Functional and structural neuroimaging in Huntington's disease

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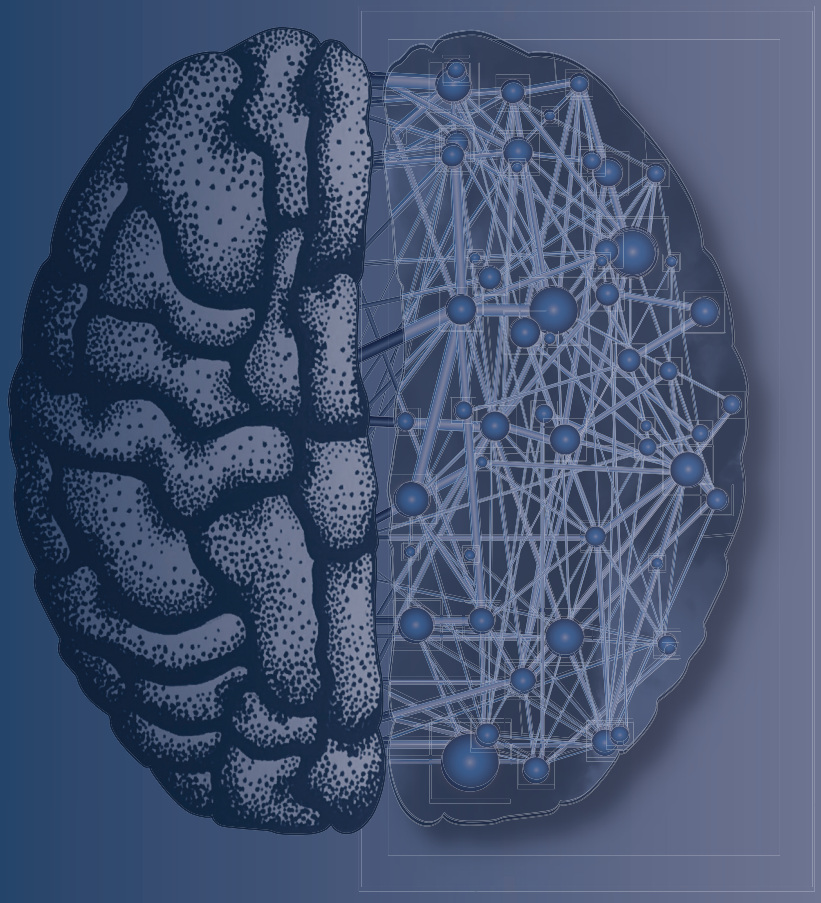

General introduction

CHAPTER 1

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Huntington's disease (HD) is a relentlessly progressive autosomal dominant neurodegenerative disorder with a broad spectrum of clinical features, characterized by a triad of motor, cognitive and psychiatric signs and symptoms. The disease is caused by a mutation in the Huntingtin gene (*HTT*) on the short arm of chromosome 4.[1] The mutation consists of an expanded cytosine-adenine-guanine (CAG) trinucleotide repeat, with variable penetrance in the range of 36-39 and full penetrance in repeats of 40 and higher.

We have gained a great deal of knowledge on the basis and natural course of HD since the publication of one of the earliest medical descriptions of the "hereditary chorea" by George Huntington in 1872.[2] Unfortunately, there still is no known cure or neuroprotective therapy for the disease and only symptomatic medication is available at present. Huntington's statement about the disorder still holds true: "Once it begins it clings to the bitter end".

The mean age at which the adult form of the disease becomes manifest is between 30 and 50 years.[3] Its course runs for 15-20 years following clinical onset, after which death occurs.[4] The term "manifest" in HD is currently reserved for individuals exhibiting characteristic motor symptoms of the disease. Before this manifest phase, there is a "premanifest" phase, where people do not exhibit evident motor signs of the disease and are seemingly healthy, but can have subtle psychiatric and or cognitive signs and symptoms. The disease is unique among neurodegenerative disorders, as individuals destined to develop the disease can be identified through genetic testing before symptom onset. This provides a window of opportunity for an intervention that could potentially delay or even prevent disease manifestation.

There is an inverse correlation between CAG repeat length and the age of onset of manifest disease, explaining up to 60% of age of motor onset variability.[5] As such, age of onset is not solely explained by the mutation, but also by other yet unknown factors. The disorder exhibits genetic anticipation in the paternal line of inheritance. Anticipation means that the onset of symptoms can occur earlier and often more severely in consecutive generations.[6] After the discovery of the causative mutation for HD in 1993, presymptomatic testing became available for the first time in an autosomal dominant disorder.[7] This major milestone in the history of HD understandably led to hopeful expectation for rapidly finding therapy for the disease and considerable effort has indeed been devoted to understanding the pathophysiology of HD and to find disease-modifying therapies.

More than 25 years after the mutant gene discovery, the first safety studies with potentially promising disease-modifying effects at the gene transcription level have been performed. In September 2015, the first-in-human study looking into the safety of IONIS-HTT_{Rx} (RG6042), an intrathecally administered antisense oligonucleotide (ASO) therapy to reduce mutant HTT (mHTT) protein, was launched in 46 early manifest HD patients (ClinicalTrials.gov Identifier:

NCT02519036). In 34 patients assigned to receive the ASO, the drug proved to be safe and the intended mHTT lowering was demonstrated in a dose-dependent manner, passing the phase II trials.[8] After this initial step, larger studies are now commencing in different stages of the disease to examine whether there indeed is a desirable disease-modifying effect.

In order to measure the effects of these potential therapies, we need to have sensitive markers that correlate with disease state and progression. If the therapeutics have a positive effect on the course of the disease, one would expect these markers to be influenced in a way that reflects slower disease-associated change. Currently used clinical measures, such as the Unified Huntington's Disease Rating Scale total motor score (UHDRS-TMS) and total functional capacity (UHDRS-TFC), are useful in measuring disease-related clinical and functional decline. These are, however, fairly crude semi-quantitative measures with substantial intra- and inter-rater variability, and are not sensitive in detecting subtle changes over short periods of time and certainly not before disease onset.[9-11] Although previous neuroimaging studies have shown potential markers, findings remain inconsistent or lacking association with disease state. For instance, findings from previous longitudinal diffusion magnetic resonance imaging reports are contradictory.[12-14] As such, further exploration of neuroimaging techniques is of great relevance.

In the present work, we aim to find robust parameters/markers corresponding with disease state and measuring progression in different stages of HD in a well-defined population, which can be used as suitable objective surrogate clinical trial endpoints. We put special emphasis on longitudinal study designs, as these provide the most useful clinical progression and parameter change associations. Rapid advances in diagnostic methods in the medical field coupled with advances in analysis methods and ever-increasing computational power provides us with the opportunity to explore different and more complex biological markers (biomarkers). A computational approach to tackle the increasing amount of data generated from functional and structural brain scans increases the likelihood of finding biomarkers specific for the disease. For that reason, we will employ different state-of-the-art approaches to evaluate the potential usefulness of specific markers. Such biomarkers are crucial in order to objectively assess expected disease-modifying properties of a potential therapeutic intervention.

With well-designed large longitudinal international studies aimed at finding biomarkers in HD, such as TRACK-HD and PREDICT-HD, our understanding of the premanifest stage has grown considerably, to the point that we now understand that subtle signs and symptoms in all three above-mentioned clinical domains of the disease are measurably present, sometimes decades before the classic disease signs become manifest.[11,15] Although chorea is the characteristic clinical motor presentation of HD and the striatum is considered to be primarily affected within the histopathological profile, the disease affects a myriad of other neurological functions and should be viewed as a multisystem neurodegenerative disorder of the brain.[16] Even though changes in behaviour, cognition, as well as motor skills often precede the onset of the manifest motor symptoms by decades, sensitive and robust longitudinal markers are still largely lacking in this

phase. The methods we employ in this study are expected to yield useful information about the premanifest stage and the progression towards manifest disease. Finding such markers in these subjects is of particular interest, as they have yet to present clinically with the hallmark motor symptoms of HD. Evidence from HD mice models point to the existence of neuronal dysfunction that is reversible through reduction of mHTT load, which leads to phenotypic and histopathological improvements.[17-20] As such, a strategy focusing on both brain function as well as structure to identify biomarkers in HD seems promising.

Aims and outline of the thesis

The general aim of this thesis is to quantify functional and structural disease-related brain aberrations in Huntington's disease, with the goal of exploring biomarker potential of these different parameters for use in clinical trials. It is important to do so for both the premanifest as well as the manifest stage in order to better understand the "functional and structural natural history" of the disorder and to potentially help guide a therapy aimed at slowing or halting disease progression.

As HD symptoms are most likely a consequence of dysfunctioning brain networks, rather than simply being "striato-centric", we aim to explore which regions or circuits in the network are affected in different stages of the disease and how these may change over time. In Chapter 2, we use this network approach on "resting state" functional magnetic resonance imaging (RS-fMRI) activity patterns of the brain, a method generating spatial covariance patterns of blood oxygenation level dependent (BOLD) signal fluctuations by using independent component analysis. The patterns acquired with this technique are usually referred to as "functional connectivity". We hypothesize that greater changes in functional connectivity occur longitudinally in premanifest gene carriers compared to healthy controls over a follow-up period of three years. As this method is data-driven and lacks a priori assumptions regarding potential disturbances to brain connectivity, it is well suited to explore the earliest signs of functional disturbances before manifest disease occurring in the brain as a whole. This approach may potentially reveal changes in brain function ahead of the occurrence of structural changes. Given the importance of the striatum in the histopathological profile of HD, we additionally include a hypothesis-driven part to the analysis by using a region of interest approach examining a potential striatal functional connectivity change relative to the network.

In Chapter 3, we examine microstructural brain abnormalities occurring in different stages of HD in a two-year follow-up period using diffusion tensor imaging (DTI). As microstructural abnormalities naturally occur before macrostructural abnormalities become evident, we expect this technique to provide more sensitive biomarkers compared to volumetric MRI methods. This diffusion MRI technique quantifies water diffusion in tissue and provides indirect information about the microstructural organization of brain tissue. We use an automated histogram analysis method to assess cross-sectional as well as longitudinal changes occurring within two years of

diffusivity measures in whole-brain white matter, grey matter and the striatum. The choice for an automated method is made consciously, as a straightforward, standardized, fully automated and objective approach for interrogating imaging data will be needed in large clinical trials.
As the network of structural brain connectivity is expected to degrade with disease progression, we use a graph theoretical approach to analyse longitudinal diffusion MRI data (**Chapter 4**). A graph theoretical analysis (GTA) is a powerful mathematical framework for quantifying topological properties of networks, which is able to characterize regional and global structure of networks. We expect this integrated approach to provide new insights into the organization of whole-brain structural connectivity in relation to clinical and cognitive functions in HD over a two-year period, potentially providing usable markers of disease progression. This will be the first-of-its-kind study in HD.

In **Chapter 5** we focus on the evolution of *in vivo* microstructural properties of the occipital cortex in different stages of HD, something which has not been a primary focus in HD research to date. We expect to find measurable abnormalities occurring in a two-year time frame in HD and provide a new region of interest for biomarker research and a measure of disease progression in HD clinical trials. Although the striatum is known to be progressively affected during the disease, it is less well established if other specific regions of the brain are also preferentially impacted in a longitudinal manner. Mounting evidence from whole-brain MRI analysis suggest that the occipital regions are altered early on in the disease.[21-27] Furthermore, post-mortem studies have shown atrophy of the occipital lobe to be most pronounced compared to other cortical areas and histologically the absolute nerve cell numbers of the occipital lobe were found to be reduced.[28,29] Given this evidence of early and preferential involvement of the occipital regions in HD, we set out to study this region using diffusion MRI with a fully automated procedure.

Shifting our focus from MRI investigations to electrophysiological markers, in **Chapter 6** we assess the potential of electroencephalography (EEG) as a biomarker in HD using machine learning automatic classification. EEG abnormalities are known to occur in HD.[30] Through registration of physiologic activity of neurons, quantitative electroencephalography (qEEG) provides objective parameters assessing possible (sub)cortical dysfunction occurring prior to or concomitant with motor or cognitive disturbances observed in the disease. Given the progressive functional deficits seen with disease advancement, it is expected that EEGs of HD patients are different from healthy subjects. To test this hypothesis, automatic analysis methods for such complex data are desirable in order to provide objective and reproducible results. In this cross-sectional study, we use a machine learning method with the aim of automatically classifying EEGs as belonging to HD gene carriers versus healthy controls. Furthermore, we aim to derive qEEG features that correlate with commonly used clinical and cognitive markers in HD research to evaluate biomarker potential.

It is likely that a multimodal approach is needed to have a comprehensive understanding of neuropathology in HD, as any one modality is always limited by its intrinsic properties. In **Chapter 7** we use a multimodal approach to characterize the visual network in HD using different MRI

modalities and visual evoked potentials as an electrophysiological modality. This is done in the light of considerable evidence showing that the visual cortex is one of the first cortical regions in HD to be affected by neuronal loss, as was described above.

In **Chapter 8** we provide summarizing remarks together with potential directions for future research.

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