5)	44.1 (2.6)[§]
Total Functional Capacity Mean (SD)	12.9 (1.9)	12.6 (0.8)	10.2 (1.9)*[§]
UHDRS - Motor Mean (SD)	2.5 (2.5)	2.4 (1.4)	20.3 (11.0)*[§]
Expected disease onset (years) Mean (SD)	n/a	11,6 (4.4)	n/a
Disease duration (years) Mean (SD)	n/a	n/a	6.8 (7.4)

N = number of participants, SD = Standard deviation, n/a = not applicable, UHDRS – Motor = Unified Huntington's Disease Rating Scale – total motor score, * significantly different to controls at $p< 0.05$, [§] significantly different to premanifest gene carriers at $p< 0.05$.

In both premanifest gene carriers and early manifest HD the same regions in the left frontal lobe and the right parietal lobe displayed reduced connectivity with the NOI1 (medial visual network), as compared to controls ($0.05>p>0.0001$). The area in the left frontal lobe comprised the grey matter near the pre-central and middle-frontal gyri. The area in the parietal lobe was localised in the post-central gyrus (figure 1). Premanifest gene carriers only, also displayed reduced connectivity bi-laterally of the cingulate gyrus with NOI1 compared to the controls. This area of reduced connectivity was not found in the early HD group. The manifest HD group demonstrated additional areas of reduced connectivity with NOI1 that were not observed in the premanifest gene carrier group. These areas were located bi-laterally within the superior occipital lobe, within a large field in the deep grey matter, including the putamen, globus pallidus, thalamus, and bi-laterally in the cortex of the frontal orbital region (figures 1).

The connectivity of the left parietal lobe, the pre-frontal cortex in both hemispheres, and regions of grey and white matter in the both temporal lobes with NOI5 (the default mode network) was reduced in early HD only as compared to controls ($0.05>p>0.0001$) (figure 1). Connectivity of a small region in the thalamus and the left supramarginal gyrus with NOI6 (executive control network) was reduced in manifest HD as compared to controls ($0.05>p>0.0001$) (figure 1). No differences between any of the study groups were found in the connectivity with the other NOIs.

Figure 1. Group comparisons of functional brain connectivity shown in three orientations.



Green areas show the voxels encompassing the network of interest (NOI) with which the connectivity decreases are present. Blue - light blue areas show the areas of reduced connectivity with the NOI between premanifest gene carriers and controls, red-yellow areas show the areas of reduced connectivity with the NOI between early manifest HD and controls. Some are of blue and red overlap is present, here the functional connectivity is reduced in both premanifest and manifest HD.

Discussion

Reductions in intrinsic functional connectivity are apparent in both premanifest gene carriers and patients with early HD. The earliest areas to show a reduction in connectivity are regions within the left frontal and right parietal and bilateral visual lobes. These areas also demonstrated reduced connectivity in the early manifest group. Further connectivity reductions were also apparent in many other brain regions in early HD such as subcortical grey matter and the occipital lobes. These observed differences to healthy controls are not explained by brain atrophy.

In premanifest gene carriers our findings show reduced connectivity of the medial visual network (NOI1) with the left frontal, right parietal and bilateral cingulate gyrus during rest. The only known other report of RS fMRI in HD is a methodological report

describing the stability and suitability of RS fMRI over a one year follow up period in premanifest gene carriers. No group differences in any of their prespecified interregional fMRI correlations were found[44]. The prespecified two seed regions used for interregional correlation were different to the networks applied in the current paper, and this methodological dissimilarity may explain the variation in outcome. Carriers of genes resulting in neurological diseases other than HD have been found to show aberrant intrinsic functional connectivity in the absence of disease signs[32;45], thus supporting the occurrence of functional brain changes prior to a disease manifestation. Results from other studies using task based fMRI in HD also support our findings[23;24;46-48]. These studies show disrupted activation (either increased or decreased) in areas that do not form an identical spatial match to our results, but do show great similarity of involved brain areas. Some regions with altering between region connections in this study, such as the left frontal lobe, specifically in the middle frontal gyri and pre-central gyrus, have also shown locally decreased task related fMRI activation[23;24;46-48]. Task based fMRI studies also demonstrated the implication of the post central gyrus[48], and the bilateral cingulated cortex[23;24;48]. Further support for our finding is found in results using a different imaging technique that measures blood perfusion during rest. Cerebral blood flow was found to be altered in premanifest gene carriers in prefrontal brain regions[49]. Our results demonstrate that early reductions in intrinsic functional connectivity are present prior to the clinical manifestation of HD. This is an important finding as therapeutic interventions may wish to monitor the functional impact of a compound on the brain in the absence of clinical outcome measures.

In the early HD group, our findings of reduced connectivity encompass more and larger regions in the brain than of premanifest gene carriers. Some, but not all of these regions have previously been shown to show disturbed activation during task based fMRI. The disrupted activation was reported in the same brain areas with which we found reductions in connectivity of the medial visual network (NOI1); left frontal lobe[20;50], right parietal lobe[20;50], superior occipital[20] and frontal orbital[22] regions in both hemispheres, and specific subcortical structures such as the putamen[22;48]. However, no previous literature describes involvement of the globus pallidus, or thalamus. The brain regions demonstrating reduced connectivity with the default mode network (NOI5) were also reported to show altered activation during performance, such as with the left parietal[50], and bilateral prefrontal cortices[21;48] and temporal lobes[22]. The reduction of connectivity of the left supermarginal gyrus and thalami with the executive control network (NOI6) during rest, does not find support in other studies of connectivity or brain activation during task execution. Despite the different nature of RS fMRI versus task based fMRI, our current findings do seem complementary to the task-based fMRI results. With RS fMRI overall brain connectivity is examined that is not limited to task related brain regions, and we have demonstrated that the connectivity of multiple brain networks is affected in HD.

The brain regions demonstrating reduced connectivity as compared to healthy controls showed overlap between premanifest gene carriers and early HD patients, possibly indicating progressive functional deficits. The regions demonstrating reduced connectivity generally occur bilaterally and throughout the brain, especially in manifest HD.

It is unknown whether reduced connectivity patterns reflect connectivity that is limited or non-existent due to neuronal death or whether such results reflect intact but abnormally functioning neurons in HD. The results from this current study suggest that the latter may be a more accurate reflection, given that atrophy reflects (advanced) volume loss as a result of neuronal death, and that our results remain valid when taking into consideration MRI detectable regional atrophy. Therefore, it is not likely that reductions in functional connectivity can be explained solely by neuronal death in HD.

The strengths of this study lay in the comprehensive and exploratory nature of the fMRI analysis. As this study was performed in a single sample of strictly selected premanifest and early manifest HD the results reflect varying stages of disease progression. Furthermore, by taking atrophy into consideration the potential influence of cell loss on connectivity results was reduced. Examination of brain networks encompassing almost the entire brain allowed for a hypothesis generating approach. Therefore the brain regions found to display reduced connectivity may be targeted in future studies of HD. The limitations of this study lay in the potential for the influence of motion artefacts due to chorea. However, every effort was made to prevent motion during scanning and furthermore strict quality control was applied to prohibit the inclusion of poor quality scans. This resulted in the exclusion of three scans from the manifest HD group. Also, the influence of excessive motion was reduced by including a strictly selected early HD group where chorea is generally limited. Other limitations are the novelty of the technique and the cross-sectional design. To further understand if connectivity patterns are indeed affected by the progressive nature of this degenerative disease, study reproduction and longitudinal follow-up is essential in all study groups. Longitudinal follow-up using RS fMRI has the advantage over task-based fMRI that it is easier to standardise for cross-site, cross-cultural studies. In the multi-site follow up of the TRACK-HD study the evaluation of RS fMRI as a biomarker for HD is ongoing.

In conclusion, we have demonstrated that in the absence of processes that put demand on the brain, the HD brain functions differently. These findings are not explained by the presence of cerebral atrophy. We have shown that these functional differences are present not only after disease manifestation but also in the preceding ‘premanifest’ phase. Functional connectivity measures can potentially be used for early disease detection and for monitoring of disease modifying compounds.

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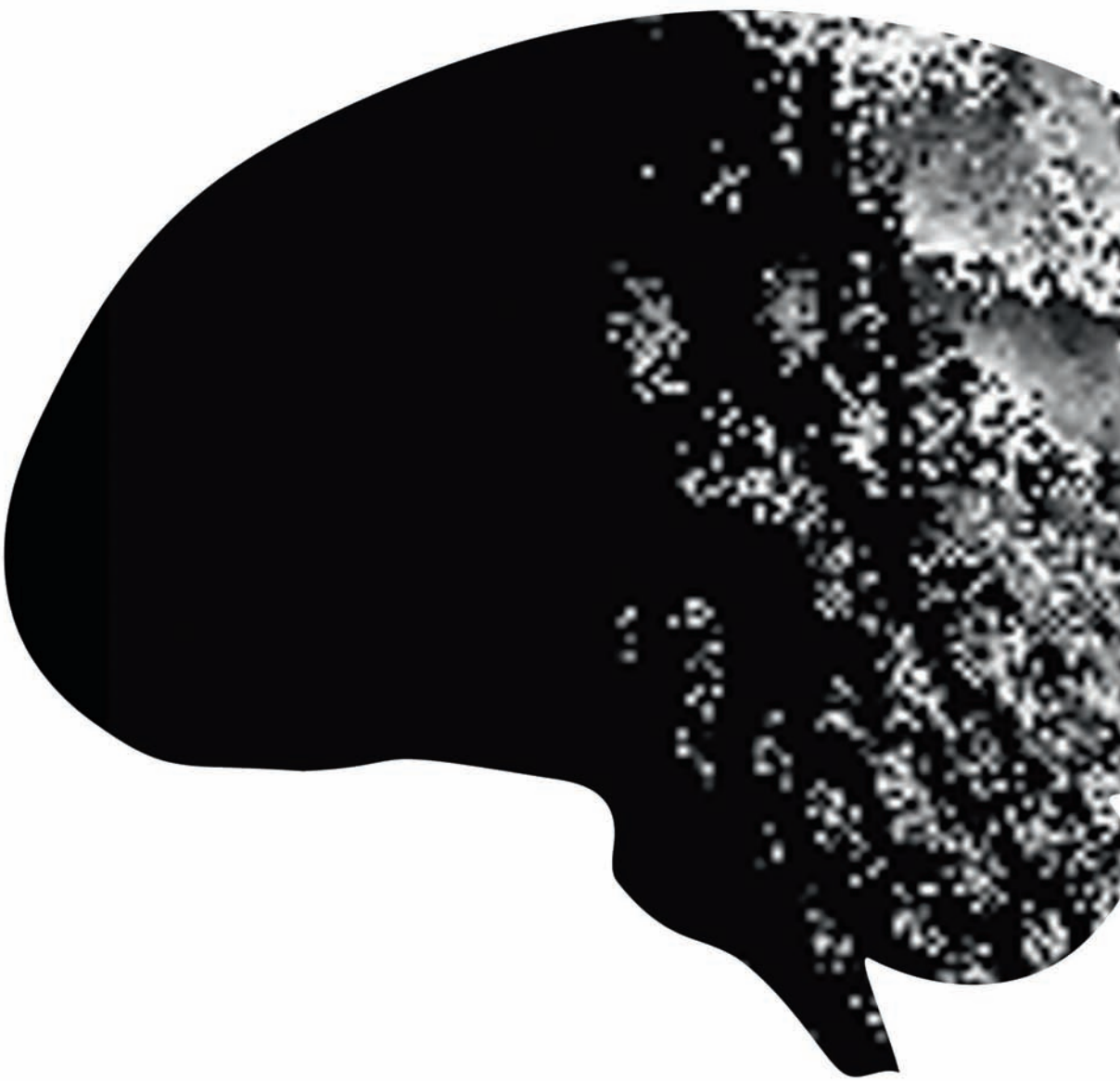

Chapter 9

Conclusions and future perspectives

Conclusions

The aims of this thesis were to gain further insight into specific disease processes in HD and to identify promising biomarkers. To achieve these aims, cognitive functioning, structural brain characteristics and intrinstic functional brain connectivity of premanifest and early HD subjects were examined.

Understanding disease processes

Following a review of the current literature on cognitive functioning in premanifest and manifest HD, we concluded, in chapter 2, that cognitive deficits show a differential progression. During late disease stages all cognitive domains show moderate or severe disturbances. The global profile of cognitive functioning in premanifest HD is characterised by subtle deficits of psychomotor speed, negative emotion recognition and some executive functions. In manifest HD these deficits progressively worsen and are eventually accompanied by memory dysfunction. Global cognitive abilities and language capacities are the last to show deterioration. Finally, these cognitive deficits in HD result in a generalised dementia.

In chapter 3 we demonstrated that visuospatial working memory is not only deficient in early manifest patients but also in premanifest gene carriers. Contrary to our expectations we found worsened accuracy and performance speed in early HD patients. Patients did not slow their performance in order to maintain accuracy. The absence of this 'speed accuracy trade off' and the presence of a 'worse-worse' phenomenon generates the hypothesis that advice such as 'pace yourself, take your time' may not have the desired effect in HD. Furthermore, it suggests that patients may need more time to integrate visually presented information.

The frontostriatal circuit encompasses the brain structures thought to be involved in the performance of complex cognitive processes such as visuospatial memory[1;2]. The frontal cortex, the striatum, and the white matter connecting the two comprise the frontostriatal circuit[3]. In this thesis, these brain structures were examined in terms of structure (chapters 4 and 7), metabolism (chapter 5) and function (chapter 8).

Since the earliest assessments of brain changes in HD, distinct grey matter deterioration or loss of striatal volume have repeatedly been found in HD[4-6]. In chapter 4, we concluded that atrophy of subcortical nuclei show a differential deterioration profile. In premanifest gene carriers within approximately 11 years to onset we found atrophy of the hippocampus, accumbens nucleus, globus pallidus, thalamus, and brainstem, alongside the well established atrophy of the caudate nucleus and putamen[7;8]. In manifest HD, further

atrophy of these structures was observed, with marked loss of hippocampal volume. Interestingly, atrophy of the brainstem and thalamus was found to correlate with whole brain atrophy, and therefore the volume loss of these grey matter structures may not reflect an accelerated degenerative process, as seems to be the case for the hippocampus, accumbens nucleus, thalamus, caudate nucleus and putamen.

Ex vivo studies have demonstrated iron accumulation in the caudate nucleus and putamen[9]; where cellular structure deterioration in HD was also shown[5;10]. The relationship between atrophy and iron accumulation in vivo has not been previously examined. In chapter 7 we demonstrated that iron accumulation is present in the early stages of HD and that iron accumulation and atrophy seem to reflect independent disease processes. Increased magnetic field inhomogeneities suggestive of elevated iron accumulation were found in the putamen and caudate nucleus of early HD patients. In premanifest gene carriers elevated iron accumulation was not found in any of the examined subcortical structures. The thalamus, hippocampus, globus pallidus, amygdala and accumbens nucleus were also not affected in early manifest HD. After assessing the volume of the subcortical structures, we examined the relationship of atrophy to iron accumulation in these structures. Both volume is lost and iron accumulates in the caudate nucleus and putamen, however, we established that these processes are independent.

In chapter 5 magnetic resonance spectroscopy was used to examine metabolic levels in the caudate nucleus, putamen, thalamus, hypothalamus, and frontal lobe. The caudate nucleus and putamen show reduced creatine in early manifest HD suggestive of reduced energy metabolism[11]. Furthermore, N- acetylaspartate reductions in the caudate nucleus and putamen suggests that the integrity and vitality of neurons is negatively affected in early manifest HD[11] . This finding is reinforced by the reduced structural integrity, as measured with DTI, of the caudate nucleus (chapter 6). Therefore, not only the absolute volume of the subcortical structure, but also the function and integrity of the remaining tissue is reduced in early HD.

Structural and functional connectivity of brain regions are of importance as adequate brain functioning relies on their extensive interactions[12]. Disturbed integrity proved to be relevant not only for grey matter structures, but also along functional pathways connecting these structures to the rest of the brain. In premanifest gene carriers the integrity of the white matter pathway of the sensorimotor cortex was reduced (chapter 6). In line with these findings we also found evidence that the intrinsic functional connectivity of left middle frontal and pre-central gyri, and right post central gyrus with the medial visual network was reduced prior to disease onset (chapter 8). In manifest HD, a similar but more widespread pattern of reduced integrity of white matter pathways (chapter 6) and functional connectivity of cortical regions was observed (chapter 8).

When all structural and functional brain changes are considered together, a broad picture of multi-level deficits begins to emerge. Cortical, subcortical and the intermediate white matter brain tissue show evidence of structural and functional decline. This is supported by previous findings demonstrating cortical thinning[13;14], similar subcortical atrophy[6] and reduced integrity in selected white matter regions[15-17]. We found evidence that several disease processes, such as altered metabolism, excessive iron accumulation and cell loss, play a role in the observed changes. We conclude that changes occur throughout the brain from the earliest disease phase onwards. Hence, both premanifest and manifest HD should not be regarded as a disorder of the basal ganglia, but as a disease affecting the whole brain.

Identifying promising biomarkers

By reviewing the existing literature on cognitive functioning in HD we concluded that the most promising cognitive biomarkers are measures from the domains of working memory, psychomotor speed, recognition of negative emotions, and attention or visuospatial executive functions (chapter 2). We showed that a measure of visuospatial working memory has good cross-sectional sensitivity for distinguishing premanifest gene carries and early HD groups from controls. When assessing the value of visuospatial working memory as a biomarker over a 12 month follow-up period, the measure was sensitive to deterioration in patients in stage two of the disease (chapter 3). For potential therapeutic trials aiming to improve cognitive capacities, such a measure may be useful from stage two HD onwards.

Iron levels were examined for their potential as a biomarker. We conclude that iron is not suitable as an early biomarker but has good potential as a marker of disease state (chapter 7). Metabolic changes in HD are also apparent from the manifestation of the disease onwards (chapter 5). For this reason reductions in creatine and N-acetylaspartate may also be good markers of disease state in HD.

We have shown that several measures reflect early changes in the brain, prior to the appearance of overt clinical signs. We demonstrated that the process of early cell loss in the brain encompasses numerous subcortical structures (chapter 4). Therefore, in addition to the caudate nucleus and putamen, volume loss observed in the accumbens nucleus and pallidum are potentially sensitive as biomarkers from ten years prior to disease onset onwards (chapter 4). In the premanifest phase of the disease, other markers also reflect early changes in the way brain regions interact. Reduction in integrity of a white matter pathway (chapter 6) and reduced intrinsic functional connectivity of similar regions (chapter 8) was demonstrated. These changes in integrity and functional connectivity were found to encompass more brain regions in manifest HD (chapters 6 and 8), thereby

reflecting the progressive nature of the disease.
In summary, candidate biomarkers that have the potential to objectively reflect the early changes and the progressive nature of the disease are measures of subcortical atrophy, integrity of white matter pathways and of intrinsic functional brain connectivity. Iron, creatine, and N- acetylaspartate concentrations in the caudate nucleus and putamen may prove to be most useful as markers of disease state for objectifying transitional disease processes from premanifest to manifest HD. Visuospatial working memory could be applied as a state marker for stage two HD.

Future perspectives

HD is a disease that affects the whole brain. Therefore, to further understanding of HD, future research should take a comprehensive approach. Furthermore, this may provide more insight into the relationship between structural changes and functional disturbances.

The therapeutic interventions that are currently being developed aim to reverse the destructive processes of HD. However, it has not been established whether the brain changes observed in premanifest gene carriers result from the slow onset of pathofysiological processes, or whether the brains of HD gene carriers develop differently. If brain changes are developmentally determined, reversal is futile. Prior to disease onset, gene carriers experience a healthy life, however, little is known about the impact of mutant huntingtin during the development of their brains. We do not know whether the structural and especially functional differences are already present in very young gene carriers.
Due to the ethical limitations posed by genetically testing at-risk children, developmental research is challenging. However, if a solution overcame these challenges, observational developmental research should be performed. In the absence of such a solution, larger groups of premanifest gene carriers must be observed in varying stages of proximity to disease onset. The premanifest gene carriers examined in these studies were all within approximately two decades of disease manifestation. Future research could include the youngest premanifest gene carriers available; those furthest from estimated disease onset. Such groups must be observed over time as they transition through phases of premanifest HD to manifest HD to establish the most sensitive measure for early disease detection. This also requires longitudinal observation.

Measures of disease change need to be sensitive in both premanifest and manifest HD. Hence, although focus on premanifest gene carriers is important, observational research should always include the fullest range of gene carriers possible, also including manifest HD patients.

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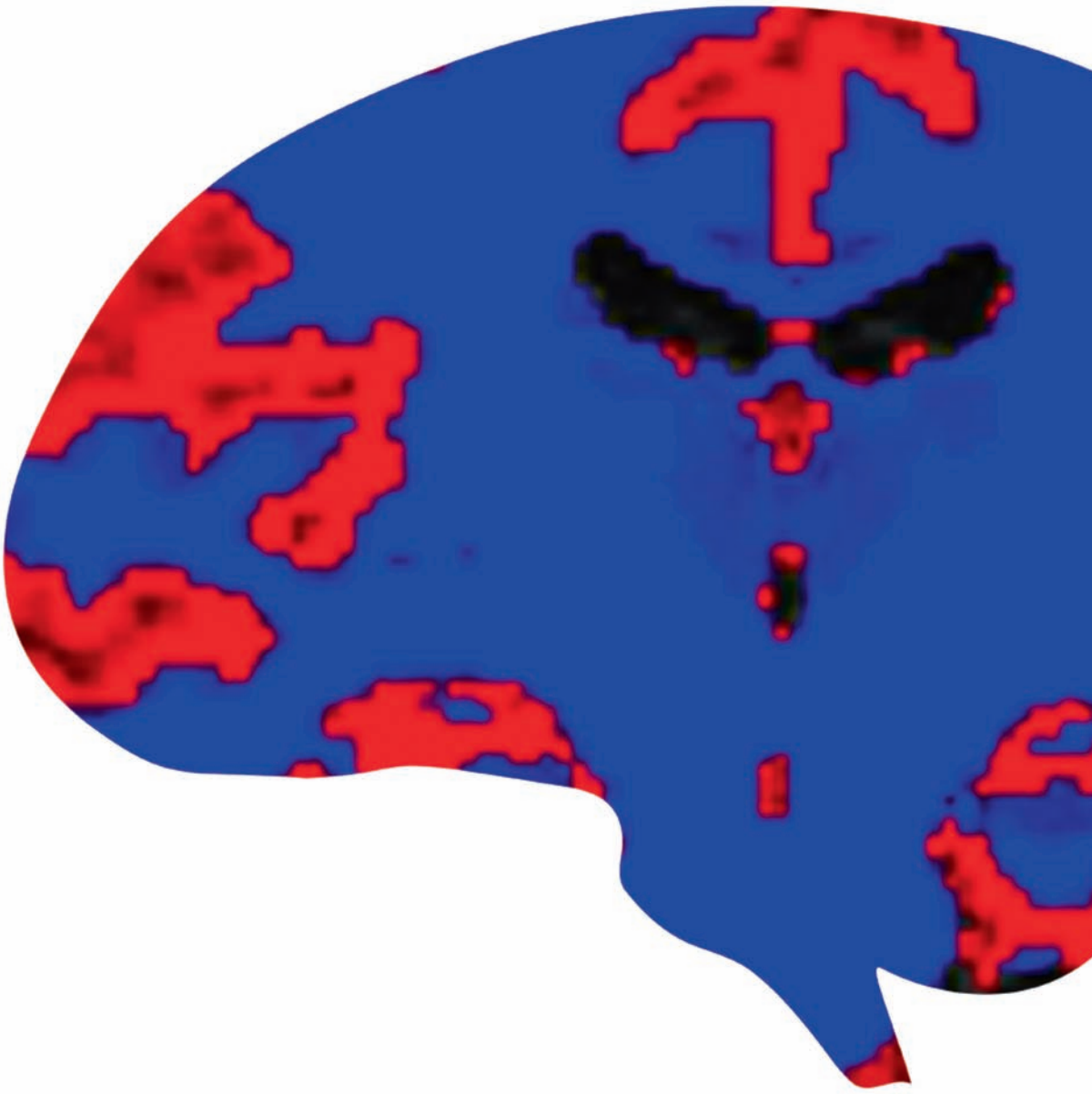

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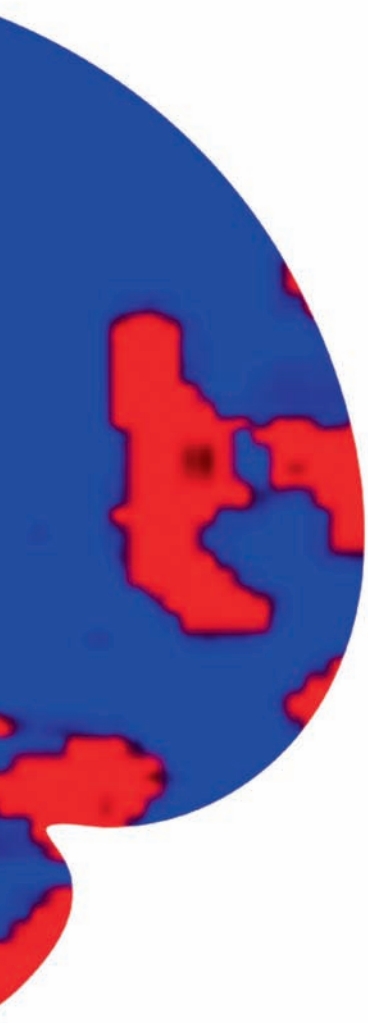


Summary

The aims of this thesis were to gain further insight into specific disease processes in HD and to identify promising biomarkers. To achieve these aims, cognitive functioning, structural brain characteristics and intrinstic functional brain connectivity of premanifest and early HD subjects were examined.

In **chapter 2**, from a review of the existing literature, we concluded that cognitive deficits show a differential progression. During late disease stages all cognitive domains show moderate or severe disturbances. The global profile of cognitive functioning in premanifest HD is characterised by subtle deficits of psychomotor speed, negative emotion recognition and executive functions. In manifest HD these deficits progressively worsen and are eventually accompanied by memory dysfunction. Global cognitive abilities and language capacities are the last to show deterioration. Eventually, these cognitive deficits in HD result in a generalised dementia.

In **chapter 3,** we demonstrated that visuospatial working memory is not only deficient in early manifest patients but also in premanifest gene carriers. Contrary to our expectations we found a 'worse-worse' phenomenon with worsened accuracy and performance speed in early HD patients. Over a period of twelve months, patients with stage two HD showed deterioration in visuospatial working memory.

In **chapter 4**, we discussed that atrophy of subcortical nuclei shows a differential deterioration profile. In premanifest gene carriers we found atrophy of the hippocampus, accumbens nucleus, globus pallidus, thalamus, brainstem, caudate nucleus and putamen. In manifest HD, further atrophy of these structures was observed, with marked loss of hippocampal volume.

In **chapter 5** magnetic resonance spectroscopy was applied demonstrating that the caudate nucleus and putamen show reduced creatine in early manifest HD suggestive of reduced energy metabolism. Furthermore, N- acetylaspartate reductions in the caudate nucleus and putamen suggests that the integrity and vitality of neurons is negatively affected in early manifest HD.

In premanifest gene carriers reduced integrity of the white matter pathway of the sensorimotor cortex, as measured with DTI, was demonstrated in **chapter 6**. In manifest HD, a more widespread pattern of reduced integrity of white matter pathways was observed.

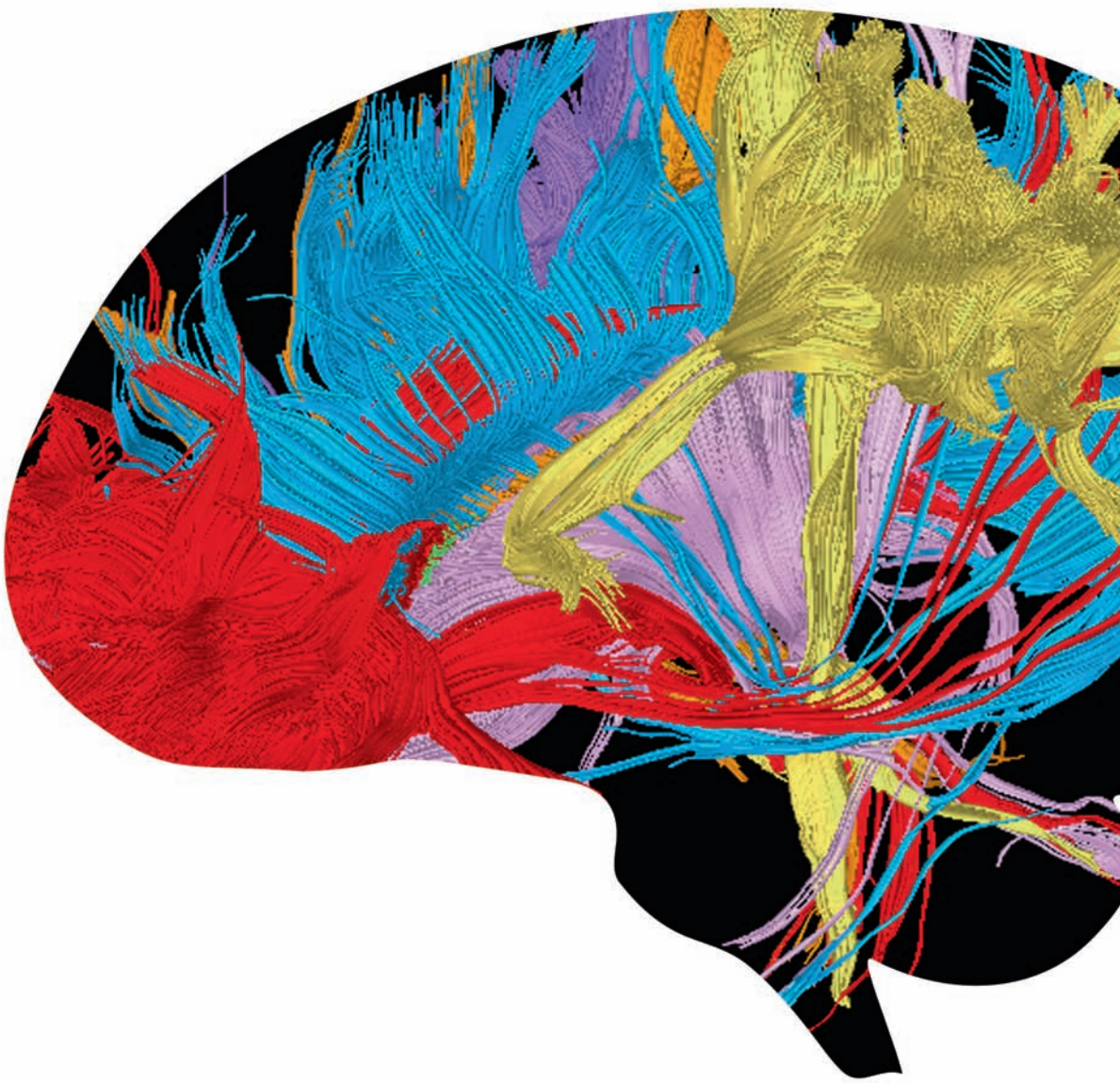
In **chapter 7** we demonstrated that iron accumulation is present in the early stages of HD and that iron accumulation and atrophy seem to reflect independent disease processes. Increased magnetic field inhomogeneities suggestive of elevated iron accumulation were found in the putamen and caudate nucleus of early HD patients. In premanifest gene carriers elevated iron accumulation was not found in any of the subcortical structures examined.

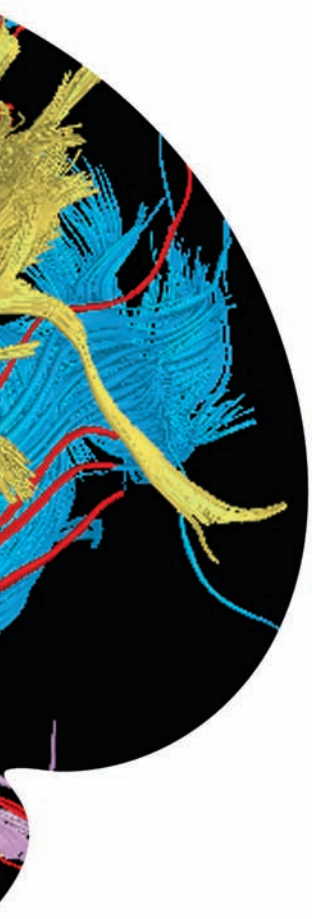
In **chapter 8** we found evidence for reduced intrinsic functional connectivity of left middle frontal and pre-central gyrus, and right post central gyrus with the medial visual network in premanifest gene carriers. In manifest HD, a similar but more widespread pattern of reduced intrinsic functional connectivity was observed.

When all structural and functional brain changes are considered together, a broad picture of multi-level deficits begins to emerge. Cortical, subcortical and the intermediate white matter brain tissue shows evidence of structural and functional decline. We found evidence that several disease processes, such as altered metabolism, excessive iron accumulation and cell loss, play a role in the observed changes. We conclude that changes occur throughout the brain from the earliest disease phase onwards. Hence, both premanifest and manifest HD should not be regarded as a disorder of the basal ganglia, but as a disease affecting the whole brain.

Candidate biomarkers that have the potential to objectively reflect the early changes and the progressive nature of the disease are measures of subcortical atrophy, integrity of white matter pathways and of intrinsic functional brain connectivity. Iron, creatine, and N- acetylaspartate concentrations in the caudate nucleus and putamen may prove to be most useful as markers of disease state for objectifying transitional disease processes from premanifest to manifest HD. Visuospatial working memory could be applied as a state marker for stage two HD.

As clinical trials for treatment of HD become more frequent, the need for objective and sensitive biomarkers becomes more significant. Longitudinal establishment of promising biomarkers is needed. To further understanding of the complexities of HD a comprehensive approach is important for future research. Observational research of young premanifest gene carriers is important for understanding the impact huntingtin has on the developing brain. Nonetheless, observational research should always include the fullest range of gene carriers possible.





Dutch Summary

Samenvatting

De doelstellingen van het onderzoek beschreven in dit proefschrift waren om meer inzicht te krijgen in de ziekte processen van de ziekte van Huntington (HD) en kandidaat biomarkers te identificeren. Om deze doelstellingen te behalen zijn zowel de cognitieve functies als structurele en functionele aspecten van de hersenen onderzocht van premanifeste gendragers en patiënten in een vroeg stadium van de ziekte.

Een overzicht van bestaande literatuur **(hoofdstuk 2)** toonde aan dat de deficiënties in de verschillende cognitieve domeinen een gedifferentieerd beloop laten zien. In de latere stadia van de ziekte laten alle cognitieve domeinen in meer of mindere mate afwijkingen zien. Het cognitieve profiel van premanifeste gendragers laat subtiele deficiënties zien in de psycho-motore snelheid, negatieve emotie herkenning en executieve functies. In de manifeste stadia van de ziekte verergeren deze afwijkingen en worden er tevens stoornissen van het geheugen waargenomen. Globale cognitieve en taalvaardigheden zijn de laatste domeinen die een achteruitgang tonen. Uiteindelijk leiden deze cognitieve deficiënties bij HD tot een gegeneraliseerde dementie.

Het visueel-spatieel werkgeheugen toonde niet alleen afwijkingen aan bij HD patiënten in een vroeg stadium maar ook bij premanifeste gendragers **(hoofdstuk 3)**. In tegenstelling tot onze verwachtingen vonden wij bij patiënten in een vroeg stadium van de ziekte een 'worse-worse' fenomeen met een verminderde accuratesse en een verminderde snelheid. Over een periode van twaalf maanden laten stadium twee HD patiënten een aantoonbare achteruitgang zien in visueel-spatieel werkgeheugen.

Door gebruik te maken van geautomatiseerde MRI analyses demonstreerden wij dat atrofie van de subcorticale grijze stof een gedifferentieerd profiel laat zien **(hoofdstuk 4)**. Hierbij vonden wij dat er bij premanifeste gendragers sprake is van atrofie van de hippocampus, nucleus accumbens, globus pallidus, thalamus, hersenstam, putamen en nucleus caudatus. In de vroeg manifeste stadia van de ziekte vonden wij meer atrofie van deze hersenstructuren, waarbij de sterk toegenomen atrofie van de hippocampus opvallend was.

Met behulp van 'magnetic resonance spectroscopy' toonden wij aan dat zowel de nucleus caudatus als het putamen een reductie laten zien in de hoeveelheid aanwezige creatine **(hoofdstuk 5)**. Dit suggereert dat er sprake zou kunnen zijn van verminderd energetisch metabolisme. Ook suggereert de afname van N-acetylaspartaat in deze kernen dat de integriteit en vitaliteit van neuronen in vroeg manifeste HD is aangedaan.

Een afname in de integriteit van de witte stof banen van de sensorimotor cortex in premanifeste gendragers werd aangetoond met behulp van 'diffusion tensor imaging'

(hoofdstuk 6). In vroege manifeste HD observeerden wij een uitgebreider patroon van integriteitafname in meerdere witte stof banen.

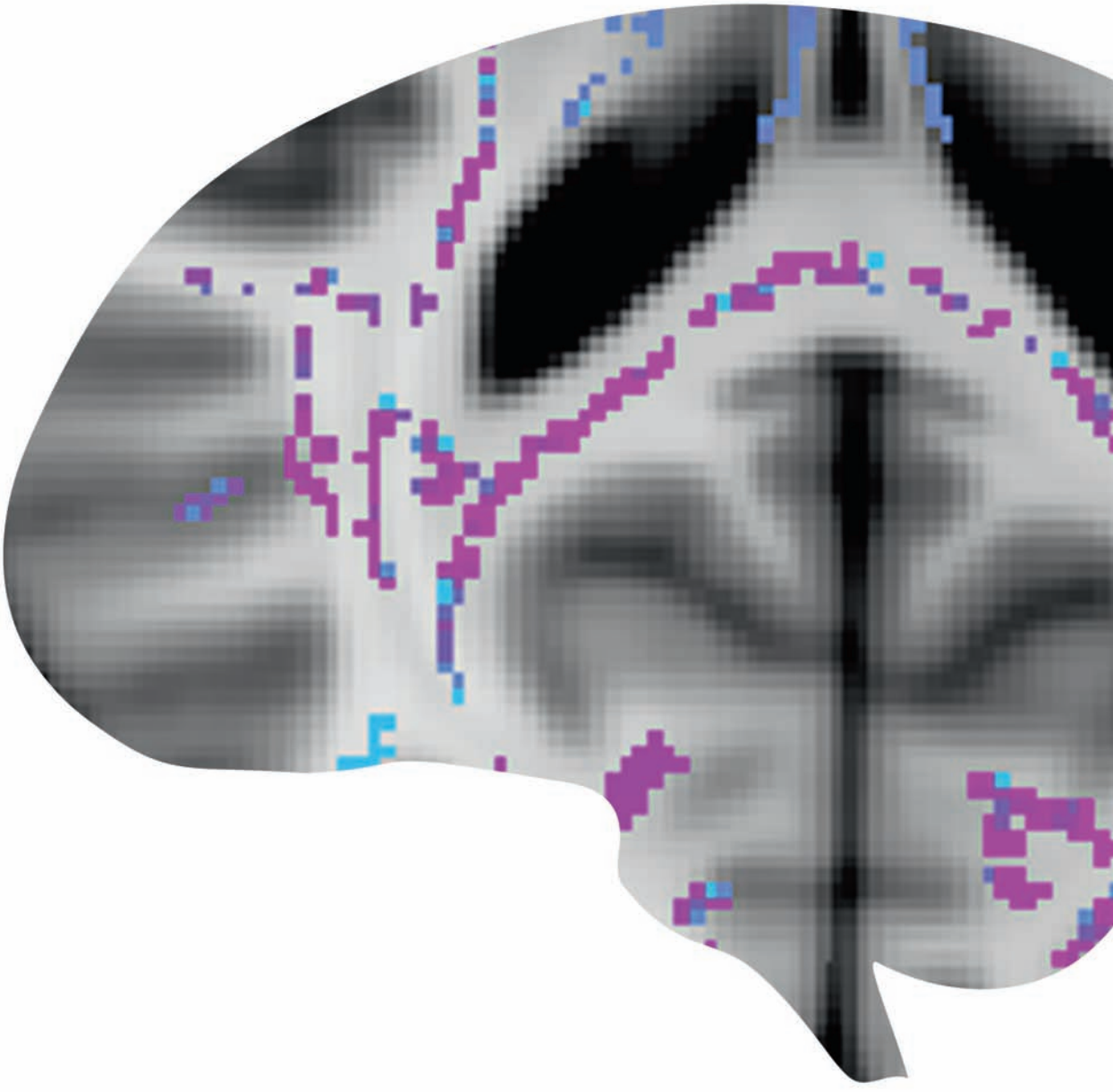
Toegenomen inhomogeniteiten van het magnetische veld, duidend op toegenomen ijzer, werden aangetoond in het putamen en de nucleus caudatus in vroeg manifest HD **(hoofdstuk 7)**. Deze verhoogde ijzerconcentratie lijkt een onafhankelijk proces te reflecteren van atrofie van deze kernen. In premanifeste gendragers werd er in geen van de onderzochte subcorticale hersenstructuren een verhoogde ijzerconcentratie gevonden.

Door gebruik te maken van 'resting state fMRI' toonden wij een afname aan in intrinsieke functionele connectiviteit van; het mediale visuele netwerk met de linker mediale frontale en precentrale gyri, en de rechter postcentrale gyrus in premanifeste gendragers **(hoofdstuk 8)**. In vroeg manifeste HD werd een vergelijkbaar maar uitgebreider patroon van afgenomen intrinsieke functionele connectiviteit gevonden.

Wanneer al deze structurele en functionele veranderingen samen worden genomen, vormt zich er een breder zicht op de deficiënties op meerdere niveaus. Corticaal, subcorticaal en de tussenliggende witte stof laten aanwijzingen zien voor functionele en structurele achteruitgang. Wij vonden dat meerdere ziekteprocessen, zoals veranderd metabolisme, toegenomen ijzer en verlies van cellen, een rol spelen in de waargenomen veranderingen. Wij concluderen dat veranderingen in de hersenen voorkomen vanaf het vroegste moment in het ziekteproces. Daarom zou premanifeste en manifeste HD niet moeten worden beschouwd als een ziekte van de basale kernen alleen, maar als een ziekte die de gehele hersenen aangaat.

Kandidaat biomarkers die het vermogen hebben om op een objectieve manier de vroege veranderingen en de progressieve aard van de ziekte te weerspiegelen zijn maten van subcorticale atrofie, integriteitsmaten van de witte stof banen en van intrinsieke functionele connectiviteit. IJzer, creatine, en N-acetylaspartaat concentraties zouden toepasbaar kunnen blijken voor het objectiveren van de transitie van premanifeste naar manifeste HD. Visueel-spatieel werkgeheugen zou kunnen worden toegepast als een maat voor stadium twee HD.

Er zullen waarschijnlijk meer klinische interventie studies voor HD komen en daarom wordt de behoefte aan objectieve en sensitieve biomarkers steeds relevanter. Om beter begrip te krijgen in de complexe ziekteprocessen van HD is het van belang om in toekomstig onderzoek een alomvattende aanpak toe te passen. Observationeel onderzoek van jonge premanifeste gendragers is belangrijk om de gevolgen van het mutante huntingtin-eiwit op de ontwikkelende hersenen beter te begrijpen. Daarom is het van belang om bij observationeel onderzoek het breedste scala aan gendragers te includeren.



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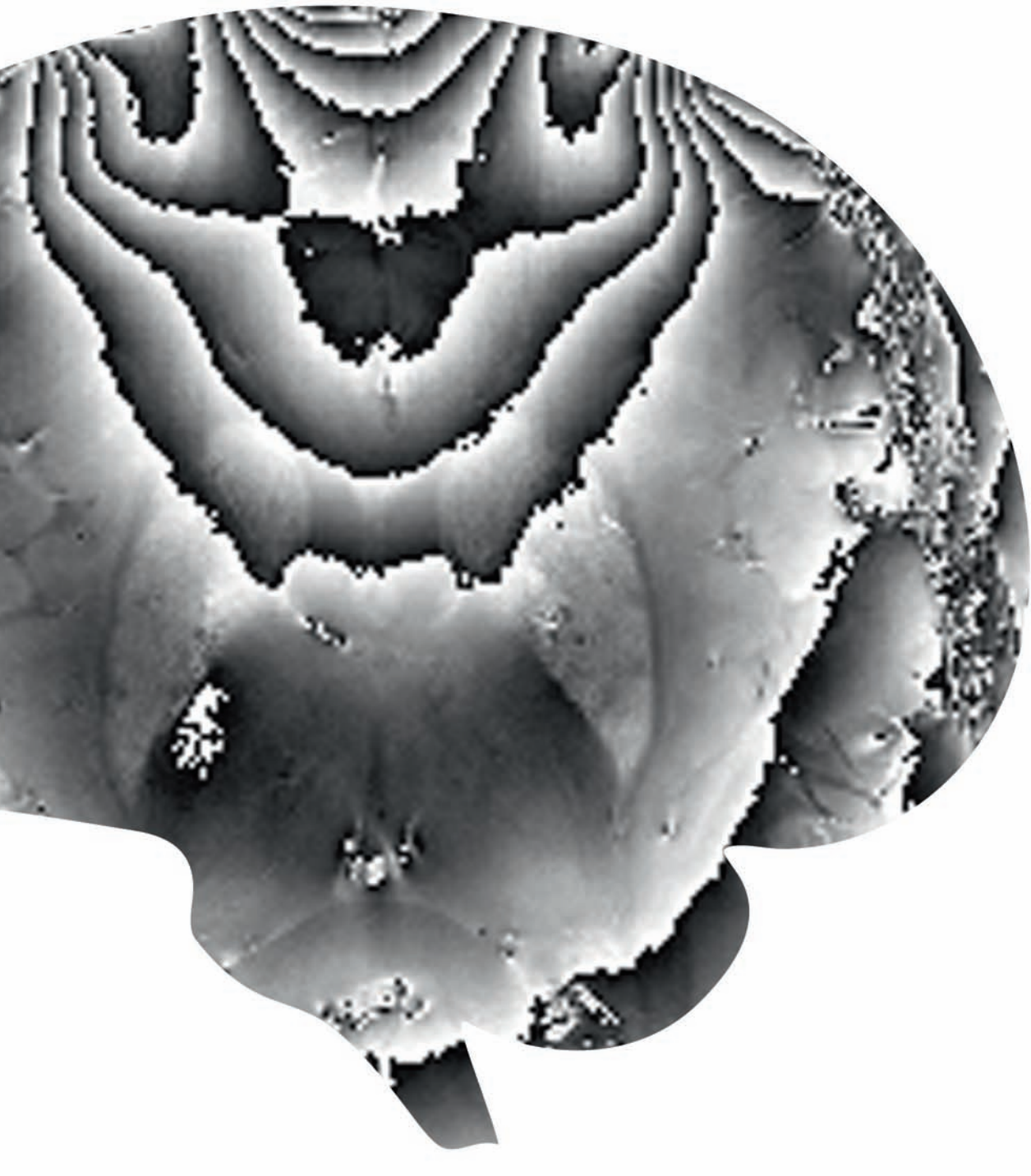

Curriculum Vitae

Eve Marie Dumas werd geboren op 19 september 1982 in Rotterdam. Zij woonde op meerdere plaatsen binnen Europa voordat zij in 1995 aan Engelstalig middelbaar onderwijs begon in Abu Dhabi in de Verenigde Arabische Emiraten. In 1999 behaalde zij haar General Certificates of Secondary Education op The English College in Dubai, waarna zij in 2001 haar Advanced Level General Certificates of Education behaalde op The British School in the Netherlands in Voorschoten. In datzelfde jaar begon zij aan de studie Psychologie aan de Universiteit Leiden en in 2002 aan een deeltijdopleiding aan de Koninklijke Academie van Beeldende Kunsten in Den Haag. In deze tijd werkte zij als student-lid van het faculteitsbestuur van de Faculteit der Kunsten van de Universiteit Leiden. In 2007 behaalde zij haar Master in de Klinische Neuropsychologie en studeerde zij tevens af als autonoomdriedimensionaal beeldend kunstenaar. Aansluitend richtte zij haar bedrijf in de creatieve vormgeving op. In de afrondende fase van haar Master deed zij werkervaring op bij de functieafdeling Neuropsychologie van de afdeling Neurologie van het LUMC. Vervolgens werkte zij onder andere mee aan het Registratie onderzoek van het European Huntington's Disease Network (EHDN).

In 2008 begon zij aan een promotietraject gekoppeld aan het TRACK-HD onderzoek naar biomarkers bij de ziekte van Huntington. In aanvulling hierop was zij betrokken bij het opzetten van het 7Tesla MRI Huntington project. Sinds begin 2011 is zij als neuropsycholoog betrokken bij onderzoek naar hersenveranderingen bij jongens met de ziekte van Duchenne.

In november 2012 is Eve begonnen aan de postmaster opleiding tot gezondheidszorgpsycholoog bij Marente in Warmond en de Praktijk voor Neuropsychologie in Katwijk.