Concluding remarks and future perspectives
INTRODUCTION

Important neurodegenerative diseases, such as Alzheimer’s, Parkinson’s, and Huntington’s disease (HD) share several similarities: they are disorders of protein misfolding, caused by a combination of genetic and environmental factors, becoming apparent after an asymptomatic period during which the underlying disease process is already active. Between these important progressive brain diseases, HD stands out in several ways. Although far less prevalent than Alzheimer’s or Parkinson’s, HD seems the most straightforward to understand: it is a monogenetic disorder with full penetrance, the responsible mutation protein can be identified before the onset of disease and its repeat length is an important determinator of the onset of the disease. Therefore, insights in HD pathophysiology during the premanifest phase could affect scientific strategy in tackling problems which arise with recognition and possible treatment of Alzheimer’s and Parkinson’s disease in the future.

In this thesis, we studied the value of imaging, laboratory and neuropsychological tests to reflect the pathophysiology in that most crucial stage of the HD process: the transition period just preceding the onset of symptoms and signs of the disease. An in vivo test that gives valid and quantifiable information of the progression of these early neuropathological processes in HD is called a biomarker. Such a marker is useful for efficient selection of subjects for therapeutic trials, for detection of therapeutic effects, and for improving insight in the disease process.

A relevant biomarker for HD must at least show differences between premanifest mutation carriers (PMC) and controls- otherwise it lacks sensitivity. However, if it would clearly differentiate all PMC from controls, it would be an indicator more of disease state than of disease stage (cf. the presence of the HD mutation). An ideal quantifiable biomarker would thus be expected to show values in the control range for at least part of PMC.

Striatal dopamine D\textsubscript{2} receptors, metabolism, and volume in preclinical Huntington disease

In Chapter 2, we showed that PMC indeed have significantly lower striatal dopamine D\textsubscript{2} receptor availability, glucose metabolism, and volume compared to controls. Of these three, assessment of dopamine D\textsubscript{2} receptor availability with \textsuperscript{11}C-raclopride PET (RAC) turned out to have the highest proportion of abnormal results, followed by FDG and then by MRI. However, the majority of PMC still showed normal imaging results. This is in line with the perception that mutation carriers can be completely ‘normal’ for decades and these will be present in an unselected cohort of PMC like ours.

Other PET studies in PMC use either FDG or RAC with several studies using controls that were not specifically scanned for the study, or lacking blinding of investigators to gene
In order to compare different techniques that hold promise as a biomarker, it is important to compare their performance in the same study or at least under the same circumstances. A study by Antonini and co-workers used FDG, RAC and MRI to study 10 PMC and compared results to control values that were obtained previously. They found reduced metabolism but no change in dopamine receptor status in caudate nucleus, no change in a measure of caudate atrophy, and no correlation between FDG and RAC. In putamen, they observed reduced values for FDG and for RAC. In our larger series, we found similar changes in putamen for FDG and RAC, but also demonstrated significant decline in RAC uptake in caudate nucleus. Moreover, we could demonstrate changes in striatal volume. These differences may reflect the larger statistical power of our study, the use of a dedicated control group and of more sophisticated MRI measures.

In terms of specificity, an HD biomarker should reflect the cumulative amount of change that the mutant protein has caused in a mutation carrier: the ‘dosage’ effect. We correlated the product of age and CAG repeat length, a measure of cumulative disease load, with results of each of the imaging techniques. Here, PMC with the higher exposition to the mutant protein showed lower striatal volumes, lower putaminal glucose metabolism, and lower striatal dopamine D$_2$ receptor availability. Again, these changes were most distinct with RAC, indicating that changes in receptor status exceed the changes caused by atrophy or hindered metabolism. This suggests that changes in RAC do not just reflect the effects of total striatal cell death (which would obviously lower striatal volume and glucose consumption), but also measure the selective decline of a relevant subpopulation of D$_2$ receptor bearing cells. Alternatively, these findings may indicate that in PMC, neuronal dysfunction on a receptor level (RAC) precedes alterations in cell metabolism (FDG) and is ultimately followed by cell death, resulting in atrophy. This implies that some of the damage inflicted by mutant huntingtin is reversible, and this has been suggested in an animal model.

In this light it is striking that a large and very elaborate study in PMC failed to detect MRI features that correlate with changes in clinical or cognitive function over two years, assumably because of compensatory changes in neuronal function. In recent years, larger longitudinal observational studies like PREDICT-HD and TRACK-HD have relied on MRI rather than on PET for neuroimaging in premanifest HD and MRI clearly has advantages in availability, operational reliability, radiation exposure, user friendliness and cost. However, PET data could be more sensitive than MRI as suggested by our study. In terms of availability and reliability of tracer production, RAC remains a logistically challenging investigation but FDG PET scan facilities have become available in many hospitals because of their implementation in routine oncological care.
Changes in striatal dopamine D2 receptor binding in pre-clinical Huntington’s disease

We then set up a blindly assessed, controlled cohort of PMC with several promising imaging measures from the baseline study described in Chapter 2. However, even changes that are clearly related to the exposition to mutant HD-protein and thus seem specific for the disease process (such as striatal PET and MRI measures), reflect the alterations and accumulation of damage of a lifetime. In order to gain insight in the pathophysiology that just precedes the onset of signs and symptoms of HD, longitudinal data are needed.

Because changes in dopamine D$_2$ receptor availability, as measured by RAC-PET, turned out to be the most sensitive parameter in our baseline study, we gathered longitudinal data on RAC-PET with a follow-up duration of at least 2 years (Chapter 3).

During the study, putaminal RAC activity remained abnormal in almost half of PMC and decreased significantly at 2.6% per year. However, this change did not reach significance when compared to that observed in controls (1.8%). Again, changes were more pronounced in putamen than in caudate.

In the literature, prospective RAC normal values are scarce, with a calculated decline in striatal RAC of 0.4-0.6% per year in one study with healthy volunteers after age 36; in younger subjects, the rate of decline was higher but not calculated.20 The study by Schlosser et al.21 found a decrease of 0.36% per year, but this study had fewer control subjects than ours, at half the follow-up length. Still, one cannot exclude a systemic bias occurring during the course of an advanced imaging study conducted over several years; in fact, this only stresses the importance of the continuing use of a control group. This makes it hard to compare our RAC data to those of another relatively large prospective study22 that found decreases over time in striatal RAC, but did not include longitudinal control data, making it impossible to correct for aging or systemic bias.

As noted previously, a cohort of PMC will typically consist of some people decades away from disease onset and of some that are nearer to it. A correlation of RAC with the ‘lateness’ of the premanifest stage, would strengthen its performance as a biomarker. The Langbehn model23 permits calculation of the probability that a PMC will develop clinical HD within the next five years. Indeed, we found putaminal RAC to be inversely correlated with this modelled probability i.e., PMC closer to disease onset tended to have lower RAC in putamen. This finding has been replicated later in a smaller study.22

TRACK-HD is a large international prospective biomarker study of HD patients and PMC that does use a dedicated control group, but does not include PET studies.19 In their 2 year follow-up study of 117 PMC, the TRACK-HD investigators found decreases of MRI striatal volume ranging from one to 2.5% per year for caudate, to two to three percent per year for putamen.17 These anatomical changes were small but still more prominent than those
in a large number of clinical and neuropsychological tests. This unexpectedly low yield of longitudinal premanifest findings prompted the investigators to extend the study beyond the planned 2 year follow-up (supplemental data in 24).

During the study period, three of the 18 PMC in our cohort started to show possible signs of clinical HD, mainly oculomotor changes, and are of particular interest. Their neuropsychological assessment remained normal. At baseline, RAC in putamen was below the median of the other PMC for all three and in the lowest range for two of them. Interestingly, the annual rate of decline was 0.8% in these three subjects and 2.9% for the other PMC. Although these are very small numbers, this finding pleads against acceleration of the disease process in the late-premanifest phase and is concordant with the absence of a correlation between the rate of putaminal decline and the modelled proximity to disease onset. This paradox of correlation with decreased putaminal D2 receptor density, but not with the rate of this decrease, has been replicated in another PET study. MRI data from the much larger TRACK-HD study 24 month data are also concordant: while loss of volume of putamen was larger than of caudate in all PMC, in those PMC who showed disease progression, putaminal volume was not decreasing significantly. Taken together, these data are more in support of a fixed proportional (exponential) cell loss, which leads to smaller absolute decreases at the approach of the threshold below which clinical HD sets in.

**Striatal metabolism and psychomotor speed as predictors of motor onset in Huntington's disease**

Many studies in premanifest HD including ours have used age and genetic information (CAG repeat length) to try to estimate the risk that PMC would convert to HD in the near future and compared the performance of potential biomarkers against that risk.

The CAG repeat length is an important predictive factor, accounting for 47 to 72% of the variance in age of onset, especially in younger age groups. In a recent prospective study in over 1000 PMC however, repeat length contributed 53% of variance of age of onset, with wide ranges spanning several decades around the predicted age of onset for a given CAG repeat number.

Using risk estimations based on genetic information is an important and efficient way of preselecting PMC to study promising markers of the HD pathophysiology but eventually the appraisal of appropriate biomarkers can only come from studies that can set off their performance to the actual onset of clinical HD.

Re-assessing the HD status of PMC after a 10-year follow-up enables us to determine which individuals have actually converted to clinical (motor) HD and ascertain they were already ‘near’ to disease onset at baseline. In Chapter 4, we describe premanifest imaging and neuropsychological profiles of these ‘near’ (n=8) and ‘far’ (n=9) PMC.
We grouped neuropsychological test scores into the cognitive domains of executive function, memory and psychomotor speed (PmS). In each of these domains, we termed the results ‘abnormal’ when below two standard deviations of the control mean and ‘low average’ when between one and two standard deviations below the control mean.

Underlining the truly premanifest composition of our PMC group, analysis of the cognitive measures did not reveal significant differences between PMC and controls in these cognitive domains. In accordance with results in Chapters 2 and 3, PMC had decreased striatal (but not frontal) metabolism and this did not change significantly over 2 years when compared to controls. PMC ‘close’ to actual onset of clinical HD tended to have lower putaminal metabolism than PMC ‘far’ ($p = 0.04$).

Two years after baseline, 45% of PMC ‘far’ still had a confidently normal FDG putaminal metabolism (above control mean minus one standard deviation). Strikingly, none of the PMC ‘close’ had a truly normal putaminal FDG at that time. This means that none of the PMC with such a normal putaminal metabolism had developed motor symptoms of HD 8 years later, suggesting a very high negative predictive value of normal putaminal metabolism for development of clinical HD in the next 8 years.

At baseline, the combination of glucose metabolism of the putamen and a composite score for psychomotor speed turned out to be a powerful and better predictor of modelled disease onset than each measure separately. This dual test combination accounts for two-thirds of variance in the calculated probability of motor onset within 5 years and was validated for measurements obtained at 2-year-follow-up.

Shortly after the publication of our follow-up study (Chapter 3), results of PREDICT-HD were published. This is a large international study investigating measures that best predict onset of clinical HD. This multicenter study found striatal volume to have the best correlation with predicted onset of disease of all clinical, imaging and cognitive variables, but striatal volume explained only 23% of this variation and PET studies were not performed. Metabolism in putamen and psychomotor speed in our study explained 44 and 45% respectively, adding up to 67% in a composite score.

In a recent final report of that same study group, now with over 1,000 PMC participating, Stroop word test, which is part of the psychomotor speed composite score in our study, and volume of putamen came up as best predictors of clinical disease onset. These are very similar to the most sensitive measures in our study. In PREDICT-HD even a composite score of total motor score, which is rather self-evident for onset of clinical disease, putaminal volume and Stroop word test did not further improve prediction. In view of the large population studied, this is unlikely to be due to the shorter follow-up period in that study compared to ours. Although interobserver variance and profound changes in MRI hardware during the PREDICT-HD study may play a role, this difference is also in line with our observation...
that PET functional imaging of putamen in HD pathophysiology is a more sensitive measure than volume of that structure (Chapters 2 and 3). Possibly, network analysis of cerebral metabolism offers additional information on the HD pathophysiology and seems more sensitive than MRI, although changes in RAC precede those in the FDG network analysis in PMC.30

The TRACK-HD study investigators recently published the 3 year follow-up results.24 Again, volume of striatum, symbol digit modality test and Stroop word reading were among the strongest baseline predictors of progression to clinical HD. These modalities kept their association with phenoconversion when corrected for age and CAG repeat length. Unfortunately, no suggestion was found of an ‘absolute’ threshold MRI or clinical marker of proximity to HD onset, similar to the absence of progression to clinical HD during almost a decade in PMC with truly normal putaminal metabolism on PET as suggested in our study.

**Hsp40 expression in peripheral mononuclear blood cells in premanifest Huntington’s disease.**

Descriptions of symptoms and signs of HD have always been heavily dominated by alterations of movement, behaviour and cognition31 and clinical criteria are pertaining to brain dysfunction only.25 Still, HD is not strictly a brain disease. The exact function of huntingtin is unclear, but it is expressed in many tissues outside the brain and is essential for development of all three embryonic germ layers32 and for normal bone marrow function.33 Aggregates of mutant huntingtin, which characteristically accumulate in brain tissue in HD, can also be found in peripheral tissue of PMC and of subjects with HD34 and in recent years, the range of pathology outside the nervous system has come to attention.35,36

HD neuronal inclusions consist of misfolded huntingtin, components of the cell proteasome degradation pathway and chaperones. Some of these chaperones are molecules that can be up-regulated as a reaction to heat or other stress conditions and are called heat shock proteins. They can prevent the aggregation and misfolding of intracellular proteins, are important in protein quality control37 and are involved in the process of mutant huntingtin aggregation in the cell nucleus.38 Heat shock proteins from the Hsp40 family, also called DNAJ39 are of particular interest in HD, since they are the most effective group of chaperones in preventing aggregation of huntingtin polyglutamine repeats.40 Pathological mechanisms of HD are at work years before symptoms and signs of HD arise.29 Given the widespread expression of mutant huntingtin outside the brain and the role of HSp40 in mutant huntingtin aggregate formation, we speculated that Hsp40 might be up-regulated in premanifest HD as a cellular defence mechanism. We therefore measured the expression of Hsp40 in peripheral blood cells of PMC (Chapter 5).

Mononuclear Hsp40 levels corrected for GAPDH expression were not significantly higher in PMC and did not correlate with cognitive test results. Correlation analysis of Hsp40 up
regulation with putaminal imaging parameters appeared to follow the rank of sensitivity levels described in Chapter 2: stronger for RAC than for FDG, followed by MRI, but these relations did not reach significance.

Higher Hsp40 levels were however associated with the cumulative disease load as expressed by the Langbehn formula, suggesting that progression to the later stages of premanifest HD coincides with increased intracellular stress levels. Because Hsp40 expression in the total group of PMC was similar to controls, the mere presence of mutant huntingtin is not causing an up regulation of Hsp40 per se. For the future, this means that determining levels of chaperones like Hsp40 only makes sense when information on the proximity to onset of clinical HD is available. To find out if Hsp40 responses could serve as a biomarker for HD, longitudinal studies extending to the clinical phase are required.

Recent studies have shown that increasing the levels of Hsp40 in mice expressing mutant huntingtin protects against huntingtin aggregate formation and motor disease. These results underline the importance of the cell's normal mechanisms of function involving Hsp40. These mechanisms may be partly responsible for the differences in disease onset that cannot be explained by the CAG repeat length.

**1H magnetic resonance spectroscopy in preclinical Huntington disease.**

Because functional changes in brains of HD mutation carriers are likely to precede structural ones, other imaging modalities than structural MRI have to be considered as a biomarker. 1H magnetic resonance spectroscopy (MRS) can measure in vivo indications of energy metabolism (through measurement of creatine) or neuronal integrity (through N-acetyl aspartate (NAA)). In HD patients, several studies have found metabolite changes such as decreased striatal NAA. In premanifest HD however, MRS has yielded inconclusive results.

After MRS became available at our institution, we consecutively studied 19 PMC and 8 controls with MRS (Chapter 6). Hence they formed an unselected part of our study population at large. We calculated relative metabolite concentrations of creatine, choline and NAA in putamen and thalamus and found no significant differences between PMC and controls. We found only a weak correlation of decreasing NAA in putamen relative to thalamus with increasing disease load.

Since our publication on MRS others have studied MRS in PMC with considerable heterogeneity in selection of PMC, data acquisition or analysis. Gomez-Anson et al. found no changes in striatal metabolites. Padowski and co-workers found normal absolute concentrations of caudate metabolites and decreased relative concentrations only of glutamine/glutamate. However, the mutation carriers in this study consisted of a mix of PMC and HD patients, which had not all been assessed clinically with UHDRS, and the voxel...
studied contained large amounts of white matter and even ventricle. Sturrock et al.\(^{47}\) found lower putaminal NAA in PMC, but used a large voxel that contained white matter. Their results could not be replicated in a later study with a more advanced MRI machine and well-positioned voxels.\(^{48}\)

In premanifest HD, this lack of discriminative power could either mean that no biological changes have yet occurred in most PMC, or that MRS is not very sensitive to detect these changes. Unlike recent reports of MRS in premanifest HD,\(^ {47,48}\) we are in a position to compare the results of MRS to those of other imaging techniques. Since we found clear changes in structural MRI and especially in PET parameters in the same cohort of PMC, our negative MRS study of putaminal metabolites pleads strongly against MRS being a more sensitive biomarker for HD than PET or even MRI.

Two important caveats to this conclusion apply however. Firstly, our series is small, although we are aware of only one slightly larger MRS study in premanifest HD.\(^ {47}\) In larger cohorts, significant changes may still be found for statistical reasons but it is very unlikely that these changes will be larger than those already found with PET in the smaller cohort. Secondly, we have used MRS at 1.5 Tesla field strength while investigators recently have had access to 3\(^ {47}\) or even 7 Tesla machines,\(^ {48,54}\) which can improve spatial and spectral resolution.\(^ {55}\)

**Looking ahead**

In conclusion, knowledge and understanding of HD mechanisms have taken a great flight over the last 25 years. A reliable genetic test has become available, insight in the genetics and molecular biology of HD has accumulated, credible animal models expressing mutant huntingtin have been developed and researchers can count on well-organised patient organisations and on funded multicenter research collaborations. Yet none of the clinical trials, several after promising results in animal models, have brought any result towards a real therapy for HD which is so badly needed.

Several clinical trials are under way, including studies involving deliverance to the brain of antisense oligonucleotides or RNA interference agents that reduce the levels of huntingtin messenger RNA.\(^ {56}\) In addition, more conventional trials are testing inhibitors of phosphodiesterase 10A (PDE10A). This enzyme is involved in intracellular signal transduction and is mainly present in the striatum with increased levels in medium spiny neurons in an animal HD model.\(^ {57,58}\) In mice expressing mutant huntingtin, inhibitors of PDE10A delayed the onset of motor disease and reduced the typical neuropathological abnormalities.\(^ {59}\) Phase 2 randomised clinical trials are conducted in human HD subjects.\(^ {60,61}\)

Looking for a disease modifying drug in HD patients and not in PMC may seem logical, because pathology is overt and progression is real. Yet in many neurodegenerative diseases, alteration of the disease process may have to be installed before a certain amount of
damage is done in order to be effective.

Brain atrophy as measured with MRI has become the most frequently used imaging parameter over the last years, probably mostly for practical reasons, but it still has not qualified as a useful biomarker, let alone a surrogate endpoint.56 This means that even with all research effort deployed, we cannot be certain that we would recognise a meaningful modifier of premanifest HD even if it was studied in a trial. As outlined above, we feel that PET imaging both with RAC and FDG has better credentials to act as a biomarker than MRI, but has only rarely been compared directly in other studies. Because FDG has become a standard hospital procedure with reliable tracer production infrastructure, as opposed to RAC, we feel that a larger trial comparing FDG to MRI in “late” PMC with clinical endpoints is warranted.
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