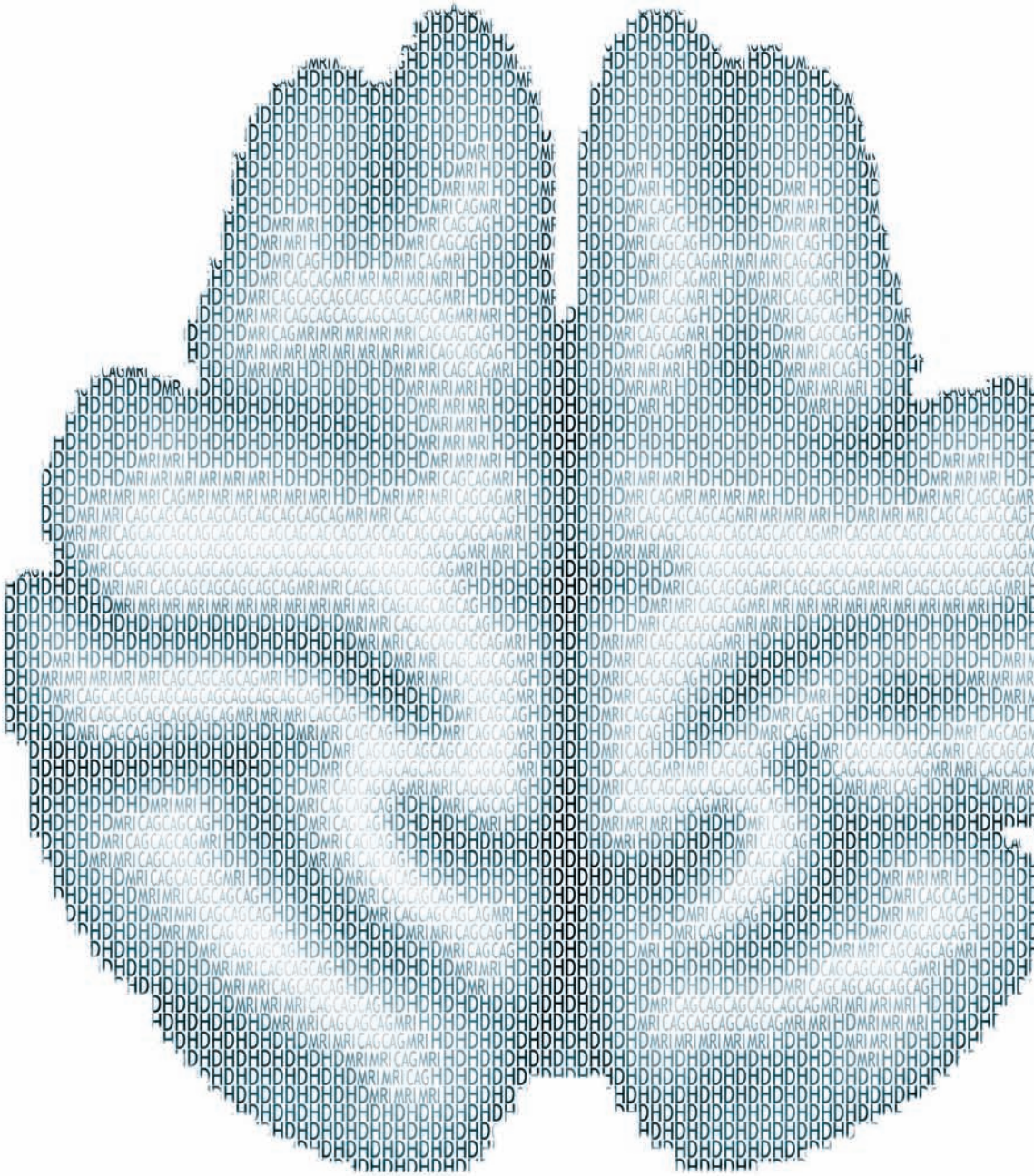
HD
MRI
CAG

Chapter 1

General Introduction

Huntington's disease

Huntington's disease (HD) is a progressive neurodegenerative disorder first described by George Huntington in his paper *On Chorea*[1] in 1872. He described a form of chorea he called *hereditary chorea,* evident in some families living on Long Island, USA, which seemed different to the chorea occurring in children. As an acknowledgment of his accurate description, the disease was later named after him.

The observations by George Huntington still hold true to this day. Besides giving a detailed description of chorea, he also described the hereditary nature of the disease, a tendency towards insanity and suicide, and onset in adult life[1]. More recent clinical descriptions elaborate on the symptoms and signs describing motor-, cognitive-, and psychiatric disturbances, and also autonomic nervous system impairment, weight loss and sleep disturbances[2;3].

Motor symptoms are the most visibly explicit symptoms and consist of chorea, dystonia, loss of postural reflexes, bradykinesia and rigidity[2]. Cognitive decline is also evident with impairment in memory, psychomotor speed, negative emotion recognition and executive functioning[4]. Among the psychiatric symptoms a high risk of depression, suicide, obsessive compulsive disorders, irritability and aggression exists[2;5]. Symptoms originating from the autonomic nervous system are gastrointestinal, urinary, cardiovascular, temperature regulation, and sexual problems[3]. Also weight loss and sleep disturbances are important features of the disease[2;6;7]. Notably the most overt physical signs, such as chorea, do not necessarily have the most impact on quality of life of both patients and family members. Patients report that psychosocial factors influence quality of life more than physical factors[8]. The mean age of disease onset is between 35-44 years of age, with a wide range from early infancy to 90 years of age[9]. The duration of illness, from clinical diagnosis until death ranges from 15-30 years[10]. The initial symptom varies between individuals and can start with problems in any of the above described domains. Chorea and balance impairments are easily noticed by patients. However, family members may notice psychiatric or cognitive symptoms many years prior to diagnosis[10].

Genetics and pathofysiology

The genetic defect responsible for HD was discovered in 1993[11].The gene defect is located on the short arm of chromosome 4 and consists of an expanded Cytosine-

Adenosine-Guanine (CAG) repeat in the *HTT*-gene. The normal gene produces *huntingtin (HTT)* protein. A normal repeat length is up to 26 repeats; extended CAG-repeat lengths of 27-35 do not lead to disease but have a potential to expand >35 within one or more generations. CAG-repeat lengths above 36 will eventually lead to the development of the disease, with repeat lengths of 36-39 with reduced penetrance and ≥40 with full penetrance[12]. Sixty to seventy percent of the variability in the age of onset can be explained by the length of the CAG repeat, leaving a reasonable amount of unknown factors[9]. Expected age of onset can be reasonably predicted by the formula devised by Langbehn *et al.* (2004,2010)[13;14]. However, due to various limitations, this formula is usually only used for research purposes and not in clinical practice.

The exact pathofysiological mechanism responsible for the neurodegeneration is still not understood. The following five neurodegenerative mechanisms have been described. First, direct neurotoxic effects of mutant *HTT*-protein. Second, impaired energy metabolism due to mitochondrial disturbances. Third, transcriptional dysregulation of multiple genes, encoding for neurotransmitter receptors, enzymes and proteins involved in neuronal structure, stress responses and axonal transport. Fourth, disrupted dynamic intracellular processes, including trafficking of vesicles from the Golgi apparatus and within the cytoskeleton, transport along microtubules in axons, and synaptic disruptions. Fifth, excitotoxicity based on increased sensitivity to neurotransmitter mediated stimulation, causing overstimulation and cell death[9;15-17]. In recent reports, substantial evidence has been found for a role of these mechanisms in the neurodegeneration observed in HD. It is not likely that any of these mechanisms are solely responsible. Rather, a combination of mechanisms could be responsible with interactions between these different pathogenic pathways. This interaction theory is highlighted when reviewing the evidence for N-methyl d-aspartate receptor (NMDAR) involvement[9]. Evidence exists for the transcriptional dysregulation of genes involved with the NMDAR. Also selectively activated NMDARs in animal models resulted in a HD neurodegenerative pattern. Finally, mitochondrial dysfunction has been shown to sensitize cells to NMDAR mediated cell death[9]. Hence, these theories describe the complex interaction between multiple pathways in the pathogenesis of HD.

Neuropathology

George Huntington stated in his opening sentence “chorea is essentially a disease of the nervous system”, which of course, as we know now, is certainly the case. However, he hypothesized that the cerebellum was responsible for disease

symptoms. Later neuropathological findings pointed towards the striatum being the most affected brain regions. Jelgersma was the first to correlate atrophy of the caudate nucleus with HD in 1908 and since then many other reports emerged on pathologic findings in HD[18]. In 1985 a grading system was proposed by Vonsattel et al, describing the amount of striatal involvement in relationship to clinical disability[19]. The pathologic spectrum however extends greatly beyond the striatum[18].

On gross examination, atrophy of the striatum and whole brain is apparent, with the frontal lobe being more affected than others[18]. Microscopic findings include neuronal nuclear inclusions of mutant *HTT*, which occur in the striatal, neocortex and allocortex neurons[20;21]. In the striatum reactive fibrillary astrocytosis, increased density of oligodendrocytes and reactive microgliocytes can be detected. Mild to marked neuronal loss is also seen in globus pallidus, thalamus, subthalamic nucleus, substantia nigra, white matter and cortex, usually without reactive gliosis. This widespread atrophy is especially evident in grades 3 and 4 of the Vonsattel grading[18;19]. Overall, striatal abnormalities are most evident with other brain regions becoming affected at a later stage. Interestingly, involvement of the cerebellum, as first thought of by George Huntington, is the one region still heavily debated[18].

The neuropathologic findings are generally limited to end-stage HD patients. It does not tell us when, how and where changes begin in premanifest HD gene carriers. Nor is a neuropathological outcome suitable for making statements regarding the success of a therapeutic strategy. These findings do point out the regions most interesting for further study.

Biomarkers

As our understanding of the pathofysiology of the disease increases, the development of therapeutic strategies becomes possible. For therapeutic strategies the point of application within the pathogenic pathway from mutant gene to neuronal loss is vast. Possibilities include gene therapy, post-transcriptional alterations, metabolic alterations, neuroprotective agents and more[16]. Many promising strategies are being examined, however no successful compound has been found to date. To measure possible effects of such compounds, biological markers (biomarkers) are used. A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”[22]. The application of biomarkers is divers; they can be used in early efficacy and

safety evaluations and “proof of concept” applications, mostly for in vitro or in vivo studies. Furthermore biomarkers can be used as either a diagnostic tool, for disease staging, as indicators of disease progression or prediction and monitoring of clinical response to an intervention. The term surrogate endpoint is a specific application of biomarker but is often used interchangeably. A surrogate endpoint is defined as: “a biomarker that is intended to substitute for a clinical endpoint”. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathofysiologic, or other scientific evidence[22].

The need for robust and sensitive biomarkers in HD is highlighted by the fact that several problems are present in measuring effectiveness in HD. First of all HD has a very heterogenic clinical profile. We can roughly predict the age of onset, however it is not exactly known when or with what set of symptoms the disease will start. Motor symptoms are most overt and usually lead individuals to seek medical attention. Cognitive decline can develop slowly and even go unnoticed for many years. Psychiatric illness can appear as a first symptom of HD or are not related to HD at all. A second challenge is the slow rate of disease progression, taking up more than a decade from diagnosis to death[2]. This makes medication trials long lasting and very expensive. Thirdly, it is difficult to measure any clinical effect in the premanifest stage of the disease, as no symptoms are present. Conversely, this group is most likely to benefit from any potential therapeutic strategy when the neuronal loss is still limited.

For manifest HD many potential biomarkers could be appropriate, such as the quantification of motor or cognitive symptoms. To grade clinical severity the Unified Huntington’s Disease Rating Scale (UHDRS) is the most widely applied measure[23]. Premanifest HD-gene carriers can display *subtle* motor, cognitive or psychiatric abnormalities that are not captured by this scale. Therefore this scale is unsuitable as a biomarker in the premanifest phase.

Alternative biomarkers to clinical outcome measures are sought whereby the most obvious candidates are magnetic resonance imaging (MRI) parameters. These could serve as biomarkers individually or in combination with other measures such as chemical markers (blood, urine or cerebral spinal fluid) or other imaging techniques such as positron emission tomography. The choice for imaging techniques such as MRI seems logical as they allow for direct examination of the damaged structure, namely the brain. Furthermore MRI can be applied fairly easily and objectively and the biggest advantage is that it can be used in the premanifest stage of the disease. Indeed, early MRI studies already showed striatal abnormalities in at risk HD gene carriers[24;25].

In this thesis the term MRI biomarkers refers to indicators of disease progression with the purpose of disease monitoring. Ultimately, these measures can serve as surrogate endpoints in clinical trials. As a first step, prior to use in clinical trials, it is absolutely vital to determine the 'natural history' of disease changes as measured by MRI biomarkers.

Magnetic Resonance Imaging

MRI can give *in vivo* insight in HD pathology. Conventional MRI sequences, such as T1- and T2-weighted sequences, are based on protons bound to water molecules. Relaxation properties of these protons in different tissues provide contrast between grey and white matter. A more recently introduced sequence of diffusion weighted imaging (DWI) makes use of the displacement or diffusion properties of protons. This technique can be extended to diffusion tensor imaging (DTI), creating a 3 dimensional image per voxel of the principal movement direction of protons. This is especially useful for visualizing white matter tracts, using a technique commonly referred to as tractography.

Protons bound to other molecules than water, have different resonance characteristics, creating possibilities to quantify these molecules. Magnetic resonance spectroscopy (MRS) is a technique for quantifying concentrations of certain metabolites in the brain. An example is N-acetylaspartate (NAA), a metabolite considered a marker of neuronal integrity. Magnetization transfer imaging (MTI) is based on the exchange of protons between a pool of protons bound to macromolecules and a pool of free protons water.

Functional MRI makes use of the magnetic properties of oxygen-rich and oxygen-depleted blood in the brain. Oxygen is carried by the molecule hemoglobin, and deoxygenated hemoglobin is more magnetic than oxygenated hemoglobin, which is virtually nonmagnetic. Utilizing these properties it is possible to map neuronal activity.

These different MRI techniques show the many possibilities MRI has to offer to the field of biomarker research. MRI techniques are subject to fast development and are still further developing. Techniques such as T2*-weighted imaging, susceptibility weighed imaging, asymmetric spin echo and other techniques are of importance, however will not be discussed in this thesis.

Aims and outline of the thesis

The primary aim of this thesis is to search for MRI biomarkers for HD. The value of different techniques as biomarkers will be explored for the different stages of HD. Neuropathologic findings and previous MRI studies are starting points for the study of potential biomarkers. Known genetic and clinical factors will be taken into consideration. Finally, based on our observations, a recommendation will be given regarding MRI as potential biomarker in HD.

An overview of all current literature on MRI research in HD is given in chapter 2. An extensive volumetric MRI analysis of the main structures of the HD brain is described in chapter 3. More structures than striatum alone will be addressed providing an overview of the atrophy of subcortical grey matter. To gain insight into the localized changes in these nuclei, shape analysis has been performed on these subcortical structures and is discussed in chapter 4. The more recent MRI techniques of MTI, DTI and MRS are discussed in chapters 5, 6 and 7 respectively. The technique of MRS diverts somewhat from structural MRI scanning as it measures metabolites which can be both markers of neuronal integrity as well as part of dynamic processes. How structural integrity changes occur over time as measured by MTI is outlined in chapter 8 and how metabolites change over time using MRS is discussed in chapter 9. A general discussion of findings, final conclusions and future perspectives are outlined in chapter 10.

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