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CHAPTER 1

Introduction
HUNTINGTON’S DISEASE

Clinical features of Huntington’s disease (HD) include motor, neuropsychiatric and cognitive symptoms[1]. HD is an autosomal dominant inheritable neurodegenerative disorder. This implies that, if one of the parents is afflicted, their offspring has a 50% probability of developing HD. Although neuropsychiatric and cognitive symptoms often precede the manifestation of motor abnormalities of HD, the definite diagnosis of HD is usually made when the first motor symptoms occur. Although the age of onset is wide (ranging from early childhood to senescence), it is generally between 30 and 50 years of age. The duration between the time of diagnosis and death is around 20 years[2]. Motor symptoms may initially be subtle and might, at first, escape the awareness of the patient. Chorea is the most prominent motor symptom in HD and is characterized by involuntary irregular movements of the head, trunk and limbs. Other movement disorders, such as dystonia, bradykinesia, hypokinesia and postural instability, also occur[3]. In advanced stages of the disease, dysphagia and dysarthria may also develop[4].

NEUropsychiatric symptoms in HD

In HD, the most prevalent neuropsychiatric symptoms are depressed mood, irritability and apathy. The prevalence of depressed mood ranges from 33-69%. Apathy and irritability occur frequently in HD with a reported prevalence of 34-76% for apathy and of 33-73% for irritability[1].

Apathy is defined as a disorder of motivation with diminished goal-directed behavior, cognitions and emotions[5]. Apathy is positively correlated with cognitive decline, male sex, the presence of depression, and the use of psychotropic medication[6]. Longitudinally, although new-onset apathy was shown to occur in 14% of HD gene expansion carriers over a two-year study period, apathy also remitted in 14% during that same time period[7], indicating the potential for remittance. This suggests that HD patients with apathy should be evaluated for treatable causes of apathy.

Irritability can be characterized as a dysphoric mood state that predisposes toward both verbal and non-verbal expressions of aggression in a non-adaptive manner that complicates the relationship between the patient and the caregiver and/or partner[8]. Even before motor symptoms are present, irritability can cause severe distress to HD patients and their caregivers.

In pre-motor symptomatic disease stages, cognitive dysfunction mostly comprises deficits in attentional and executive functions, semantic verbal fluency and visual working memory. Deficits in memory become apparent around the time of onset of motor symptomatic disease. In the advanced disease stage, patients often exhibit severe impairments, particularly in executive functioning[9].
PATHOPHYSIOLOGY

The causal genetic mutation was discovered in 1993 and comprises an expanded cytosine-adenine-guanine (CAG) trinucleotide repeat coding for the huntingtin (\textit{htt}) protein on the short arm of chromosome 4 (4p16.3)[10]. Persons with an expansion of >39 CAG repeats will develop the disease, although an expansion of 36-39 CAG repeats may also result in symptomatic HD (‘reduced penetrance’). The expanded gene codes for mutant huntingtin (\textit{mhtt})[11]. A greater CAG repeat length is associated with an earlier age of onset of symptoms.

Neurodegeneration is a prominent feature of HD. In HD, there is neuronal cell loss in the brain, particularly in the caudate nucleus and the putamen, but also in other brain regions, including the cortex. This loss of neurons is already detectable in pre-motor symptomatic HD gene expansion carriers[12]. In physiological circumstances \textit{htt} is expressed in all cells (with the highest concentrations in the brain and testes) and plays a key role in transcription, cell signaling and intracellular transporting[13]. However, much remains unknown about the physiological function of \textit{htt} as well as the exact pathophysiology by which \textit{mhtt} causes cerebral tissue damage. Several mechanisms are likely to play a role in the neurodegenerative process, including increased excitotoxicity, mitochondrial damage, free radical formation, and immune activation[14].

 IMMUNE SYSTEM AND INFLAMMATION IN HD

Aberrant immune activation is one of the proposed underlying mechanisms for neurodegeneration and, subsequently, the development of the characteristic symptoms in HD[14]. Microglia, the main immunocompetent cells in the central nervous system, play a key role in immune processes in the brain[15]. Activated microglia have been demonstrated in post-mortem samples of patients with HD and on cerebral positron emission tomography (PET) scans[16, 17]. \textit{Mhtt} may play an important role in the activation of microglia by directly activating the nuclear transcription factor-kB (NF-kB), thereby initiating the first steps of the acute-phase response [18]. Also, \textit{mhtt} can activate the IkB kinase complex, thereby enhancing activity of the nuclear transcription factor NF-kB[18]. NF-kB plays a key role in the regulation of the immune response to infection[19]. Alternatively, cell damage caused by \textit{mhtt} through other mechanisms may activate the acute-phase response. The NF-kB pathway is a major inducer of the pro-inflammatory cytokines interleukin (IL)-1\beta, IL-6 and tumor necrosis factor (TNF)-\textit{\alpha}. Among these cytokines, IL-6 is the most potent inducer of the acute-phase response[20], a systemic reaction aimed at restoring physiological homeostasis under physiologic circumstances[21]. This response is regulated by several other pro-inflammatory and anti-inflammatory cytokines such as IL-8 and IL1-ra, IL-5 and IL-10, respectively. As part of this systemic reaction, the production of certain proteins is upregulated or downregulated to the benefit of the injured organism[22, 23]. As such, the production of C-reactive protein (CRP), that plays a prominent role in the complement cascade, is
upregulated and its circulating level can be thought of as a positive acute-phase protein, whereas the production of albumin is downregulated and its circulating level can therefore be thought of as a negative acute-phase protein.

**BIOMARKERS OF DISEASE STATE IN HD**

Biomarkers are important because they may provide an early indication as to whether (or not) a potential therapeutic intervention is beneficial. A useful biomarker needs to be readily quantifiable, robust and reproducible [12, 24, 25]. In HD, biomarkers of several modalities have been employed to observe differences between HD gene expansion carriers and controls, and to observe differences between HD gene expansion carriers at different disease stages, or even to observe differences in HD gene expansion carriers before disease onset[12].

Clinical measures are regularly used as markers of disease stage in HD investigations. One of the most frequently used clinical rating scales is the Unified Huntington’s Disease Rating Scale (UHDRS) which comprises subscales on several domains, such as daily functioning and motor symptoms. In general, the UHDRS scale measures changes in symptomatic HD over time, but is not able to detect changes in pre-motor symptomatic HD. In addition, neuropsychiatric measures, such as the Problem Behaviours Assessment (PBA) scale, are used to assess specific neuropsychiatric features of the disease.

Structural magnetic resonance imaging (MRI) is widely used to define cerebral biomarkers in HD gene expansion carriers. Cross-sectionally, MRI can show disease-related atrophy of the striatum and white matter of HD gene expansion carriers compared with controls. This atrophy can be demonstrated years before the onset of clinical disease. In addition, using MRI, progressive neurodegenerative changes can be demonstrated in early HD and pre-motor symptomatic HD gene expansion carriers.

However, information from biochemical biomarkers might be obtained more rapidly (and at less cost) compared with imaging markers. In addition, biochemical markers that are in close proximity to the underlying pathology may be more sensitive to disease progression and might show reversal in response to therapeutic interventions. Cytokine levels in the plasma are potential biochemical biomarkers in HD. Increased levels of several cytokines have been reported in cross-sectional studies among HD gene expansion carriers[26-30]. Most consistently, plasma IL-6 levels were higher in motor symptomatic HD gene expansion carriers than in matched controls. Also, increased plasma levels of sIL-2R, sTNF-α and IL-8 were found compared with levels in controls.

*Cytokines and acute-phase proteins in relation to neuropsychiatric symptoms in HD*

The acute-phase response involves a systemic reaction of the immune system as reflected in activation of monocytes, which is regulated by several cytokines[20, 21]. In addition, metabolic
changes occur, such as the production of certain acute-phase proteins and the inhibition of production of other molecules [22, 23]. These changes, which are readily quantifiable in the blood, may be accompanied by behavioral changes that favor the survival of the organism when the homeostasis is disturbed. In mouse models, behavioral changes (such as psychomotor retardation, anorexia, sleep disturbances and lethargy) have been called ‘sickness behavior’. Changed levels of cytokines in the brain may also cause part of these behavioral effects in humans [31]. Reciprocal connections exist between the peripheral immune system and the brain [31-34]. Cytokines in the peripheral plasma can pass the blood-brain barrier under certain conditions and can activate nerve cells that stimulate immune cells in the brain to produce cytokines.

Given this bidirectional association between plasma and brain cytokines and behavior, several studies have examined the connection between cytokines and neuropsychiatric symptoms in non-HD populations. The association between cytokines, acute-phase proteins and depression has been investigated the most extensively [35-37]. Consistently increased levels of IL-6 and CRP were found in (non-HD) depressed patients. In addition, the association between cytokines and cognitive decline, as well as dementia, yielded similar results [38]. The association between cytokines and irritability and apathy has also been investigated in non-HD populations, but with inconsistent results [37, 39-44].

In HD, neuropsychiatric symptoms (in particular psychosis and irritability) and motor symptoms are frequently treated with antipsychotic medications [45]. However, antipsychotics can induce symptoms that can mimic apathy, depressive mood and cognitive decline [46]. Through several (hepatic) mechanisms, antipsychotics may adversely influence plasma levels of cytokines and acute-phase proteins [47-49]. In addition, metabolic disturbances are a well-known side-effect of antipsychotics, which is associated with low-grade inflammation [50]. Therefore, the use of antipsychotics is a potential confounder of the relationship between cytokine levels and acute-phase proteins on the one hand and neuropsychiatric symptoms on the other, and should be taken into account when investigating these relationships.

AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis was to get a better understanding of the incidence and course of neuropsychiatric symptoms in HD, particularly irritability that is a core behavioral symptom. Also, we aimed to investigate the relationship between activity of the immune system and presence of neuropsychiatric symptoms. We expected to find a relation between neuropsychiatric symptoms and increased pro-inflammatory activity of the immune system, and, given the findings of earlier studies, we expected this activity to further increase in more advanced disease stages. This aberrant immune state drives neuroinflammation and in turn causes neuronal dysfunction and in the end, cell-death. We hypothesized that these pathological changes would be reflected in occurrence of neuropsychiatric symptoms. Therefore, we expected to find that neuropsychiatric
symptoms would increase as activity of the immune system increased as measured by levels of cytokines in the plasma. In Chapter 2, the psychometric properties of the Irritability Scale were assessed in order to reliably measure and detect irritability in HD. Also, because environmental factors play an important role in HD psychopathology, in Chapter 2 and Chapter 3, we investigated sociodemographic and clinical characteristics that correlated with irritability, or could predict irritability. The role of some parts of the innate immune system was investigated using both plasma acute-phase proteins and cytokines in relation to neuropsychiatric symptoms and cognitive dysfunction in HD. In Chapter 4, the relation between acute-phase proteins and neuropsychiatric symptoms, and the use of psychotropic medication in HD, were examined. The studies in Chapter 5 investigated the role and usefulness of immune system parameters as biomarkers in HD. In a longitudinal study design, we aimed to investigate whether cytokine levels correlated with disease stage and whether cytokine levels changed in conjunction with changes in disease stage. In Chapter 6, plasma cytokine levels were investigated in relation to neuropsychiatric symptoms and cognitive dysfunction in HD. Finally, in Chapter 7, our findings are summarized and considered within the current perspective, methodological and clinical implications are discussed, and suggestions are made for further research.
REFERENCES

Introduction

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