1

General introduction and aims of the thesis
Huntington’s disease (HD) is an autosomal dominant disease, characterised by progressive disturbances of cognition, movement and behavior. It is caused by an expanded CAG trinucleotide repeat in the HD gene on chromosome 4, leading to the production of the mutant huntingtin protein. The typical onset of HD is in middle age but symptoms can start at any time between infancy and very old age. Thus, most mutation carriers are free of symptoms and signs of the disease for decades. Reflecting increasing neuronal brain cell loss, HD is a progressive disease that will become manifest sooner or later in mutation carriers, depending for 60-70% on the length of the mutation’s CAG repeat expansion, and on other factors.

Brains of HD patients show widespread changes with early and pronounced bilateral atrophy of caudate nucleus and putamen, reflecting neuronal cell loss, especially of the medium spiny neurons, which express dopamine receptors. In premanifest or early stages of the disease, these histological changes are most evident in the striatum (putamen and caudate nucleus).

Although symptomatic treatment, genetic counseling and family support can be offered, no cure is available and patients typically continue to deteriorate with a mean disease duration of 15 to 20 years. HD leads to complete dependency and imposes an enormous burden on patients and their families.

Ever since discovery of the mutation, researchers have hoped that unraveling the pathophysiology of HD might lead to a rational treatment. Reliable genetic testing is available and mutation carriers can be neurologically unharmed for decades, so directly targeting the disease process holds the promise of slowing or preventing HD related brain changes.

Assuming that reversing the effect of significant HD neuronal cell loss is not realistic, the ultimate goal in HD research is to prove that a treatment strategy not only will stop or slow disease progression in people with overt HD, but will also prevent or postpone its occurrence in premanifest carriers (PMC) of the HD mutation. This requires trials showing slowing or prevention of progression of clinical impairment in treated mutation carriers.

In order to implement such a pivotal trial, several important conditions have already been met: researchers can count on a reliable genetic test, organised and motivated mutation carriers and on funded multicenter research collaborations with investigators trained to use an internationally accepted standardized clinical rating scale. Researchers have gained considerable knowledge from studies on rodent brains and promising therapeutic agents from animal HD models are likely to continue to emerge, but even in this situation, trial design faces two important difficulties: the selection of an apt study population and of
optimal outcome measures.

Firstly, if HD mutation carriers were to be randomly randomized for such a trial, the large majority of subjects would not develop disease manifestations if a trial were to last for five or ten years due to the relatively slow rate of progression of the underlying disease process. Even the effects of a powerful intervention would thus be diluted and difficult to document. The CAG-repeat number is an important predictor of the age of onset, but the length of the asymptomatic or premanifest period cannot be established in individual cases. It is not possible to reliably identify individuals that are close to the onset of clinical HD. Indeed, the attempt to identify ‘late’ or ‘pre B’ HD subjects has become an important part of recent research and trial efficiency would greatly increase if researchers could use a biomarker identifying ‘late’ PMC.

Secondly, clinical HD signs such as chorea and disturbances of behavior and personality can vary from day to day and are difficult to quantify and compare in a multicenter trial. Demonstrating a preventive effect on HD onset in PMC would require thousands of study subjects, lasting many years.

An ideal biomarker would both reliably reflect and quantify the HD-related changes that occur in the brain of PMC. This would enable us to select ‘late’ PMC and measure alterations in the natural course of HD pathology, thus greatly increasing trial efficiency with obvious advantages in reduction of cost and burden of trial participation and, most importantly, time to the availability of a ‘real’ HD therapy.
AIMS OF THE THESIS

With these observations in mind, the general objective of the thesis was to identify a robust HD biomarker in PMC. We also tried to increase our understanding of the nature of changes that occur in premanifest HD, and of their rate of progression.

Our baseline study (Chapter 2) describes the selection of a cohort of PMC (i.e., people carrying the disease mutation but without signs of clinical HD), while mutation-negative individuals from HD-families form a control group. We aim to detect changes in at least part of PMC using three different imaging techniques: brain MRI, and PET studies with $^{18}$Fluorodeoxyglucose (FDG) and $^{11}$C-raclopride (RAC). This enables us to study striatal morphology, glucose metabolism and dopaminergic changes respectively. MRI, FDG and RAC are all known to indicate progressive changes disease that are compatible with pathology of the disease (e.g. pronounced in striatum) in HD patients$^{19-21}$ and in PMC$^{22-24}$

With these imaging techniques, we try to find biomarker candidates that detect changes in at least part of PMC, preferably correlating with the cumulative burden of exposition to the mutant protein, ideally with a threshold predicting irrevocable progression to manifest HD within a limited time frame.

In a dynamic disorder like HD, it is crucial to characterise changes over time, rather than differences between fixed groups. In Chapter 3, we aim to find changes over a follow-up period of over 2 years in this well-characterized group of PMC for RAC-PET imaging. If these changes over time were to surpass any changes in the control group (e.g., because of age), such changes in the striatal dopaminergic neurons could then be scrutinised for influences of the disease load, which is a function of age and the length of the CAG repeat expansion. The results of imaging and neuropsychological assessment of any subjects developing signs of clinical HD during the study period would be of particular interest.

Re-assessing the HD status of PMC after a 10-year follow-up enables us to ascertain which individuals have actually converted to clinical (motor) HD and were retrospectively ‘close’ to disease onset at baseline. We aim to review premanifest imaging and neuropsychological profiles of these ‘close’ and ‘far’ PMC and try to find discriminating factors (Chapter 4).

The mutant huntingtin protein is also expressed outside the brain and alterations outside the central nervous system, e.g. in blood cells, can be found in HD$^{25}$ We speculate that systemic cell stress could be partially compensated for by the heat shock protein (HSP) machinery. Therefore, we propose to measure HSP levels in systemic blood cells in PMC and controls and report the results in Chapter 5.

We later added a fourth imaging technique that became available at our institution: MR spectroscopy. In part of our PMC and controls, we intend to find out if neuronal metabolic
stress in the basal ganglia of PMC could precede or accompany the neuronal cell damage inflicted by the mutant huntingtin. We therefore study the striatal levels of the neuron-specific molecule N-acetyl aspartate and of other metabolites in Chapter 6.

The studies described in this thesis (Chapters 2 to 6) required collection and analysis of clinical, neuropsychological, MRI, MRS, FDG-PET and RAC-PET data at different time points with a follow-up of up to 10 years and have taken a long time to complete. In the meantime, the field of HD research has been dynamic. In Chapter 7, we summarise our findings from Chapters 2 to 6 and pay special attention to recent developments in HD research that have taken place since publication of our research papers.
REFERENCES


