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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