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Neuropsychiatric symptoms and immune system parameters in Huntington's disease

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Neuropsychiatric symptoms and immune system parameters in Huntington's disease

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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 (*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 (*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 *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 *htt* as well as the exact pathophysiology by which *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]. *Mhtt* may play an important role in the activation of microglia by directly activating the nuclear transcription factor- κ B (NF- κ B), thereby initiating the first steps of the acute-phase response [18]. Also, *mhtt* can activate the I κ B kinase complex, thereby enhancing activity of the nuclear transcription factor NF- κ B[18]. NF- κ B plays a key role in the regulation of the immune response to infection[19]. Alternatively, cell damage caused by *mhtt* through other mechanisms may activate the acute-phase response. The NF- κ B pathway is a major inducer of the pro-inflammatory cytokines interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α . 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 IL-1 α , 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.

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CHAPTER 2

Irritability in Huntington's disease

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ABSTRACT

Irritability is a frequent neuropsychiatric symptom in patients with Huntington's disease (HD). The Irritability Scale (IS) and the irritability factor of the Problem Behaviours Assessment (PBA) was used to assess irritability among 130 HD mutation carriers and 43 verified non-carriers. The IS was tested using receiver operating characteristic analysis against different cut-offs of the PBA irritability factor. A robust IS cut-off score of ≥ 14 points was found indicating that 45 (35%) of the 130 mutation carriers were irritable vs. 4 (9%) of the 43 non-carriers ($P=0.001$). The level of agreement between self-report and informant-report IS was of moderate strength (intraclass correlation=0.61). Using univariate and multivariate regression analyses, independent correlates of irritability were: being married/living together ($P=0.02$), CAG repeat length ($P=0.01$), and use of benzodiazepines ($P=0.008$). Using the same model with the informant's irritability score, use of benzodiazepines was the only significant independent correlate of irritability ($P=0.005$). Irritability is a prominent symptom of HD and can be reliably assessed with the IS using a cut-off score ≥ 14 points. Although it is unclear whether benzodiazepine use causes irritability, or irritability leads to the prescription of benzodiazepines, clinical evaluation with respect to the use of benzodiazepines in HD warrants attention.

INTRODUCTION

Huntington's disease (HD) is a progressive autosomal dominant neurodegenerative disorder resulting from an expanded trinucleotide cytosine-adenine-guanine (CAG) repeat in the huntingtin (*HTT*) gene on chromosome 4 (Huntington's Disease Collaborative Research Group, 1993). Clinical features of HD include motor disturbances, cognitive deterioration and a variety of psychiatric symptoms (Walker, 2007). Psychiatric symptoms such as depressed mood, perseverative behaviour, and irritability are frequently reported, and often precede the manifestation of motor abnormalities of HD (Paulsen, 2001; Duff, 2007; van Duijn, 2007).

Despite its frequent occurrence, negative clinical consequences for mutation carriers and its heavy burden for caregivers, irritability has scarcely been studied in HD. The term 'irritability' has been used as a description of behavior ranging from bad temper to violent outbursts, but is also defined as a mood state characterized by a reduction in control over temper possibly but not necessarily resulting in verbal or behavioral outbursts (Snaith, 1985; Craig, 2008).

Few reliable data on the true prevalence of irritability in HD exist due to small sample sizes, use of different methodologies, and lack of control groups. Reported prevalence rates for irritability range from 38–73% (van Duijn, 2007). This variation in prevalence can be explained by the use of different assessment methods with varying definitions and different study populations. No follow-up studies have covered a long period of time. Irritability may occur in all stages of HD, even before motor symptoms are present, and may cause severe distress to mutation carriers and their families, determining the need for admittance to a nursing home.

The instruments used to assess the presence and severity of irritability in HD include the Neuropsychiatric Inventory (NPI) (Paulsen, 2001; Kulisevsky, 2001). The behavioural section of the Unified Huntington's Disease Rating Scale (UHDRS-b) (Murgod, 2001), the Irritability Scale (IS) (Chatterjee 2005; Klöppel, 2010), and the Problem Behaviours Assessment (PBA) (Craufurd, 2001; Kingma 2008). However, no gold standard (cut-off) for assessing the presence of irritability is available.

The present study uses the IS and the PBA to assess irritability in HD. The aim was to investigate the psychometric properties of the IS against the irritability factor of the PBA, in order to establish reliable cut-off scores for irritability with high sensitivity and specificity. Prevalence rates of both self-reported and informant-reported irritability in HD and their correlates were assessed.

METHODS

Participants

Between May 2004 and August 2006, HD mutation carriers and first-degree non-carriers were recruited from the outpatient department of Neurology and Clinical Genetics of the Leiden

University Medical Centre (LUMC) and from a regional nursing home. Subjects with a CAG repeat length of 36 or more repeats were considered to be HD mutation carriers. Details of the study design are described elsewhere (Kingma, 2008). A second measurement was conducted two years after the baseline visit.

Since the use of the IS was introduced while the first wave was already underway, subjects for this study comprised (non-overlapping) 130 mutation carriers and 43 non-carriers from both the first and second waves. Additional information was available from 120 informants of the 130 mutation carriers and from 38 informants of the 43 non-carriers. Of the 120 mutation carriers' informants, 70 were spouses or partners, 4 were neighbours or friends, 10 were first-degree family members, 15 were specialized caregivers, and the status of 21 informants was unknown. The study was approved by the Medical Ethical Committee of the LUMC, and all participants gave informed consent.

Instruments

Assessment of irritability

The IS was used to assess irritability (Figure 1); this scale has previously been used to assess irritability in HD (Chatterjee, 2005; Klöppel, 2010). The IS poses 14 questions about the presence of various phenomena of irritability in the two weeks prior to the interview. Each question has four answer categories scored on a 4-point Likert scale: 'not at all', 'slightly', 'some', and 'a lot'. The total sum score of the IS ranges from 0 to 42 points, with higher scores indicating more severe irritability. The participant was asked to rate the self-report version of the IS (IS-self) and the informant was asked to rate the irritable behaviour of the participant with the identical informant-report version of the IS (IS-informant). Until now, no studies have assessed the psychometric properties of the IS.

The PBA is a reliable instrument to assess neuropsychiatric symptoms in HD (Craufurd, 2001; Kingma, 2008). The severity and frequency of each of the 36 items of the PBA are rated on a scale from 0 to 4 points, with higher scores indicating more psychopathology. The interrater reliability of the PBA was 0.82 (95%CI=0.65–1.00) for severity scores and 0.73 (95%CI=0.47–1.00) for frequency scores (Kingma, 2008). A factor analysis of the PBA revealed three symptom clusters (factors): irritability, depression, and apathy. The irritability factor of the PBA consists of five items: 'irritability', 'aggression', 'verbal outbursts', 'inflexibility', and 'self-centeredness/demanding behaviour' (Kingma, 2008). The irritability factor score is obtained by the sum of the multiplied frequency and severity scores of the five items (range 0–80 points). We chose to validate the IS against the PBA irritability factor, as we consider the PBA to be the best measurement tool available to assess irritability in HD.

Since the UHDRS-b is widely used in HD studies, we also scored the severity and frequency of the 11 neuropsychiatric items of this scale (Huntington Study Group, 1996). Severity and frequency are rated on a scale from 0 to 4 points, with higher scores indicating more psychopathology.

Irritability Scale Self-report version					
	not at all	slightly	some	a lot	ICC*
1. Are you easily irritated?	0	1	2	3	0.49
2. Do you pout if things don't go your way?	0	1	2	3	0.37
3. Do you have good control of your temper with the family (or persons living with you)?	3	2	1	0	0.31
4. Do little things cause you to fly off the handle?	0	1	2	3	0.44
5. Do you adjust well to a change in plans?	3	2	1	0	0.43
6. When you lose your temper, do you have a hard time calming down again?	0	1	2	3	0.38
7. Do you insist on having your own way?	0	1	2	3	0.28
8. Are you easily agitated by minor problems?	0	1	2	3	0.38
9. Can you discuss problems together and agree to a reasonable solution?	3	2	1	0	0.47
10. Do disagreements often lead to arguments?	0	1	2	3	0.40
11. Can you appreciate a different point of view from yours?	3	2	1	0	0.30
12. Do you yell a lot?	0	1	2	3	0.59
13. Are you able to control your temper with persons outside the family?	3	2	1	0	0.25
14. Do you consider yourself to be irritable?	0	1	2	3	0.45

Figure 1 Irritability Scale (self-report version) with the level of agreement between self-reported and informant-reported level of irritability.

* Level of agreement for each score between IS-self and IS-inf as determined by ICC with a 1-way random effects model with single-measure reliability.

ICC = Intraclass Correlation Coefficients;

IS-self = Irritability Scale self-report;

IS-inf = Irritability Scale informant-report.

Sociodemographic and clinical characteristics

Information on sociodemographic and clinical characteristics of mutation carriers and controls was collected in a standardized manner. Global daily functioning was assessed with the Total Functional Capacity (TFC) scale of the UHDRS (Shoulson, 1979). The TFC consists of five questions assessing employment, capacity to handle financial affairs, capacity to manage domestic chores, capacity to perform activities of daily living, and the care level provided, with higher scores indicating better functional capabilities. The neurological examination was performed by a neurologist with experience in HD, blinded for the genetic status of the subject. Motor functioning was assessed according to the motor section of the UHDRS (UHDRS-m) (Hun-

tington Study Group, 1996). Mutation carriers with UHDRS Confidence Level score 0 or 1 were considered pre-motor symptomatic and mutation carriers with Confidence Level score >1 were considered motor symptomatic. Estimated duration of disease was calculated by the estimated age of onset according to the equation of Vassos et al.: $\ln [\text{age of onset (years)}] = 6.18 - 0.054 * [\text{CAG repeats (number)}]$ minus the current age (Vassos, 2008).

Assessment of cognitive functioning

The Mini-Mental Status Examination (MMSE) (Folstein, 1975), Symbol Digit Modalities Test (SDMT) (Smith, 1968), Verbal Fluency Test (VFT) (Hodges, 2009), and Stroop tests (Stroop, 1935) were administered to assess both global and frontal executive cognitive functioning.

Assessment of psychiatric disorders

The Dutch translation of the computerised version of the Composite International Diagnostic Interview (CIDI, version 2.1) (Robins, 1989) was used to assess the presence of a depressive disorder according to the criteria of the Diagnostic and Statistical Manual of mental disorders, 4th edition (DSM-IV) (American Psychiatric Association, 2000). The CIDI was not administered in subjects with MMSE score <18 points, since the CIDI cannot be reliably administered to patients with severe cognitive dysfunction.

Statistical analyses

Data are presented as n (%), mean ($\pm$ standard deviation (S.D.)), or median (inter-quartile range (IQR)) when appropriate. Chi-square tests for categorical data, t-tests for independent samples with normal distribution, or nonparametric Mann-Whitney U tests were conducted to compare mutation carriers and non-carriers. Convergent validity was assessed by Spearman's correlation coefficient. Kruskal-Wallis tests were conducted to compare IS-self and IS-informant scores among the three groups of non-carriers, pre-motor symptomatic carriers, and motor symptomatic mutation carriers. Cronbach's alpha was assessed as a measure for internal consistency of the IS-self and IS-informant.

Because no known cut-off score exists for the presence of irritability as assessed with the IS, Receiver Operator Characteristic (ROC) analyses were performed against different cut-offs (i.e. 10, 15, and 20 points) of the irritability factor of the PBA that yielded optimal sensitivity and specificity. The area under the ROC curve (AUC) was used as an indicator of the discriminatory power of the IS to distinguish between irritable and non-irritable subjects according to the irritability factor of the PBA.

Using univariate logistic regression analyses, mutation carriers with IS score ≥ 14 points were compared to those with IS score <14 points to determine correlates of irritability. Odds ratios (OR) and their corresponding 95% confidence interval (CI) were computed. Because of a non-normal distribution of TFC, UHDRS-m and MMSE scores, these data were dichotomized using a median split. To yield the independent correlates of irritability (IS-self), multiple lo-

gistic regression analysis with a forward selection procedure was used, selecting the following univariate correlates with $P < 0.10$: being married/living together with a partner, CAG repeat length, TFC, use of benzodiazepines, and Stroop interference test. This model was adjusted for age and sex (i.e., forced into the model). In addition, the same variables were entered using the IS-informant score as the dependent variable. In sensitivity analyses, models were repeated using different cut-off scores of the IS.

Agreement between IS-self and IS-informant scores was assessed using one-way random, single measure intraclass correlation coefficients (ICCs). The same analysis was performed to assess the level of agreement on each of the 14 items of the IS. All tests were two-tailed with $P < 0.05$ denoting statistical significance. The SPSS version 16.0 (SPSS Inc., Chicago, USA) was used for the analyses.

RESULTS

Sociodemographic, clinical, and neuropsychiatric characteristics

Table 1 presents the sociodemographic, clinical and neuropsychiatric characteristics of the 130 (39 pre-motor symptomatic and 91 motor symptomatic) HD mutation carriers and the 43 non-carriers. Mutation carriers were older and less often married/living together with a partner than non-carriers. Mutation carriers had significantly higher irritability scores (both IS-self and IS-informant) than non-carriers, and 45 (35%) of the 130 mutation carriers were irritable according to an IS-self score ≥ 14 points, compared to 4 (9%) of the 43 non-carriers. Although the CIDI assessment was not possible in 11 mutation carriers due to severe cognitive impairment (MMSE < 18 points), there were 13 (10%) mutation carriers, of whom 8 had a major depressive disorder vs. one (2%) non-carrier with a psychiatric disorder.

Psychometric properties of the Irritability Scale

Cronbach's alphas were 0.90 for the IS-self and 0.93 for the IS-informant. There was some evidence for convergent validity with the irritability item of the UHDRS-b indicated by Spearman's correlation coefficient of 0.56 ($P = 0.001$, $n = 32$ with complete data for both scales). Using ROC analysis, a score of ≥ 14 points on the IS-self was identified as a robust indicator for irritability according to all three different cut-off points (10, 15, and 20 points) of the irritability factor of the PBA; the three cut-off points corresponded to prevalence rates of 33%, 22% and 12% of irritability (Table 2; Figure 2). The IS cut-off score of ≥ 14 points yielded an acceptable sensitivity and high specificity for all three cut-off points.

Level of agreement between the IS-self and IS-informant scores

The overall ICC for IS-self and IS-informant scores was 0.61 (95%CI=0.50–0.72, $P < 0.001$) (Figure 3A). The ICC for IS-self and IS-informant was higher (ICC=0.75) when spouses/partners were

Table 1. Sociodemographic and clinical characteristics of HD mutation carriers and non-carriers.

	Mutation carriers (n = 130)	Non-carriers (n = 43)	p-value*
Sociodemographic and clinical characteristics			
Male (n, %)	58 (45)	20 (47)	0.83
Age, years (mean $\pm$ SD)	49 $\pm$ 11	41 $\pm$ 11	< 0.001
Higher level of education ^a (n, %)	78 (60)	31 (72)	0.17
Married/living together (n, %)	81 (62)	35 (81)	0.02
Excessive use of alcohol (n, %)	13 (10)	1 (2)	0.14
CAG repeat length (mean $\pm$ SD)	44 $\pm$ 3	22 $\pm$ 4	< 0.001
Neuropsychiatric characteristics			
IS-self (median, IQR)	9 (3 – 17)	5 (2 – 9)	0.01
IS-self with cut-off $\geq$ 14 (n, %)	45 (35)	4 (9)	0.001
IS-inf (median, IQR)	11 (5 – 19)	4 (2 – 10)	0.01
IS-inf with cut-off $\geq$ 14 (n, %)	51 (39)	5 (12)	0.001
PBA irritability (IQR)	7 (1 – 16)	1 (0 – 5)	< 0.001
Any psychiatric disorder ^b (n, %)	13 (10)	1 (2)	0.10
Major depressive disorder ^b (n, %)	8 (6)	0 (0)	0.09

Data are presented as n (%), mean ($\pm$ Standard Deviation (SD)), or median (Inter Quartile Range (IQR)) when appropriate.

* P-values by chi-square tests for catechorical data, by t-test for independent samples with normal distributions, or non-parametric Whitney-U tests.

^a Higher education $\geq$ 12 years of education;

^b The presence of psychiatric disorders in the last two weeks are diagnosed with the Composite International Diagnostic Interview;

IS-self = Irritability Scale self report;

IS-inf = Irritability Scale informant report;

PBA = Problem Behaviours Assessment.

Table 2. ROC analysis for IS-self scores among 152 HD mutation carriers against three different cut-off scores for PBA irritability factor.

	PBA irritability cut-off score		
	> 10 points	> 15 points	> 20 points
Prevalence of irritability	33%	22%	12%
Optimal IS-self cut-off	13 – 14	13 – 14	13 – 14
Sensitivity	0.58	0.69	0.88
Specificity	0.84	0.81	0.78
AUC	0.80	0.84	0.87

ROC = Receiver Operator Characteristic;

IS-self = Irritability Scale self-report;

PBA = Problem Behaviours Assessment;

AUC = Area Under the Curve.

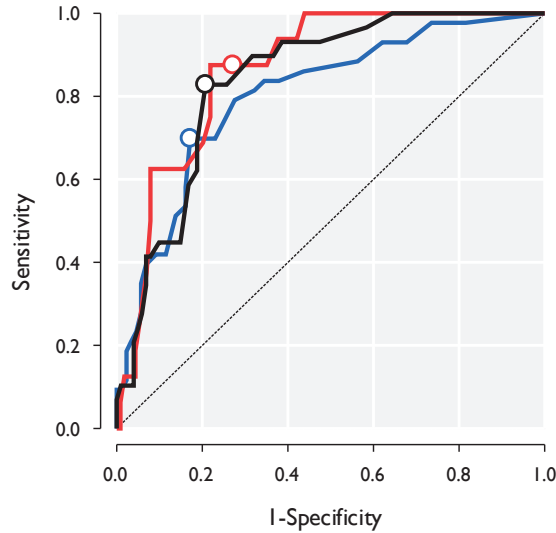


Figure 2 ROC curves for the irritability scores among 152 HD mutation carriers according to the PBA scores.

The blue line is with a cut-off score of 10 points (resulting in 33.1% with irritability). The black line is with cut-off score of 15 points (resulting in 22.3% with irritability). The red line is with a cut-off score of 20 points (resulting in 12.3% with irritability). The open dots indicate the sensitivity and 1 – specificity for a cut-off score of ≥ 14 points for the irritability scale.

ROC = Receiver Operator Characteristic;

PBA = Problem Behaviours Assessment.

their informants, than when others (e.g. family members, friends, or nurses) were informants ($ICC=0.48$). On item level, ICCs were highest for question 12: “Do you yell a lot?” ($ICC=0.59$, $P<0.001$) and question 1: “Are you easily irritated?” ($ICC=0.49$, $P<0.001$), and lowest for question 7: “Do you insist on having your way?” ($ICC=0.28$, $P<0.001$) and question 13: “Are you able to control your temper with persons outside the family?” ($ICC=0.25$, $P<0.001$) (Figure 1).

Using a cut-off of ≥ 14 points for both IS-self and IS-informant, 33 (28%) mutation carriers were considered irritable according to both IS scales, whereas 60 (50%) mutation carriers were found not irritable according to both IS scales. For the remaining 27 (23%) mutation carriers, there was disagreement between participants and informants, with the majority of participants ($n=18$; 67%) not rating themselves as irritable whereas their informants did.

Presence and severity of irritability in non-carriers and HD mutation carriers

There were important group differences for the IS-self and IS-informant scores among the 43 non-carriers, 39 pre-motor symptomatic, and 91 motor symptomatic carriers. Both for the IS-self ($P=0.02$) and for the IS-informant ($P=0.004$) there was a trend for increasing irritability scores from non-carriers, pre-motor symptomatic, to motor symptomatic carriers (Figure 3B). In post-hoc comparisons, non-carriers had lower levels of irritability than both groups of mutation carriers.

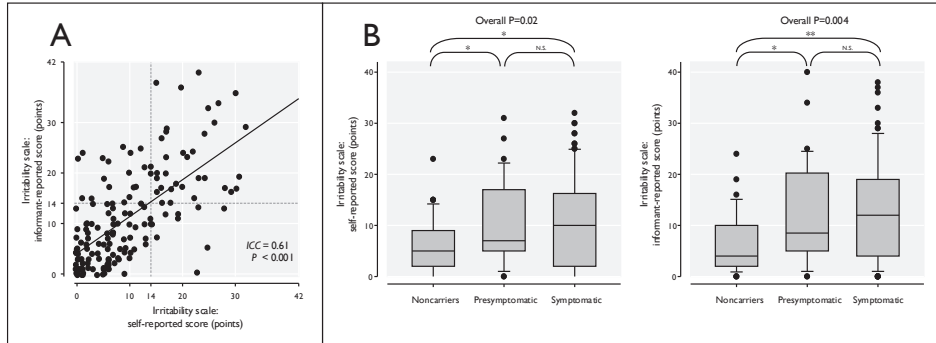


Figure 3 Scatter plot representing the intercorrelation between IS-self and IS-inf scores; B. IS-self and IS-inf scores (median, IQR) in non-carriers, pre-motor symptomatic carriers and motor symptomatic mutation carriers

A. Univariate regression line is shown, with the intraclass-correlation coefficient (ICC). Dotted lines indicate the cut-off score of ≥ 14 points being indicative of the presence of irritability.

B. The line within the box represents the median and the boundaries of the box represent the interquartile range, while the error bars represent the 10th and 90th percentile values (P_{10} and P_{90}).

* $p < 0.05$; ** $p < 0.005$, n.s. = non-significant.

Correlates of irritability in HD mutation carriers

Table 3 shows that mutation carriers with IS-self ≥ 14 points had a higher mean CAG repeat length (OR=1.16 per CAG triplet, 95%CI=1.02–1.30, $P=0.02$), a lower TFC score (OR=2.12, 95%CI=1.02–4.48, $P=0.04$), and more often used benzodiazepines (OR=2.67, 95%CI=1.20–5.92, $P=0.02$) compared to those with IS-self score < 14 points.

In the multivariate logistic regression model, being married/living together (OR=2.85, 95%CI=1.19–6.83, $P=0.02$), CAG repeat length (OR=1.20 per CAG triplet, 95%CI=1.04–1.39, $P=0.01$), and the use of benzodiazepines (OR=3.28, 95%CI=1.36–7.89, $P=0.008$) were independent correlates of self-reported irritability (Table 4). Using the same model with the dichotomized IS-informant score as the dependent variable, the use of benzodiazepines was the only significant independent correlate of irritability (OR=3.54, 95%CI=1.45–8.64, $P=0.005$).

In sensitivity analyses, being married/living together and the use of benzodiazepines remained independent correlates of self-reported irritability when cut-off scores of IS-self ≥ 12 points and ≥ 16 points were used. However, CAG repeat length was no longer an independent correlate.

Table 3. Sociodemographic, clinical, and neuropsychiatric characteristics as correlates of irritability in HD mutation carriers.

	No irritability (n = 85)	Irritability* (n = 45)	Univariate logistic regression OR (95%CI)	p-value
Sociodemographic characteristics				
Male (n, %)	38 (45)	20 (44)	0.99 (0.48 – 2.05)	0.98
Age, years (mean ± SD)	49 ± 11	48 ± 12	1.00 (0.97 – 1.03)	0.80
Higher level of education ^a (n, %)	53 (62)	26 (58)	0.83 (0.40 – 1.73)	0.61
Married/living together (n, %)	48 (57)	33 (73)	2.12 (0.96 – 4.66)	0.06
Clinical characteristics				
Excessive use of alcohol (n, %)	6 (7)	6 (13)	2.03 (0.61 – 6.69)	0.25
CAG repeat length (mean ± SD)	44 ± 3	45 ± 3	1.16 (1.02 – 1.30)	0.02
TFC < 8.5 points (n, %)	37 (44)	28 (62)	2.12 (1.02 – 4.48)	0.04
UHDRS-m > 19 points (n, %)	38 (45)	26 (58)	1.69 (0.82 – 3.51)	0.16
Use of psychotropics (n, %)	39 (46)	29 (64)	2.14 (1.02 – 3.51)	0.16
- Antidepressants (n, %)	27 (32)	18 (40)	1.43 (0.68 – 3.04)	0.35
- Antipsychotics (n, %)	22 (26)	10 (22)	0.82 (0.35 – 1.92)	0.65
- Benzodiazepines (n, %)	17 (20)	18 (40)	2.67 (1.20 – 5.92)	0.02
Neuropsychiatric characteristics				
IS-self (median, IQR)	5 (1 – 8.5)	20 (16.5 – 25)	-	-
IS-inf (median, IQR)	7 (3 – 12)	19 (12.5 – 27.5)	-	-
Major depressive disorder ^b (n, %)	3 (7)	5 (6)	1.15 (0.26 – 5.08)	0.85
MMSE < 28 points (n, %)	46 (54)	30 (67)	1.82 (0.85 – 3.90)	0.13
SDMT	0.08 (1.05)	-0.12 (0.90)	0.80 (0.55 – 1.15)	0.23
VFT	0.01 (0.94)	-0.02 (1.12)	0.98 (0.68 – 1.40)	0.89
Stroop word	0.14 (1.04)	-0.26 (0.86)	0.66 (0.45 – 0.97)	0.11
Stroop colour	0.10 (1.05)	-0.19 (0.88)	0.75 (0.51 – 1.08)	0.12
Stroop interference	0.11 (1.06)	-0.21 (0.86)	0.73 (0.50 – 1.05)	0.09

Data are presented as n (%), mean (± Standard Deviation (SD)), or median (Inter Quartile Range (IQR)) when appropriate. Odds Ratios (ORs) with 95% Confidence Intervals (CI) and p-values by univariate logistic regression.

*Irritability was considered present if IS-self ≥ 14 points, range 0 – 42 points.

^a Higher education ≥ 12 years of education;

^b The presence of major depressive disorder in the last two weeks are diagnosed with the Composite International Diagnostic Interview+

TFC = Total Functioning Capacity;

UHDRS-m = Unified Huntington's Disease Rating Scale motor section;

IS-self = Irritability Scale self-report;

IS-inf = Irritability Scale informant-report;

MMSE = Mini-Mental State Examination;

SDMT = Symbol Digit Modality Test;

VFT = Verbal Fluency Test;

SDMT, VFT, and Stroop tests scores are in standardized z-scores.

Table 4. Independent correlates of self-reported and informant-reported irritability in HD mutation carriers.

	Self-reported (n = 130)		Informant-reported (n = 120)	
	OR (95%CI)	<i>p</i> -value	OR (95%CI)	<i>p</i> -value
Age	1.01 (0.98 – 1.06)	0.48	1.00 (0.96 – 1.04)	0.87
Male	0.98 (0.43 – 2.21)	0.96	1.73 (0.77 – 3.87)	0.18
Married/living together	2.85 (1.19 – 6.83)	0.02	1.75 (0.75 – 4.06)	0.19
CAG repeat length	1.20 (1.04 – 1.39)	0.01	1.14 (0.99 – 1.32)	0.06
Use of benzodiazepines	3.28 (1.36 – 7.89)	0.008	3.54 (1.45 – 8.64)	0.005

Odds Ratios (ORs) with 95% Confidence Intervals (CI) and *p*-values by multivariate logistic regression.

DISCUSSION

Using the optimal cut off score of IS ≥ 14 points, the prevalence of irritability in HD mutation carriers was 35%. There was a moderate level of agreement between mutation carriers and their informants in reporting irritability, with a tendency for mutation carriers to underestimate their level of irritability. Being married/living together, a higher CAG repeat length, and the use of benzodiazepines were independent correlates of self-reported irritability, whereas the use of benzodiazepines was the only independent correlate of both self-reported and informant-reported irritability.

Since there is no gold standard or formal criteria for the assessment of irritability, any cut-off point remains somewhat arbitrary. Therefore, we investigated the psychometric properties of the IS against the irritability factor of the PBA, an instrument especially developed for the assessment of behavioural problems in HD. ROC analysis showed that a cut-off score of ≥ 14 points was robust over three PBA cut-off scores. This cut-off score had face validity, since we considered it likely that irritable subjects would score at least 1 point on each of the 14 questions of the IS. In an earlier study using the IS ($n=53$), the median IS-self score of 15 points was used as a cut-off, defining irritability by IS > 15 points; however, that study did not perform a ROC analysis (Chatterjee, 2005).

Whereas other (smaller) studies found prevalence rates between 38% and 73% for irritability in HD (van Duijn, 2007), we found a rather low prevalence. This may partly be explained by the different assessment tools used: the other studies all used non-specific measures for neuropsychiatric symptoms. Besides differences in methodology, sociodemographic and clinical factors – like the use of medication – may have contributed to the variation in prevalence of irritability. Unfortunately, the only two studies that used the IS do not report prevalence rates of irritability; however, these two studies report mean IS-self score of 14 points (Chatterjee, 2005), and 12 points (Klöppel, 2010), respectively. Furthermore, high levels of hostility may already be present before motor symptoms occur (Vassos, 2007), but prevalence of irritability may vary between

disease stages. So far, no significant differences between different disease stages have been found. In line with our results

Both previous studies using the IS assessed self-reported and informant-reported irritability. In the first study, agreement between (motor symptomatic) mutation carriers and informants regarding the presence of irritability (median IS-self >15 points, median IS-informant >16 points) was moderate to poor (Chatterjee, 2005). Disagreement was stronger among mutation carriers with more intact cognition. The second study assessed irritability in 15 pre-motor symptomatic mutation carriers and found no significant differences between self-reported and informant-reported irritability (Klöppel, 2010). In the present study, we found moderate agreement between self-reported and informant-reported irritability. Mutation carriers tended to underestimate their level of irritability compared to their informants, since in 18 of the 27 cases with discordant scores, mutation carriers rated themselves as non-irritable (IS <14 points), whereas their informants scored above the cut-off. This may indicate denial or a lack of awareness by mutation carriers of their level of irritability. Since we did not ask informants of non-carriers to rate the level of irritability, we cannot conclude whether or not this is related to the disease itself. On the other hand, caregiver burden may be a source of disagreement between self-reported and informant-reported irritability, contributing to a possible overestimation of irritability by informants. However, there was a higher level of agreement between IS-self and IS-informant scores when spouses/partners rated the IS, than when other informants did so, suggesting a more correct estimation by the most intimate informants.

Of all the sociodemographic and clinical characteristics, being married/living together, CAG repeat length, and use of benzodiazepines were independent correlates of self-reported irritability. While most partners and other caregivers are extremely helpful and important for mutation carriers, a higher level of irritability may become more pronounced in intimate relationships that may comprise more potential triggers of increased irritability.

The CAG repeat length of mutation carriers was also independently correlated with self-reported irritability, but not with informant-reported irritability, whereas sensitivity analysis also showed that CAG repeat length was not an independent correlate. This is in line with studies that found no relationship between CAG repeat length of the *HTT* gene and any kind of psychopathology (Weigell-Weber, 1996; Zappacosta, 1996; Ravina, 2008; Vassos, 2008).

In the present study use of benzodiazepines was independently correlated to both self-reported and informant-reported irritability. Although benzodiazepines may have been prescribed more often to irritable mutation carriers, this cross-sectional study does not allow to draw conclusions about causality. Even if the occurrence of irritability in HD is (in part) iatrogenic and induced by the use of benzodiazepines, no longitudinal studies have examined the use of benzodiazepines and their effects on irritability in HD or other neurodegenerative diseases. Nevertheless, it is established that some patients may show paradoxical 'aggressive' behaviour with benzodiazepines, or behavioural disinhibition (Bond, 1995; Weisman, 1998; Lader, 1991).

The strength of this study is the use of three different assessment methods for irritability, with standardized interviews, in a relatively large HD study population. However, some limitations also need to be addressed. First, in the absence of criteria or a gold standard for the assessment of irritability, we used the PBA for validation of the IS. Second, only subjects who volunteered to participate were included; this may have led to underestimation of the prevalence of irritability in HD, since irritable subjects were more likely to refuse to participate. Third, our study involved the analysis of cross-sectional data which precludes drawing conclusions about the direction of causality.

In conclusion, we recommend the use of the IS to assess irritability in HD in a standardized manner, since this scale proved to be a valid and easy to use instrument. Being married/living together and the use of benzodiazepines were independently associated with the presence of irritability, although only the use of benzodiazepines was also correlated with informant-reported irritability and confirmed in the sensitivity analyses. Longitudinal studies are needed to further explore these relationships. Given the strong association between irritability and the use of benzodiazepines, close monitoring of the effect of benzodiazepines is important, since clear evidence for an effective treatment of irritability in HD is still lacking (van Duijn, 2010).

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CHAPTER 3

Irritability in a prospective cohort of Huntington's disease mutation carriers

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ABSTRACT

A key symptom of Huntington's disease (HD), irritability contributes to a decline in functioning and to great distress in both patients and their caregivers. To identify mutation carriers prone to the development of irritability, this study aimed to investigate the course and temporal relationships between irritability and other neuropsychiatric symptoms. A cohort of 90 mutation carriers was followed for two years. Using the Irritability Scale, the incidence of irritability was 23% whereas irritability persisted in 70%. An increase in irritability was strongly associated with an increase in apathy.

INTRODUCTION

Neuropsychiatric symptoms, motor disturbances, and cognitive impairment are core symptoms of Huntington's disease (HD) {1-3}, an autosomal dominant, neurodegenerative disorder resulting from an expanded trinucleotide cytosine-adenine-guanine (CAG) repeat in the *HTT* gene on chromosome 4 that codes for the mutant protein huntingtin. Although under normal circumstances huntingtin is present ubiquitously, its physiologic role is not fully elucidated. The mutant huntingtin probably confers a toxic gain of function, mainly resulting in striatal cell loss. The age of onset is widespread, ranging from early childhood to senescence, and is generally about 40 years. The duration between diagnosis of motor symptoms and death is around 20 years. Neuropsychiatric symptoms, such as irritability, apathy and depressed mood often precede the onset of motor symptoms.

Irritability is a common and key neuropsychiatric symptom of HD {4-6}, which can be characterized as a mood state predisposing towards anger, hostile appraisals, impatience, intolerance, poorly controlled anger and overt aggression {7;8}. Reduced control over temper frequently occurs, resulting in verbal or behavioral outbursts. However, irritability may also be present without observable manifestation. Irritability should be distinguished from the DSM-IV-TR diagnosis of intermittent explosive disorder, since the latter has to meet strict, observable criteria and is severe in its phenotype and consequences, whereas irritability may not even be visible to an observer. The subjective experience of irritability may be either brief or prolonged. In contrast to justifiable anger, verbal or behavioral outbursts originating from irritable mood are nonadaptive, complicate the interaction between the patient and his environment, and are always unpleasant for the individual. Irritability can occur early in the course of HD, e.g. up to 10 years before the onset of motor symptoms {9} and often contributes to a decline in personal and occupational functioning {10}. Moreover, irritability of mutation carriers may also cause great distress among others, affecting the wellbeing of their families and possibly contributing to the necessity of institutionalization {11}.

The prevalence of irritability ranges from 35% to 73%, depending on its definition, the assessment tools used, and the study populations {2;9;12}. In REGISTRY (a European multi-center prospective observational study including both manifest and premanifest HD mutation carriers) a prevalence of 19.1% of overt disruptive or aggressive behavior among was found among 1468 mutation carriers {13}. Two prospective studies conducted among 12 pre-motorsymptomatic{14}- and 111 motorsymptomatic{15} HD mutation carriers showed that irritability increased over time.

Cross-sectional studies of irritability in motor-symptomatic mutation carriers have found associations with other neuropsychiatric symptoms including impulsivity{4}, obsessive compulsive symptoms{16} and suicidal thoughts{17}. Using the 14-item Irritability Scale{18;19}, we earlier demonstrated an association between irritability and a low Total Functioning Capacity (TFC) score, use of benzodiazepines and more often being married or living together{2}.

Since irritability can occur before the onset of motor symptoms, our cohort included both pre-motor symptomatic and motor symptomatic mutation carriers. Although cross-sectional associations with irritability have been investigated before {2;4;16;17}, to our knowledge, this is the first study investigating predictors of incident and persistence of irritability in HD, also this is the first study investigating correlates of change in irritability over time. Identification of mutation carriers prone to the development and persistence of irritability is required to provide adequate treatment and support of HD mutation carriers and their families. Therefore, this study investigates the course and temporal relationships between irritability and other neuropsychiatric symptoms in a cohort of 90 HD mutation carriers followed for two years.

METHODS

Participants

HD mutation carriers and first-degree non-carriers were recruited between May 2004 and August 2006 from the outpatient departments of Neurology and Clinical Genetics of Leiden University Medical Centre (LUMC) and from a regional nursing home specialized in HD. All participants underwent genetic testing and were considered HD mutation carriers with a CAG repeat length ≥ 36 repeats. A second measurement was conducted 2 years after the first measurement and a third measurement 2 years thereafter. The study design has been described in detail elsewhere{20}.

In the present longitudinal analysis, only the data of mutation carriers who participated in the second and third waves of this study are included, because the Irritability Scale {2} was introduced halfway during the first measurement. Here, the second wave is referred to as the 'baseline measurement' and the third wave as the 'follow-up measurement'. A total of 121 mutation carriers completed the Irritability Scale at baseline and irritability was also assessed in 90 mutation carriers at follow-up. In total 32 mutation carriers dropped-out (Figure 1).

The study was approved by the medical ethical committee of the LUMC and informed consent was obtained from all participants.

Instruments

Assessment of irritability

Irritability was assessed with the self-rated Irritability Scale (Appendix I) that consists of 14 questions addressing the presence of various phenomena of irritability in the two weeks prior to the interview, rated on a 4-point Likert scale {18}. The total sum score of the Irritability Scale ranges from 0 - 42 points with higher scores indicating more severe irritability. We used a cut-off score of > 13 points as indicative for the presence of irritability; this score was selected based on our previous cross-sectional study among 130 HD mutation carriers (largely overlapping with baseline data of the current study) where this cut-off provided optimal sensitivity and speci-

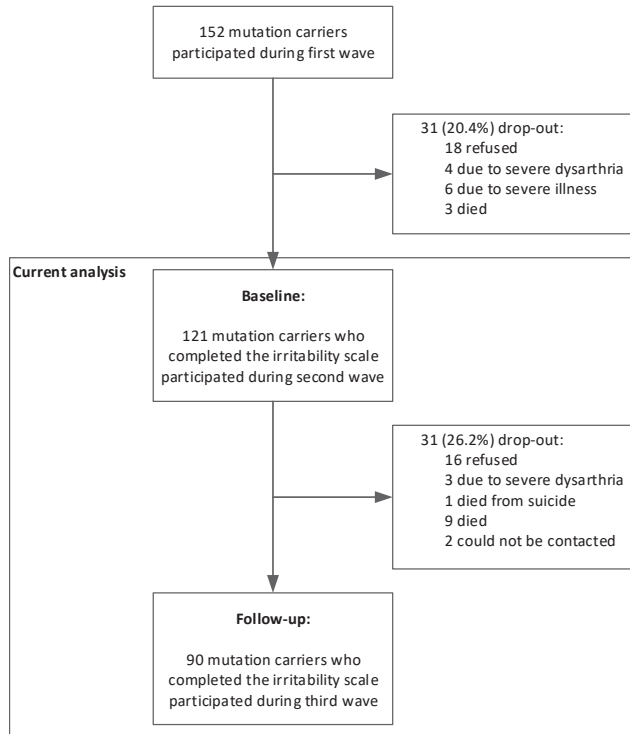


Figure 1 Flow chart of inclusion of the study participants for the present analysis

ficity according to ROC analyses {2}. The Cronbach's alpha of the irritability scale is 0.90 and its sensitivity and specificity for detecting irritability in HD mutation carriers is 0.69 and 0.81, respectively {2}. We preferred the use of the Irritability Scale over the PBA irritability subscale because the Irritability Scale was designed specifically for the assessment of irritability in neurodegenerative disease. Also, the psychometric properties of the Irritability Scale are better than those of the PBA irritability subscale (Cronbach's alpha of 0.90 vs. 0.67, respectively). Since no cut-off score for the PBA irritability subscale for the presence of irritability exists, a direct comparison is not possible. In our previous study however, we found that with a cut-off score on the PBA irritability factor of >15 points, a cut-off score of >13 point on the irritability score yielded a sensitivity of 0.69 and a specificity of 0.81.

Assessment of other neuropsychiatric characteristics

The Problem Behaviors Assessment (PBA) was used to assess depression. The PBA is a semi-structured interview assessing the frequency and severity of 36 potential behavioral problems in HD in the month before the interview {4}. The interrater reliability of the Dutch version of the PBA is 0.82 for severity scores and 0.73 for frequency scores. We used the symptom factor depression (range 0-80) that was previously estimated by factor analysis of the PBA {5}. Apathy was

assessed with the Apathy Scale (Appendix II){21}; this scale consists of 14 questions, measuring different features of apathy in the 2 weeks before the interview. Caregivers' information and the interviewers' judgment was included in rating the Apathy Scale, since patients with apathy may lack adequate insight into their own symptoms. The Apathy Scale was chosen above the apathy factor of the PBA because, although no clear preference arises from the literature, the Apathy Scale has better psychometric properties than the PBA apathy subscale (Cronbach's alpha 0.89 vs. 0.84, respectively) and because the former was specifically designed for the assessment of apathy.

Sociodemographic and clinical characteristics

Information on sociodemographic and clinical characteristics (including alcohol consumption, use of psychotropic medication and information about the household) was collected in a standardized manner. Global daily functioning was assessed using the Total Functional Capacity (TFC) scale {22} of the Unified Huntington's Disease Rating Scale (UHDRS) {23}. Motor symptoms were assessed using the motor section of the UHDRS (UHDRS-m) by trained neurologists. Mutation carriers with an UHDRS-m confidence level 0-1 were considered pre-motorsymptomatic and mutation carriers with UHDRS-m confidence level >1 were considered motorsymptomatic. Global cognitive functioning was assessed with the Mini-Mental State Examination (MMSE) {24}. Executive cognitive functioning was assessed with the Symbol Digit Modalities Test (SDMT) {25}, the Verbal Fluency Test (VFT) {26} and Stroop tests {27}. Since there is a high level of multi-collinearity between most tests (Pearson's $r > 0,80$), an aggregate variable for executive functioning, ExCog, was constructed by computing the mean of the standardized scores of the SDMT, VFT and Stroop tests.

Statistical analyses

Data are presented as n (%), mean ($\pm$ standard deviation; S.D.) or median (inter-quartile range; IQR) when appropriate. Associations between sociodemographic-, clinical- and neuropsychiatric characteristics on the one hand, and incident and persistent irritability on the other were determined using univariate logistic regression; odds ratios (ORs) were computed for the incidence or persistence of irritability. For the assessment of incident irritability, we identified HD mutation carriers without irritability at baseline who were irritable at follow-up. Similarly, for the assessment of persistent irritability we identified HD mutation carriers who were irritable at baseline and remained irritable at follow-up. A multivariate logistic regression (adjusting for sex and age that were entered into the model) was conducted with variables with $p < 0.10$ in the initial univariate analyses. To assess associations between temporal changes of clinical- and neuropsychiatric characteristics on the one hand and irritability on the other, we conducted an additional multivariate linear regression analysis. Absolute changes (i.e. delta-values) in all clinical- and neuropsychiatric characteristics between baseline and follow-up were calculated. Linear regression analysis was used to assess whether changes in scores of clinical- or neuropsychiatric

characteristics as independent variables were associated with changes in the Irritability Scale score as the dependent variable. Since our investigation is of an explorative nature and we are not aware of known correlates of changes in irritability score, we decided to let a forward selection procedure select variables to be included in our final model based upon statistical strength. In the final model, the most robust relationships with changes in both irritability and its correlates were further analysed using regression analysis, with adjustment for sex, age, and use of antipsychotics. All tests were two-tailed with $p < 0.05$ denoting statistical significance. The SPSS 20.0 Package (SPSS Inc. Chicago, IL) was used for the statistical analyses.

RESULTS

The 90 HD mutation carriers in the current study had an average age of 49 (SD 1.7 years) and 41 (46%) were males. Their mean TFC score was 8.6 (SD 4.4) and their mean UHDRS-m score was 25.3 (SD 2.47). Twenty-five (28%) mutation carriers were pre-motorsymptomatic and 64 (71%) were motorsymptomatic. UHDRS-m score and motorsymptomatic status was missing in 1 mutation carrier. The score on the Irritability Scale between baseline and follow-up showed no significant change: average increase 1.56 points; 95% confidence interval (CI) -0.23 to 2.89.

At baseline, of the 90 mutation carriers, 33 (37%) were irritable. During the 2-year follow-up, of the 57 non-irritable HD mutation carriers at baseline, 13 (23%) developed irritability and 23 (70%) with irritability at baseline were persistently irritable. Compared with mutation carriers who completed the follow-up, drop-outs had on average a lower TFC score (5.8 vs. 8.6, $p = 0.004$), higher UHDRS-m score (40.4 vs. 25.1, $p = 0.007$), lower MMSE score (23.7 vs. 26.9, $p = 0.001$) and worse executive cognitive functioning (-0.53 vs. -0.13, $p = 0.04$); however, there was no difference in their Irritability Scale score (10.1 vs. 10.6, $p = 0.78$).

Of the 57 non-irritable HD mutation carriers at baseline, the 13 mutation carriers with incident irritability at follow-up had, at baseline, a longer mean CAG repeat length, a higher UHDRS-m score, a lower MMSE score, lower scores on all executive cognitive measures including the ExCog variable, and more frequently used benzodiazepines, compared with HD mutation carriers who did not develop irritability. In a logistic regression model adjusting for sex and age, a longer CAG repeat length, a higher UHDRS-m score, a higher use of benzodiazepines, a lower MMSE score and worse cognitive functioning at baseline were all associated with incident irritability at follow-up. (Table 1) Next, we built a final multivariate logistic model adjusting for all these significant correlates. In this model, all determinants lost their significance. (Table 1).

There were no significant differences in mutation carriers who showed remission of irritability compared to those with persistent (Table 2). In the final model, we adjusted for the use of antidepressants, SDMT score and Stroop color score, since these variables had a correlation with a p -value < 0.10 in the univariate analyses. In this model, there were no significant associations.

Table 1 Predictors of incident irritability in patients with Huntington's Disease without irritability at baseline

	No irritability at follow-up (n=44)	Incident irritability (n=13)	p-value*	Adjusted for sex and age		Fully adjusted	
				OR	(95% CI)	p-value	OR
Sociodemographic characteristics							
Sex	19 (43)	7 (54)	0.50				
Age in years	49.6 ± 1.7	50.29 ± 2.32	0.82				
Married or living together	26 (59)	8 (62)	0.87	1.09	(0.30 ; 3.90)	0.90	
Clinical characteristics							
CAG repeats	43.0 ± 0.4	45.00 ± 1.21	0.04	1.34	(1.05 ; 1.72)	0.02	0.32
Motor score	20.1 ± 3.4	38.54 ± 7.91	0.03	1.03	(1.00 ; 1.06)	0.03	0.85
Antidepressants	13 (30)	7 (54)	0.11	3.20	(0.85 ; 12.03)	0.09	
Antipsychotics	8 (18)	5 (39)	0.14	3.41	(0.79 ; 14.83)	0.10	
Benzodiazepines	3 (7)	4 (31)	0.03	6.32	(1.17 ; 34.03)	0.03	0.10
Neuropsychiatric characteristics							
PBPA depression	6.86 ± 2.04	9.69 ± 4.94	0.54	1.02	(0.98 ; 1.06)	0.41	
Apathy score	5.41 ± 1.26	8.62 ± 1.93	0.22	1.04	(0.97 ; 1.12)	0.24	
MMSE score	27.7 ± 0.5	24.4 ± 1.3	0.01	0.81	(0.68 ; 0.95)	0.01	0.40
VFT	25.0 ± 2.0	15.4 ± 3.6	0.03	0.93	(0.88 ; 0.99)	0.02	
SDMT	37.7 ± 2.9	24.4 ± 6.1	0.047	0.96	(0.92 ; 1.00)	0.027	
Stroop color	60.4 ± 3.5	42.3 ± 6.4	0.02	0.96	(0.93 ; 0.99)	0.02	
Stroop word	77.9 ± 3.7	51.5 ± 8.0	0.01	0.96	(0.93 ; 0.99)	<0.001	
Stroop interference	34.6 ± 2.3	24.0 ± 3.9	0.04	0.94	(0.90 ; 0.99)	0.02	
ExCog	0.10 ± 0.13	-0.63 ± 0.26	0.02	0.33	(0.14 ; 0.76)	0.01	0.93
				0.92	(0.14 ; 5.84)		

Data are mean ± standard error of the mean or number (percentage) where appropriate. *P-value calculated with t-test or chi-square test where appropriate. Odds ratio (OR), 95% confidence interval (CI) and P-value derived from logistic regression with incident irritability in the group without irritability at baseline with irritability as dependent variable. Fully adjusted model is additionally adjusted for CAG repeats, motor score, use of benzodiazepines, and ExCog. CAG denotes cytosine, adenine, guanine; PBA, Problem Behaviors Assessment; MMSE, Mini Mental State Examination; VFT, Verbal Fluency Test; SDMT, Symbol Digit Modalities Test

Table 2 Predictors of persistent irritability in patients with Huntington's Disease subjects with irritability at baseline

	No irritability at follow-up (n=10)	Persistent irritability (n=23)	p-value*	Adjusted for sex and age		Fully adjusted			
				OR	(95% CI)	p-value	OR	(95% CI)	p-value
Sociodemographic characteristics									
Sex	2 (20)	11 (48)	0.15						
Age in years	42.9 ± 2.8	50.4 ± 2.8	0.13						
Married or living together	8 (80)	17 (74)	0.71	0.61	(0.09 ; 4.03)	0.61			
Clinical characteristics									
CAG repeats	44.1 ± 1.1	45.0 ± 0.7	0.46	1.26	(0.94 ; 1.70)	0.13			
Motor score	19.1 ± 7.2	29.3 ± 4.3	0.23	1.02	(0.98 ; 1.07)	0.34			
Antidepressants	1 (10)	10 (44)	0.09	4.69	(0.46 ; 47.29)	0.19			
Antipsychotics	0 (0)	7 (30)	0.23	2.94	(0.28 ; 30.89)	0.37			0.35
Benzodiazepines	3 (30)	7 (30)	0.98	0.89	(0.16 ; 5.04)	0.90			
Neuropsychiatric characteristics									
PBA depression	12.9 ± 4.1	11.1 ± 2.8	0.71	0.98	(0.92 ; 1.04)	0.57			
Apathy score	8.00 ± 1.76	13.30 ± 2.05	0.13	1.09	(0.95 ; 1.25)	0.22			
MMSE score	28.0 ± 0.6	26.4 ± 0.8	0.26	0.73	(0.48 ; 1.10)	0.13			
VFT	24.9 ± 4.5	22.1 ± 3.6	0.64	0.98	(0.93 ; 1.03)	0.41			
SDMT	38.5 ± 3.8	28.9 ± 3.3	0.10	0.96	(0.90 ; 1.02)	0.15		1.00	(0.90 ; 1.11) 0.94
Stroop color	62.3 ± 6.9	46.9 ± 4.3	0.07	0.97	(0.93 ; 1.01)	0.12		0.51	(0.91 ; 1.05) 0.42
Stroop word	76.4 ± 7.1	60.2 ± 5.6	0.11	0.97	(0.94 ; 1.01)	0.11			
Stroop interference	33.9 ± 3.7	25.4 ± 3.1	0.13	0.96	(0.91 ; 1.02)	0.21			
ExCog	0.11 ± 0.23	-0.38 ± 0.18	0.14	0.48	(0.17 ; 1.32)	0.15			

Data are mean ± standard error of the mean or number (percentage) where appropriate. Odds ratio (OR), 95% confidence interval (CI) and P-value derived from logistic regression with incident irritability in the group without irritability at baseline and persistent irritability in the group with irritability as dependent variable. Fully adjusted model is additionally adjusted for use of antidepressants, SDMT and Stroop color test. CAG denotes cytosine, adenine, guanine; PBA, Problem Behaviors Assessment; MMSE, Mini Mental State Examination; VFT, Verbal Fluency Test; SDMT, Symbol Digit Modalities Test

We analyzed the association between changes in the clinical and neuropsychiatric characteristics with the change in Irritability Scale score. In univariate analyses of these changes, we found that an increase in UHDRS-m score, an increase in the Apathy Scale and the use of both antipsychotics and of benzodiazepines were associated with an increase in the Irritability Scale score over time. Next, we built a final multivariate regression model, comprising sex and age at baseline, increase in UHDRS-m score, Apathy Scale score, and the change in use of both antipsychotics and of benzodiazepines. We found that the association between change in Irritability Scale score and change in Apathy Scale score persisted after adjustment for confounders (Figure 2). Furthermore the continuous use of antipsychotics was independently associated with an increase in Irritability Scale score.

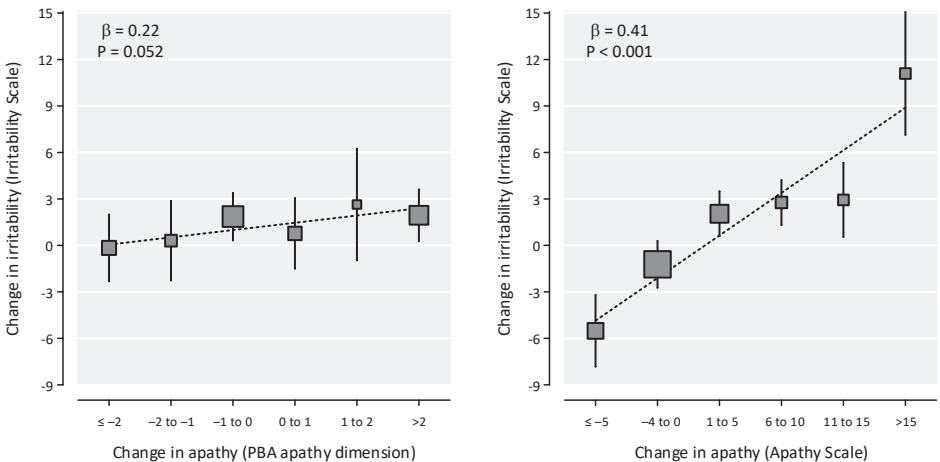


Figure 2 Fully adjusted multivariate linear regression of changes in Irritability Scale score on changes in apathy as assessed with the PBA apathy factor (left) and the Apathy Scale (right)

Table 3 Associations between changes in Irritability Scale score and changes in apathy

	Changes in Irritability Scale score	
	β (95% CI)	p-value
<i>Changes in Apathy Scale score</i>		
Crude	0.29 (0.08 ; 0.49)	0.01
Adjusted for sex and age	0.29 (0.09 ; 0.49)	0.01
Fully adjusted*	0.41 (0.19 ; 0.62)	<0.001

Beta and 95% confidence interval (CI) derived from linear regression with change in irritability score between baseline and follow-up as dependent variable. *Fully adjusted model is also adjusted for increase in motor score, use of benzodiazepines and use of antipsychotics.

DISCUSSION

In this study, we investigated the course of irritability and its associations with clinical and neuropsychiatric symptoms in a cohort of 90 HD mutation carriers who were followed for 2 years. An increasing severity of apathy and the continuous use of antipsychotics were associated with increasing severity of irritability over 2 years.

A recent longitudinal study of neuropsychiatric symptoms followed 111 HD patients for a mean of 5 years [15]. At five moments of assessment during follow-up, a minimum of 49% of HD patients had one or more symptom of irritability and a maximum of 83% had one or more symptom of irritability. However, the proportions of any of these symptoms cannot be translated easily into a prevalence rate of the syndrome of irritability which we used in our analyses. Therefore, our incidence of 23% may be a good representation of new-onset irritability at 2-year follow-up in individuals with HD.

We found a link between apathy and irritability in HD, reflected by our finding that an increase in apathy coincided with an increase in irritability over a 2-year period. At first glance this may seem paradoxical. Apathy is a disorder of motivation that is mainly characterized by a lack of goal-directed behavior and cognition and by decreased emotional responsiveness. On the other hand, irritability has been characterized as a mood state predisposing toward negative or hostile appraisals and to the experience of anger. Although irritability may lead to the overt expression of anger, it may also only bother the patient himself without overt expression of irritability. In this paradigm, an individual with apathy may well experience irritability, a hypothesis which is supported by our study. An explanation for this comorbidity of apathy and irritability in HD may be that they have a related pathophysiology since both are caused by neural degeneration in subcortical and frontal circuits [28;29], which is a key feature of HD, in particular the anterior cingulate cortex (ACC) and the orbitofrontal cortex (OFC). The ACC plays an important role in affect-regulation [30] as well as in decision making [31]. Lesions in the ACC, or in neuronal circuitry connected to it, have been associated with apathy [28] as well as behavioral inhibition, and aggression [28]. The orbitofrontal cortex is involved in impulse inhibition and lesions in the area of the OFC are associated with behavioral disinhibition and emotional lability [28]. In addition, hypoactivation of the OFC, as well as the ACC, is associated with impulsive aggression [29]. Although emotional lability and impulsive aggression may be components of overt, observable irritability, these symptoms are absent in irritability that remains out of awareness and to our knowledge, the role of subcortical circuitry has not been investigated in this type of irritability. Therefore, the comorbidity of apathy and irritability may only be partly due to a related pathophysiology. Another factor to be considered is the possibility between construct overlap of irritability and apathy as measured by the Irritability and the Apathy Scales respectively. When looking at the items of both scales, there are no obvious similarities or overlapping items. Using Spearman's correlation analyses, we found that the largest correlation coefficient was 0,44 between any of the 14 items of the Irritability scale and any of the 14 items of the Apathy

Scale. This rather low correlation on any two single items suggest that phenomenological or scale reasons cannot account for the associations we found over time between irritability and apathy .

We found an association between the use of antipsychotics and increasing irritability. Although selective serotonin reuptake inhibitors are recommended as a first-step treatment, antipsychotics are frequently prescribed for irritability and related behaviors [32]. Several underlying mechanisms may explain the association between the used of antipsychotics and irritability. On the one hand, antipsychotics may have been prescribed to treat (among others) irritability, likely resulting in confounding by indication as the treated group may on average still be more irritable than the untreated group. On the other hand, antipsychotics may have caused irritability or some other third factor may underlie the relationship. Furthermore, in some patients antipsychotics (both first-generation and second-generation) are known to cause akathisia, a state of psychomotor agitation[33]. Patients experiencing psychomotor agitation associated with akathisia, may either become irritable or may have reported positively on certain items on the Irritability Scale due to some overlap between the two constructs.

We assessed irritability using the Irritability Scale because this scale was used to assess irritability in our earlier cross-sectional study[2]. On this scale, our previously validated cut-off score of 14 points for the presence of irritability enabled us to investigate the occurrence and correlates of incident and persistent irritability.

The strengths of this study are its prospective design, a relatively large cohort of HD mutation carriers, and the use of validated instruments for the assessments of determinants and outcomes. A few limitations should also be addressed. First, although we have validated the use of the Irritability Scale in our previous study, there is no gold standard for the assessment of irritability. Second, a relatively high number of mutation carriers dropped-out and, therefore, did not participate in the current analysis; irritability at follow-up may have been a reason not to participate. Selection bias may account for an underestimation of the incidence of irritability and for the underestimation of the predictive value of some predictors because of insufficient power. Third, we cannot exclude the possibility that cognitive dysfunction in our HD patients has distorted the reliability and sensitivity of scales that assessed of neuropsychiatric characteristics.

In conclusion, we found an unexpected association between irritability and apathy. HD mutation carriers that become more apathetic over time also seem to become more irritable. The association between irritability and apathy may imply a shared underlying pathophysiology between these neuropsychiatric symptoms in HD. Since apathy may mask irritability, we recommend asking mutation carriers with apathy and their caregivers about the symptoms of irritability in order to provide adequate support and treatment.

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CHAPTER 4

Acute-phase proteins in relation to neuropsychiatric symptoms and use of psychotropic medication in Huntington's disease

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ABSTRACT

Activation of the innate immune system has been postulated in the pathogenesis of Huntington's disease (HD). We studied serum concentrations of C-reactive protein (CRP) and albumin as positive and negative acute-phase proteins in HD. Multivariate linear and logistic regression was used to study the association between acute-phase protein levels in relation to clinical, neuropsychiatric, cognitive, and psychotropic use characteristics in a cohort consisting of 122 HD mutation carriers and 42 controls at first biomarker measurement, and 85 HD mutation carriers and 32 controls at second biomarker measurement. Significant associations were found between acute-phase protein levels and Total Functioning Capacity (TFC) score, severity of apathy, cognitive impairment, and the use of antipsychotics. Interestingly, all significant results with neuropsychiatric symptoms disappeared after additional adjusting for antipsychotic use. High sensitivity CRP levels were highest and albumin levels were lowest in mutation carriers who continuously used antipsychotics during follow-up versus those who had never used antipsychotics (mean difference for CRP 1.4 SE mg/L; $P = 0.04$; mean difference for albumin 3 SE g/L; $P < 0.001$). The associations found between acute-phase proteins and TFC score, apathy, and cognitive impairment could mainly be attributed to the use of antipsychotics. This study provides evidence that HD mutation carriers who use antipsychotics are prone to develop an acute-phase response.

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease, characterized by motor abnormalities, cognitive dysfunction, and neuropsychiatric symptoms¹ including depressed mood,² apathy,^{3,4} and irritability.⁵ Although the mean age of onset is 40 years, there is a wide range in age at onset, severity, and symptomatology of HD. HD is caused by an expanded trinucleotide cytosine-adenine-guanine (CAG) repeat on chromosome 4 coding for the mutant huntingtin protein.⁶ Although the genetic defect has been elucidated, its role in the pathogenesis of HD remains unclear.

There is increasing evidence that the immune system may be involved in the pathogenesis of HD. Increased activation of components of the innate and adaptive immune systems has been found in the central nervous system (CNS)⁷⁻⁹ and peripheral tissues of HD patients.¹⁰ In particular, increased concentrations of tumor necrosis factor (TNF)- α and interleukin (IL)-6, have been found in plasma of HD mutation carriers when compared to controls.¹¹ Physiologically, TNF- α and IL-6 are pro-inflammatory cytokines which are released in the bloodstream as part of the acute-phase response.^{12,13} C-reactive protein (CRP) synthesis is upregulated in the liver under regulation of IL-6,¹⁴ whereas the synthesis of other proteins such as albumin is down-regulated. Since in the acute-phase response CRP levels increase, CRP can be thought of as a positive acute-phase protein and since albumin levels decrease, albumin can be thought of as a negative acute-phase protein.

Previously, CRP levels have been reported to be significantly increased in HD mutation carriers in advanced disease stage when compared to controls.¹⁵ Levels of CRP were significantly higher in a group of 13 HD mutation carriers when compared to 10 healthy controls. Also, levels of CRP were higher in HD mutation carriers with more advanced disease.¹⁶ A large set of markers for inflammation and innate immunity was measured in a recent study that included two cohorts comprising 81 HD mutation carriers and 40 controls, but none of the more than 20 plasma constituents were significantly different between the groups.¹⁷ Nevertheless, post-hoc tests revealed significantly lower CRP levels in HD mutation carriers with early disease versus controls, whereas premanifest HD had similar levels compared with controls.

In various other populations, there are reports indicating an association between CRP¹⁸⁻²⁰ and albumin²¹⁻²⁴ levels on the one hand, and both cognitive dysfunction and neuropsychiatric symptoms on the other hand. In a systematic review assessing the association between CRP level and stroke, cognitive disorders, and depression,¹⁸ raised CRP concentrations were associated with cognitive decline and increased risk of dementia. Low albumin levels were associated with cognitive dysfunction in two large population-based studies of the non-demented elderly.^{21,22} In a large longitudinal study with 73,131 participants from the general population, a positive association was found between CRP levels and depressive symptoms.¹⁹ Also, a meta-analysis showed a positive correlation between CRP levels and depression ($d = 0.15$; 95% confidence interval [CI] 0.10 – 0.21).²⁰ Low albumin levels were found in depressed patients compared to

healthy controls.^{23;24} Cross-sectionally, a significant association was found between CRP levels and apathy in elderly individuals.²⁵ Longitudinally, however, higher CRP levels were not associated with an increased risk of apathy in individuals aged 85 years and over.²⁶

Antipsychotics are frequently prescribed for symptomatic treatment of chorea and neuropsychiatric symptoms in HD.²⁷ It has become apparent that the use of antipsychotics may adversely affect CRP levels.^{23;28;29} In addition, weight gain is a well-known side-effect of treatment with atypical antipsychotics,³⁰ which is associated with an increase of IL-6 and other proteins associated with low-grade inflammation.³¹ The effects of antipsychotics on levels of albumin have not been thoroughly studied, although serum albumin was decreased in a group of schizophrenic patients when compared with healthy controls. However, no significant effect of the use of antipsychotics was demonstrated.³²

This study aims to investigate CRP levels as a positive acute-phase protein and albumin levels as a negative acute-phase protein in relation to clinical, neuropsychiatric, and cognitive characteristics in a cohort of HD mutation carriers. For this, we took into account factors that might have confounded or otherwise influenced these associations, in particular the use of antipsychotics. Our hypothesis was that, with disease progression, inflammatory activity would increase leading to higher CRP levels and lower albumin levels, associated with a higher prevalence of cognitive impairment and neuropsychiatric symptoms.

EXPERIMENTAL PROCEDURES

Population

Between May 2004 and August 2006, 152 HD mutation carriers and 56 controls were recruited for participation. Sources for recruitment were the outpatient departments of Neurology and Clinical Genetics of the Leiden University Medical Center (LUMC) and a regional nursing home specialized in care for HD patients. Besides HD mutation carriers, verified first-degree non-carriers participated in the study as controls. The study design is described in detail elsewhere.³³ A second measurement was conducted two years after the baseline visit (including 128 mutation carriers and 42 controls) and a third measurement two years thereafter (including 94 HD mutation carriers and 32 controls). Blood samples suitable for the determination of high sensitivity CRP (hsCRP) and albumin levels were available at the second and the third measurement. Since 6 participants did not give blood at the first biomarker measurement (defined as t1) they were excluded from the analyses, which resulted in 122 mutation carriers at t1. At the second biomarker measurement (defined as t2), 9 participants did not give blood, which resulted in 85 mutation carriers at t2.

Sample withdrawal and biochemical analyses

At t1 and t2 EDTA blood was withdrawn between the years 2008 and 2012 and subsequently stored at -80 °C within 2 h. Blood samples were stored until measurement of hsCRP and albumin levels in the fall of 2012. hsCRP was determined in EDTA plasma with an IFCC standardized method (Cat. no. 04628918190) on a COBAS INTEGRA 800 analyzer from Roche Diagnostics, according to the instructions of the manufacturer. The expected reference range for hsCRP in adult males and females is < 5 mg/L. Between-run coefficients of variation (CVs) during the study period (12 runs) were 5.8% at 4.09 mg/L, and 5.7% at 12.81 mg/L. Albumin in EDTA plasma was determined with an IFCC standardized method (Cat. no. 11970909216) on Modular P systems from Roche Diagnostics, using a BCG-based colorimetric assay with endpoint detection. This method has been standardized against the CRM 470 reference preparation. The reference range for serum albumin in adult males and females is 34–48 g/L. CVs during the study period (7 runs) were 2.94% at 37.5 g/L and 1.01% at 55.0 g/L.

Sociodemographic and clinical characteristics

Information on sociodemographic and clinical characteristics, including the potential confounders like current smoking, high alcohol consumption (defined as drinking more than 14 standardized units of alcohol a week), body weight and height, and medication use, was collected using a standardized interview. Use of antipsychotics was divided in different categories: typical antipsychotics, atypical antipsychotics, and tiapride. Tiapride was considered a separate category as it was used by many participants and cannot be classified unambiguously within one of the other categories. The estimated duration of disease was calculated by the current age minus the estimated age of onset according to the equation of Vassos.³⁴ The Total Functional Capacity (TFC) scale of the Unified Huntington's Disease Rating Scale (UHDRS)³⁵ was used to assess global daily functioning and disease stage.³⁶ Total scores range from 0 to 13 points, with higher scores indicating better global functioning.³⁷

Motor functioning was assessed by a trained neurologist using the motor scale of the UHDRS.³⁵ Total scores range from 0 to 124, with higher scores indicating worse motor functioning. The diagnostic Confidence Level (CL) of the UHDRS motor scale was used to define mutation carriers as pre-motor symptomatic (CL 0 or 1 point) or motor symptomatic (CL 2 to 4 points).

Neuropsychiatric characteristics

Neuropsychiatric characteristics were assessed by the Dutch version of the Problem Behaviours Assessment (PBA),³⁸ a semi-structured interview, assessing the frequency and severity of 36 potential behavioral problems in HD. The inter-rater reliability of the Dutch version of the PBA is 0.82 for severity scores and 0.73 for frequency scores.³⁹ In this study we used symptom factors that were previously estimated by factor analysis of the PBA, which resulted in three factors: apathy (range 0–64), irritability (range 0–80), and depression (range 0–80).³⁹ Additionally, suicidality was assessed with the PBA³⁸ by multiplying the severity and frequency score of the item

‘suicidal ideation.’ As done before, a total score > 1 point on this item was used to characterize the presence of suicidality.⁴⁰

Cognitive characteristics

The Mini-Mental State Examination (MMSE) was used to assess global cognitive functioning. Total scores range from 0 to 30 points, with higher scores indicating better global cognitive functioning.⁴¹ The cognitive scales of the UHDRS,³⁵ including the Verbal Fluency Test (VFT),⁴² the Symbol Digit Modalities Test (SDMT),⁴³ and the Stroop tests,⁴⁴ were used to assess executive cognitive functioning. For all cognitive scales, higher scores indicate better executive functioning. The ExCog, a composite variable obtained by averaging the standardized z-scores of the other executive cognitive subscales, was used as a measure for executive cognitive functioning.

Statistical analyses

Data are presented as n (%), mean ($\pm$ standard deviation [SD]), mean (95% CI), or median (interquartile range [IQR]) when appropriate.

Because of their positively skewed distribution, hsCRP levels were log transformed before being used in statistical analyses. Characteristics of HD mutation carriers and controls at t1 were compared by chi-squared tests for categorical data, t-tests for independent samples with normal distributions, or non-parametric Whitney-U tests for continuous variables without normal distributions.

Associations between hsCRP and albumin levels and clinical, neuropsychiatric, and cognitive characteristics were determined by linear regression analysis for continuous outcomes and logistic regression analysis for dichotomous outcomes. Apart from the crude model, we adjusted in the first multivariate models for the potential confounders sex, age, body mass index (BMI), smoking, and alcohol consumption at t1. Since previous literature indicated that the use of antipsychotics may affect CRP levels,^{23;28;29} a second model was built, including the variables from the adjusted model and the use of antipsychotics. Furthermore, analysis of covariance (ANCOVA) was used to compare acute-phase protein levels between participants who did not use antipsychotics, participants who started using antipsychotics during the study period, and participants who continuously used antipsychotics, with adjustment for sex, age, BMI, smoking, and alcohol consumption at t1. In an additional model comparing acute-phase protein levels at t2 between the different groups, we additionally adjusted for acute-phase protein levels at t1. As a sensitivity analysis, this ANCOVA was repeated without participants whose hsCRP level was above 10 mg/L, due to their possible association with acute infectious diseases. Since almost all participants who used antipsychotics were in later disease stages and disease stage may also be related to the acute-phase response, we performed a sensitivity analysis which only included the participants with a disease stage > 2 (TFC score < 7) at t1. As previous research showed that the effect of antipsychotic use on CRP levels is different for different kinds of antipsychotics,²⁸ we performed a mixed model analysis (i.e., multilevel regression analysis) with adjustment for age,

sex, BMI, smoking, and alcohol consumption at t1, to evaluate associations between the different categories of antipsychotics and acute-phase protein levels.

A p-value < 0.05 was considered statistically significant. p-Values presented were not corrected for multiple comparisons, since we interpreted the overall pattern of diminishing effect sizes and p-values after additional adjustment for the use of antipsychotics in multivariable models. SPSS version 20.0 was used.

RESULTS

Characteristics of mutation carriers versus controls

At t1, mutation carriers more often used psychotropic medication, had higher scores on the apathy, irritability, and depression factors of the PBA and worse scores on all cognitive tests, compared with controls (Table 1). When comparing acute-phase proteins, the hsCRP level of the mutation carriers at t1 was non-significantly higher in mutation carriers compared with controls (Table 1). At t2, the hsCRP level of mutation carriers was significantly higher compared with controls (mean: 1.94; 95% CI: 1.50–2.51 versus 0.99; 95% CI: 0.64–1.52, respectively; $p = 0.002$) (data not shown). There was a significant trend of increasing hsCRP levels across increasing disease stages at t1 and t2 (Suppl. Table 1). The albumin level of the mutation carriers was significantly lower compared with controls, both at t1 (Table 1) and t2 (mean: 46.4; 95% CI: 45.8–47.1 versus 47.6; 95% CI: 46.5–48.7, respectively; $p = 0.03$) (data not shown). However, after correction for potential confounders (sex, age, BMI, smoking, and alcohol consumption) only the hsCRP level at t2 remained significantly different between mutation carriers and controls. There was a significant trend of decreasing albumin levels across increasing disease stages at t1 and t2 (Suppl. Table 1).

Associations between acute-phase proteins and clinical characteristics at t1

Serum hsCRP level was significantly associated with TFC score, apathy score (Table 2), benzodiazepine use, and antipsychotic use (Table 3). Using multivariate linear regression analyses, with adjustment for potential confounders, the hsCRP level remained significantly associated with apathy score, Stroop interference test, and ExCog (Table 2). However, after additional adjustment for use of antipsychotics, all these associations lost their statistical significance (Tables 2 and 3).

Serum albumin level was independently associated with TFC score, apathy score, SDMT score, the Stroop test scores, ExCog (Table 2), MMSE score, use of benzodiazepines, and use of antipsychotics (Table 3). Likewise, after additional adjustment for use of antipsychotics, all these associations lost their statistical significance (Tables 2 and 3).

Table 1. Characteristics of HD mutation carriers and controls at first biomarker measurement (t1).

	Mutation carriers n = 122	Controls n = 42	p-value ^a
<i>Sociodemographic characteristics</i>			
Male gender	54 (44%)	19 (45%)	0.91
Age (years)	49.2 ± 11.5	41.0 ± 11.2	<0.001
BMI (kg/m ²)	25.6 ± 5.1	25.1 ± 4.3	0.62
Smoking	30 (26%)	12 (29%)	0.73
High alcohol consumption	11 (9%)	1 (2%)	0.30
<i>Biological characteristics</i>			
Albumin (mean in g/L, 95% CI)	47.1 (46.5 – 47.7)	48.5 (47.6 – 49.5)	0.01
hsCRP ^b (mean in mg/L, 95% CI)	1.49 (1.20 – 1.86)	1.23 (0.85 – 1.79)	0.39
<i>Clinical characteristics</i>			
CAG repeats (number)	44.1 ± 3.2	21.6 ± 4.2	<0.001
Estimated disease duration (years)	3.8 ± 11.4	NA	NA
TFC score	8 (3 – 13)	13 (13 – 13)	<0.001
UHDRS motor score	19 (5 – 48)	NA	NA
Pre-motor symptomatic	33 (28%)	NA	NA
Antidepressant use	44 (36%)	1 (2%)	<0.001
Benzodiazepine use	25 (21%)	0 (0%)	0.001
Antipsychotic use	35 (29%)	0 (0%)	<0.001
<i>Neuropsychiatric characteristics</i>			
PBA apathy factor score	2 (0 – 18)	0 (0 – 0)	<0.001
PBA irritability factor score	4 (0 – 15)	0 (0 – 2)	<0.001
PBA depression factor score	4 (0 – 12)	0 (0 – 6)	0.04
PBA suicidality	14 (12%)	1 (2%)	0.08
<i>Cognitive characteristics</i>			
MMSE score	28 (24 – 29)	29 (29 – 30)	<0.001
SDMT score	35 (12 – 50)	53 (47 – 58)	<0.001
VFT score	20 (10 – 30)	29 (25 – 37)	<0.001
Stroop colour score	50 (33 – 71)	76 (69 – 87)	<0.001
Stroop word score	71 (41 – 98)	99 (99 – 100)	<0.001
Stroop interference score	29 (16 – 40)	44 (39 – 47)	<0.001
ExCog	-0.20 ± 0.9	0.68 ± 0.3	<0.001

Data are presented as n (%), mean (± SD), or median (interquartile range [IQR]), unless otherwise specified.

BMI denotes Body Mass Index; hsCRP, high sensitivity C-reactive protein; CI, confidence interval; NA, applicable; TFC, total functional capacity; UHDRS, Unified Huntington's Disease Rating Scale; PBA, Problem Behaviour Assessment; MMSE, Mini Mental State Examination; SDMT, Symbol-Digit Modalities Test; VFT, Verbal Fluency Test.

^a p-Values by chi-squared test for categorical data, by t-test for independent samples with normal distributions, or non-parametric Whitney-U tests for independent samples without normal distributions.

^b Because of its skewed distribution, hsCRP was log transformed before the analyses.

Table 2. Cross-sectional relationships between acute-phase proteins and continuous clinical, neuropsychiatric, and cognitive characteristics in 122 HD mutation carriers at first biomarker measurement (t1).

	Crude		Model 1 ^a		Model 2 ^a	
	hsCRP ^b	Albumin	hsCRP ^b	Albumin	hsCRP ^b	Albumin
<i>Clinical characteristics</i>						
CAG repeats	0.01	0.11	0.17	-0.04	0.15	0.00
Estimated disease duration	0.12	-0.27**	0.10	-0.04	0.09	-0.01
TFC score ^b	-0.19*	0.38***	-0.15	0.28**	-0.07	0.16
UHDRS motor score ^b	0.12	-0.24**	0.14	-0.14	0.09	-0.05
<i>Neuropsychiatric characteristics</i>						
PBA apathy factor score ^b	0.21*	-0.29***	0.22*	-0.24*	0.15	-0.12
PBA irritability factor score ^b	0.06	-0.05	0.02	-0.10	0.02	-0.11
PBA depression factor score ^b	0.09	0.04	0.08	0.02	0.07	0.04
<i>Cognitive characteristics</i>						
SDMT score ^b	-0.13	0.31***	-0.16	0.20*	-0.08	0.06
VFT score ^b	-0.12	0.24**	-0.17	0.18	-0.11	0.07
Stroop Word Test score ^b	-0.14	0.27**	-0.19	0.18*	-0.12	0.08
Stroop Color Test score ^b	-0.13	0.32***	-0.18	0.24*	-0.10	0.11
Stroop Interference Test score ^b	-0.13	0.32***	-0.22*	0.24*	-0.14	0.10
ExCog	-0.14	0.30**	-0.20*	0.22*	-0.12	0.09

Data are standardized betas. Analyses performed by linear multivariate regression analyses. Model 1: adjusted for sex, age, BMI, smoking, and alcohol consumption at t1; Model 2: additional adjustment for use of antipsychotics at t1.

hsCRP denotes high sensitivity C-reactive protein; TFC, total functional capacity; UHDRS, Unified Huntington's Disease Rating Scale; PBA, Problem Behaviour Assessment; SDMT, Symbol-Digit Modalities Test; VFT, Verbal Fluency Test; BMI, Body Mass Index.

*p < 0.05.

** p < 0.01.

*** p < 0.001.

^a n=115 mutation carriers because of missing values.

^b Because of its skewed distribution, hsCRP, TFC score, UHDRS motor score, PBA factor scores, SDMT score, VFT score, and Stroop scores were log transformed before the analyses.

Associations between acute-phase proteins and clinical characteristics at t2

When analyzing cross-sectional associations between hsCRP and albumin levels on the one hand and clinical, neuropsychiatric, and cognitive characteristics on the other hand at t2, similar associations were found as at t1. Once more, significant associations disappeared after additionally adjusting for antipsychotic use (data not shown).

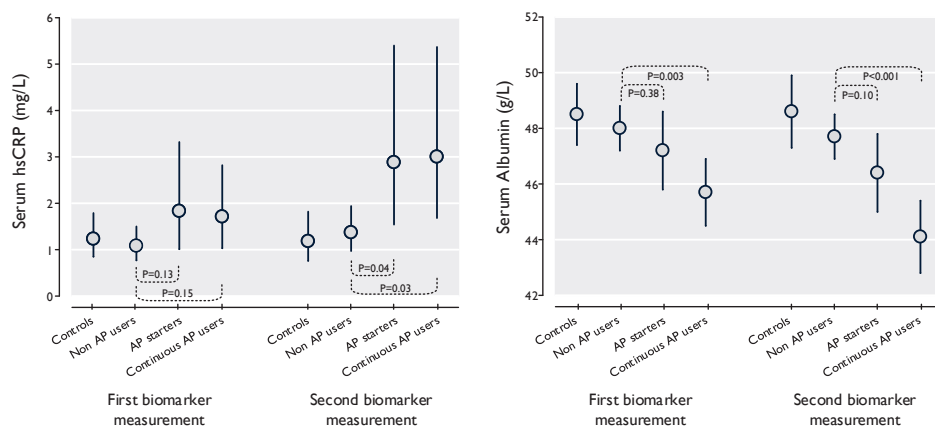


Figure 1 High sensitivity C-reactive protein (hsCRP) and albumin levels in controls and mutation carriers. For hsCRP levels the adjusted, backtransformed geometric mean values and for albumin the adjusted mean values are presented. Error bars represent the 95% confidence intervals. Mutation carriers were categorized according to antipsychotic (AP) usage into those: (1) who did not use AP at either time points; (2) who started the use of AP; and (3) who continuously used AP at both time points. Data were analyzed using analyses of covariance (ANCOVA) adjusting for sex, age, body mass index, smoking, and alcohol use at first biomarker measurement.

Use of antipsychotics and acute-phase proteins

When comparing mutation carriers who started using antipsychotics during the study period with mutation carriers who did not use antipsychotics during the study period, the hsCRP level of starters at t1 was not significantly different from non-users. At t2, however, the hsCRP level of starters had increased and was higher compared with non-users, although statistical significance disappeared after adjustment for the hsCRP level at t1 (Figure 1 and Suppl. Table 2). Compared with non-users, mutation carriers who continuously used antipsychotics had significantly higher hsCRP levels at both measurements and also had a larger increase in hsCRP level between the two measurements (Figure 1 and Suppl. Table 2).

Likewise, serum albumin levels of antipsychotic starters tended to decline, but the comparison with non-users was not significant at both measurements (Figure 1 and Suppl. Table 2). Albumin levels of continuous users were significantly lower compared with non-users at both t1 and t2, even after adjustment for the albumin level at t1 (Figure 1 and Suppl. Table 2).

After excluding participants with an hsCRP level above 10 mg/L, differences in hsCRP and albumin levels between non-users and starters and between non-users and continuous users at t1 persisted. At t2, continuous users had significantly more often hsCRP levels above 10 mg/L than non-users (21.1% versus 4.1%, respectively; $p = 0.03$). hsCRP levels remained higher and albumin levels lower in starters and continuous users compared with non-users, although the absolute differences substantially attenuated.

Table 3. Cross-sectional relationships between acute-phase proteins and dichotomous clinical, neuropsychiatric, and cognitive characteristics in 122 HD mutation carriers at first biomarker measurement (t1).

	Crude		Model 1 ^a		Model 2 ^a	
	hsCRP ^b	Albumin	hsCRP ^b	Albumin	hsCRP ^b	Albumin
<i>Clinical characteristics</i>						
Pre-motor symptomatic	1.20	0.92	1.31	0.97	1.18	1.05
Antidepressant use	1.15	0.88*	0.88	0.92	0.74	0.98
Benzodiazepine use	1.47*	0.75***	1.21	0.79*	1.08	0.84
Antipsychotic use	1.64**	0.72***	1.54	0.77**	n/a	n/a
<i>Neuropsychiatric characteristics</i>						
PBA suicidality	1.05	0.83*	0.92	0.83	0.80	0.86
<i>Cognitive characteristics</i>						
MMSE score	0.79	1.18**	0.71	1.19*	0.78	1.13

Data are odds ratios. Analyses performed by logistic multivariate regression analyses. Model 1: adjusted for sex, age, BMI, smoking, and alcohol consumption at t1; Model 2: additional adjustment for use of antipsychotics at t1.

hsCRP denotes high sensitivity C-reactive protein; PBA, Problem Behaviour Assessment; MMSE, Mini Mental State Examination; BMI, Body Mass Index.

*p < 0.05.

** p < 0.01.

*** p < 0.001.

^a n=115 mutation carriers because of missing values.

^b Because of its positively skewed distribution, hsCRP was log transformed before the analyses.

When repeating the analysis only in participants with a disease stage > 2, the differences that were found between continuous users and non-users at both t1 and t2 were comparable to those in the original analysis, while this was not the case for the differences between starters and non-users.

Using linear mixed models analyses, with adjustment for potential confounders, all three categories of antipsychotics were positively associated with hsCRP levels. Effect estimates indicated a rather similar effect sizes for typical antipsychotics (n = 32; β = 0.42), atypical antipsychotics (n = 26; β = 0.25), and tiapride (n = 28; β = 0.30). Likewise, inverse associations were found with albumin: typical antipsychotics (n = 32; β = -0.47), atypical antipsychotic (n = 26; β = -0.53), and tiapride (n = 28; β = -0.36) (data not shown).

DISCUSSION

We found strong associations between the use of antipsychotics and an elevated acute-phase response in HD. Other associations between hsCRP and albumin levels on the one hand and

TFC score, apathy, and cognitive impairment on the other hand, disappeared when adjusting for the use of antipsychotics. Also, mutation carriers who continuously used antipsychotics had significantly higher hsCRP levels and lower albumin levels compared with mutation carriers who did not use antipsychotics during the study period.

Increases in CRP levels have previously been found in HD mutation carriers when compared to controls and one study also found a correlation between disease stage and CRP levels.^{15;16} In contrast, we only found a significant increasing trend of CRP across increasing disease stages but no differences in CRP levels between the whole group of HD mutation carriers and controls. The relatively low number of HD mutation carriers in more advanced disease stages in our cohort may have accounted for this finding. This idea is in line with the finding of a study reporting that premanifest and early-stage HD mutation carriers, who are in lower disease stages, had similar or lower CRP levels respectively, when compared with controls.¹⁷

Previous publications offer several mechanisms that could explain our finding of an elevated acute-phase reaction in mutation carriers who used antipsychotics. First, the use of antipsychotics may cause symptoms associated with the metabolic syndrome which in turn induces an acute-phase response. Atypical antipsychotics are strongly associated with the development of metabolic syndrome, which is characterized by abdominal obesity, dyslipidemia, hypertension, and insulin resistance. Obesity,⁴⁵ dyslipidemia,³¹ and insulin resistance⁴⁶ may induce low-grade inflammation reflected in increased CRP levels. In rats, it has been shown that treatment with olanzapine induces weight gain and increased adipose tissue. In addition, the adipose tissue of olanzapine-treated rats had become infiltrated with macrophages and there was a 2-fold increase in the expression of the TNF- α , both indicative of low-grade inflammation.⁴⁷ Second, the use of antipsychotics may directly induce low-grade inflammation through their hepatic impact since antipsychotics are eliminated predominantly by hepatic metabolism. In the CATIE trial,²⁸ increased CRP levels were indicative of having more signs of the metabolic syndrome but were also independently associated with the use of atypical antipsychotics, in particular olanzapine. In a smaller open-label study, with 111 patients randomized to haloperidol, olanzapine, and risperidone, patients on haloperidol showed the strongest increase in CRP levels after three months of treatment.²⁹

Since the effects in our study were largely unaffected by adjustment for BMI, we assume that the use of antipsychotics directly induced an acute-phase response. Atypical antipsychotics are most often implicated in induction of inflammation but in our mixed-model analyses, we did not find a differential role for atypical antipsychotics, typical antipsychotics, and tiapride. Therefore these groups were combined.

Immune activation in HD is widespread⁴⁸ and is known to positively correlate with disease stage. IL-6, a cytokine that plays a main role in the innate immune response, was shown to be up-regulated both centrally and peripherally in HD.¹¹ One of its functions is to initiate the acute-phase response, thus subsequently increasing the synthesis of CRP while decreasing albumin levels. Neuroinflammation leads to neuronal degeneration through several mechanisms, and

neuronal degeneration in the striatum is one of the key histopathological features of HD that is associated with movement abnormalities, cognitive and several neuropsychiatric symptoms. Our findings may therefore also be explained by reverse causation meaning that neuroinflammation has caused movement abnormalities and neuropsychiatric symptoms, which in turn has led to the prescription of antipsychotics.

Although influenced by the use of antipsychotics, we did find associations between levels of acute-phase proteins and several clinical variables. We found an association for TFC, apathy, and cognitive impairment. Previous studies in non-HD populations also found associations between levels of acute-phase proteins and both apathy²⁵ and cognitive impairment.^{18;21;22} In contrast to our study, these studies did not investigate the role of antipsychotics in these associations. In our study, the elevated acute-phase reaction was strongly associated with the use of antipsychotics and all associations between acute-phase proteins and clinical variables disappeared when adjusting for antipsychotics. As previously argued, we hypothesize that the use of antipsychotics induced an acute-phase response which in turn was associated with several neuropsychiatric symptoms and cognitive impairment. But associations between acute-phase proteins on the one hand and TFC, apathy, and cognitive impairment on the other hand, may have been confounded by the use of antipsychotics. Alternatively, confounding by indication may explain our results.

To our knowledge, this is the first study investigating the role of the acute-phase response in the occurrence of neuropsychiatric symptoms and cognitive dysfunction in HD. Strengths of this study are the use of a comparison group (consisting of first-degree non-carriers), the use of validated measurement tools in a standardized interview, and the adjustment for potential confounders, including antipsychotic use, in the analyses. There are several limitations that warrant discussion. Since inflammation is associated with the neurodegenerative process in HD, relationships found in this study might not be generalized to other disorders and the general population. Given the observational rather than experimental nature of our study, it is impossible to make causal inferences about our findings.

Future research on the role of antipsychotic use on the acute-phase reaction is necessary, both in HD and in other populations. We found strong evidence for a role of antipsychotics in inducing an acute-phase response in HD mutation carriers. Although we could not make inferences regarding the direction of causation, HD mutation carriers who receive antipsychotics may be prone to the development of low-grade inflammation, which may subsequently increase their risk of cardiovascular morbidity, lower functioning capacity, apathy, and cognitive dysfunction.

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Supplementary Table 1. Levels of hsCRP and albumin at different disease stages according to TFC score at t1 and t2

Disease stage according to TFC score*	Stage I	Stage II	Stage III	Stage IV	Stage V	p-value for trend
t1:						
• No. of mutation carriers	52	19	25	10	16	
• Albumin (g/L)	48.2 ± 2.8	47.0 ± 2.3	47.1 ± 2.9	46.0 ± 3.3	44.2 ± 4.2	<0.001
• hsCRP (mg/L)	2.3 ± 3.3	2.4 ± 2.9	4.2 ± 5.2	3.2 ± 1.8	5.5 ± 6.1	0.02
• Antipsychotic use	1 (2%)	4 (21%)	13 (52%)	7 (70%)	10 (63%)	<0.001
t2:						
• No. of mutation carriers	33	16	22	7	7	
• Albumin (g/L)	47.5 ± 3.7	46.9 ± 2.5	46.5 ± 2.5	43.2 ± 0.8	43.2 ± 3.8	<0.001
• hsCRP (mg/L)	2.4 ± 2.7	5.4 ± 12.3	4.1 ± 4.9	3.3 ± 2.1	17.6 ± 25.8	0.01
• Antipsychotic use	0 (0%)	5 (31%)	15 (68%)	7 (100%)	7 (100%)	<0.001

Data are numbers, means ± standard deviation (SD), or numbers (percentages) where appropriate.

hsCRP denotes high sensitivity C-reactive protein; TFC, total functional capacity.

*: Stage I: TFC>10, Stage II: 6<TFC<11, Stage III: 2<TFC<7, Stage IV: 0<TFC<3, Stage V: TFC=0.

P-value for trend computed by linear regression analysis or chi-squared test, linear by linear term.

Supplementary Table 2. Levels of acute-phase proteins in mutation carriers according to antipsychotic use.

	Mutation carriers not using antipsychotics (n = 51; reference)	Mutation carriers who started antipsychotics (n = 15)	p-value	Mutation carriers who continuously used antipsychotics (n = 23)	p-value
High sensitivity C-reactive protein (mg/L)^a:					
<i>First biomarker measurement</i>					
- Crude	1.10 (0.76 – 1.58)	1.67 (0.79 – 3.50)	0.28	1.93 (1.27 – 2.93)	0.04
- Model 1 ^b	1.08 (0.77 – 1.50)	1.83 (1.01 – 3.32)	0.13	1.71 (1.03 – 2.82)	0.15
<i>Second biomarker measurement</i>					
- Crude ^c	1.40 (0.99 – 1.98)	2.57 (1.29 – 5.12)	0.10	3.61 (2.02 – 6.46)	0.005
- Model 1 ^d	1.37 (0.97 – 1.94)	2.88 (1.54 – 5.40)	0.04	3.00 (1.68 – 5.37)	0.03
- Model 2 ^e	1.47 (1.07 – 2.01)	2.31 (1.29 – 4.15)	0.18	2.82 (1.67 – 4.77)	0.04
Albumin (g/L):					
<i>First biomarker measurement</i>					
- Crude	48.1 (47.4 – 48.8)	47.2 (45.3 – 49.1)	0.27	45.3 (44.1 – 46.5)	<0.001
- Model 1 ^b	48.0 (47.2 – 48.8)	47.2 (45.8 – 48.7)	0.38	45.7 (44.5 – 46.9)	0.003
<i>Second biomarker measurement</i>					
- Crude ^c	47.6 (46.9 – 48.3)	46.4 (45.0 – 47.9)	0.10	44.0 (42.6 – 45.4)	<0.001
- Model 1 ^d	47.7 (46.9 – 48.5)	46.4 (45.0 – 47.8)	0.10	44.1 (42.8 – 45.4)	<0.001
- Model 2 ^e	47.4 (46.8 – 48.0)	47.0 (45.8 – 48.2)	0.55	44.7 (43.6 – 45.7)	<0.001

Data are presented as adjusted (geometric) mean values (with 95% confidence interval [CI]). P-values by *t*-tests for crude models and by analyses of covariance (ANCOVA) for model 1 and model 2.

Model 1: adjusted for sex, age, body mass index, smoking, and alcohol use at t1; Model 2: additional adjustment for acute-phase protein level (hsCRP/albumin) at t1.

^a Because of its positively skewed distribution, high sensitivity C-reactive protein was log transformed before the analyses.

^b 2 missings for non-users; ^c 2 missings for non-users, 4 for continuous users; ^d 4 missings for non-users, 4 for continuous users;

^e 4 missings for non-users, 1 for starters and 4 for continuous users.

CHAPTER 5

Disease stage and plasma levels of cytokines in Huntington's disease: a 2-year follow-up study

J.A. Bouwens, E. van Duijn, C.M. Cobbaert, R.A.C. Roos, R.C. van der Mast, E.J. Giltay

BACKGROUND

It has been shown that plasma levels of cytokines are elevated in Huntington's Disease (HD) gene expansion carriers compared to controls, and are higher in advanced disease stages (1). To test the hypothesis that plasma cytokines may serve as a biomarker of disease progression (2), levels of pro-inflammatory and anti-inflammatory cytokines were measured in different disease stages at baseline and during the 2-year follow-up.

METHODS

In a cohort of 124 HD gene expansion carriers [described in (3)], of whom 92 could be reassessed at 2-year follow-up, plasma levels of tumor necrosis factor α (TNF- α), interleukin (IL)-1b, IL-1 receptor antagonist (ra), IL-5, IL-6, IL-8, and IL-10 were measured. High sensitivity (hs) ELISA assays were used to measure TNF- α , IL-1b, IL-6, and IL-10. Coefficients of variation ranged from 5.9-64%. The diagnostic confidence level (CL) of the Unified Huntington's Disease Rating Scale (UHDRS) was used to define pre-motor symptomatic (CL 0-1) and motor symptomatic (CL 2-4) HD (5). The disease burden score (6), the Total Functional Capacity (TFC) scale (4), and the Total Motor Score (TMS) of the UHDRS were used as measures of disease progression. To assess longitudinal changes, paired-sample t-tests were used. Group comparisons were conducted using independent sample t-tests. Associations between disease stage and cytokine levels were analyzed using data from both assessments by multilevel analysis, accounting for covariance.

RESULTS

At baseline, the average age of participants was 49 years, 56% were male and 27% were pre-motor symptomatic. During the 2-year follow-up, increases were found in the levels of TNF- α [1.48 pg/mL standard error (SE) 1.05 pg/mL vs. 1.65 pg/mL SE 1.06 pg/mL, $p=0.01$] and IL-10 (0.38 pg/mL SE 1.03 pg/mL vs. 0.41 pg/mL SE 1.03 pg/mL, $p=0.04$). Motor symptomatic participants had higher levels of IL-6 than pre-motor symptomatic participants (0.98 pg/mL SE 1.06 pg/mL vs. 0.64 pg/mL SE 1.07 pg/mL, $p=0.007$). Levels of IL-5 were lower in motor symptomatic compared with pre-motor symptomatic participants (0.075 pg/mL SE 1.25 pg/mL vs. 0.151 pg/mL SE 1.25 pg/mL, $p<0.001$).

With regard to measures of disease progression, only the levels of IL-6 and IL-8 were inversely associated with the TFC score, indicating higher levels in more advanced disease stages (Table 1). After exclusion of participants who had an inflammatory disease, used anti-inflammatory agents, or had hsCRP levels >10 mg/mL (38 time points), effect sizes were not attenuated. No association was found between cytokine levels and disease burden score or TMS.

DISCUSSION

In the present study, in the total group of HD gene expansion carriers, an increase was found TNF- α and IL-10 levels during the 2-year follow-up. This may reflect a reactive elevation of anti-inflammatory cytokines after the initial pro-inflammatory response. IL-6 and IL-8 were inversely associated with the TFC score, whereas no association was found for the other cytokines and measures of disease progression. These findings are only partly in agreement with previous reports in which cytokine levels also correlated with estimated time until onset of disease (derived from the disease burden score) and with TMS (1). Therefore, currently, we cannot decisively conclude that cytokines are useful biomarkers of disease progression in HD.

A limitation of the present study is that we did not control for diurnal variation of cytokine levels. Also, the coefficients of variation were high for IL-1ra, IL-8 and IL-5, thereby increasing the probability of a type II error. Future studies should track cytokine levels in larger populations using multiple assessments in order to verify whether cytokine levels might be markers of disease progression in HD.

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CHAPTER 6

Plasma cytokine levels in relation to neuropsychiatric symptoms and cognitive dysfunction in Huntington's disease

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ABSTRACT

Background: In Huntington's disease (HD) the innate immune system is activated, as reflected by increased plasma levels of different cytokines. **Objective:** To explore whether increased cytokine levels are associated with neuropsychiatric symptoms and cognitive dysfunction in HD mutation carriers. **Method:** Plasma cytokine levels of TNF-alpha, interleukin (IL)-1ra, IL-1 β , IL-5, IL-6, IL-8 and IL-10 were assessed in 124 HD mutation carriers at two time points 2 years apart (totaling 214 observations). Using multilevel regression analysis, cytokines were analysed in relation to neuropsychiatric symptoms and cognitive dysfunction. Depressed mood was assessed with the depression subscale of the Problem Behaviours Assessment (PBA), apathy with the Apathy Scale, and irritability with the Irritability Scale. Cognitive functioning was assessed using the Mini-Mental State Examination (MMSE) and a battery of executive cognitive functioning tests, aggregated into an executive cognitive functioning (ExCog) score. **Results:** Inverse associations were found in adjusted models between IL-6 and ExCog score ($\beta = -0.114$; $p = 0.01$) and between IL-1ra and ExCog score ($\beta = -0.110$; $p = 0.02$). No associations between cytokine levels and any of the other neuropsychiatric symptom scores remained statistically significant in adjusted models. **Conclusion:** Higher plasma levels of IL-6 and IL-1ra are weakly associated with cognitive dysfunction in HD, but not with other neuropsychiatric symptoms.

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant heritable disease, characterized by neuropsychiatric symptoms, cognitive dysfunction and motor disturbances [1]. The prevalence of neuropsychiatric symptoms ranged between 33%-69% for a depressed mood, 34%-76% for apathy and 33%-73% for irritability [2, 3]. Neuropsychiatric symptoms may occur at any disease stage; only the prevalence of apathy is clearly positively associated with progression of the disease [4]. Executive cognitive impairment is a prominent component of cognitive dysfunction in HD and is positively correlated with disease progression and with whole brain volume loss. Executive cognitive impairment can become noticeable already an early stage in HD patients [1, 5]. HD is caused by an expanded cytosine-adenine-guanine (CAG) repeat in the huntingtin protein gene on the short arm of chromosome 4. This genetic defect leads to the expression of mutant huntingtin (*mhtt*). The exact pathophysiological mechanisms by which *mhtt* causes HD is not yet fully understood [6].

The role of immune activation in HD neurodegeneration is supported by several lines of evidence [6, 7]. Activated microglia – the immunocompetent cells in the central nervous system (CNS) – have been demonstrated in post-mortem samples [8] and on cerebral positron emission tomography (PET)-scans [9] of HD patients. Immune activation has also been demonstrated in plasma of HD patients and may reflect immune activation within the CNS [10]. Several cross-sectional studies have shown increased plasma levels of different cytokines in HD, such as interleukin (IL)-6, soluble (s) IL-2 receptor (R), s tumor necrosis factor (TNF)- α R, IL-4, IL-5, and IL-10. Particularly IL-6 levels were found to be consistently higher in HD mutation carriers than in matched controls [10-13]. Levels of IL-6 also correlated positively with the disease stage of HD [10].

In an earlier investigation, we studied the association between plasma acute-phase protein C-reactive protein (CRP) and neuropsychiatric symptoms including cognitive dysfunction. Since CRP is synthesized in the liver predominantly under the influence of several pro-inflammatory cytokines such as IL-6, the results of that study are relevant to our current investigation. Plasma levels of CRP were associated with disease progression, apathy, and cognitive dysfunction [14]. However, these results were likely mediated by the use of antipsychotics that may have augmented the hepatic production of CRP.

To date, plasma levels of cytokines have not been linked to the occurrence of neuropsychiatric symptoms and cognitive dysfunction in HD. In study populations other than HD, levels of plasma cytokines have been associated with neuropsychiatric symptoms like anger, hostility, irritability, apathy, depression and cognitive dysfunction [15-23]. In line with the findings in other populations, we expect positive associations between pro-inflammatory cytokines and, more speculative, inverse associations between anti-inflammatory cytokines on the one hand and neuropsychiatric symptoms and cognitive dysfunction, on the other hand. Since the highest level of plasma cytokines have been found in late disease stages [10], we expect to find the strongest associations

between cytokines and apathy and cognitive dysfunction in late disease stages. We investigated these associations using all data of up to two time points in HD mutation carriers, taking into account the covariance between both measurements that were two years apart from each other.

MATERIALS AND METHODS

Population

In total, 124 HD mutation carriers comprising both pre-motor symptomatic and motor symptomatic mutation carriers were recruited from the outpatient departments of Neurology and Clinical Genetics of the Leiden University Medical Center and a regional nursing home specialized in care for advanced HD patients. The study design is described in detail elsewhere [24]. All participants were proven HD mutation carriers with a CAG repeat length ≥ 36 repeats. Baseline measurements were conducted between May 2004 and August 2006. Two years later a second measurement was conducted and a third measurement two years thereafter (Figure 1). Blood samples suitable for determination of cytokine levels were drawn only at the second and third measurement. Thus, for the present study, data from the second and third measurement were used, referred to as t1 and t2 hereafter. The study was approved by the medical ethical committee of the LUMC and informed consent was obtained from all participants.

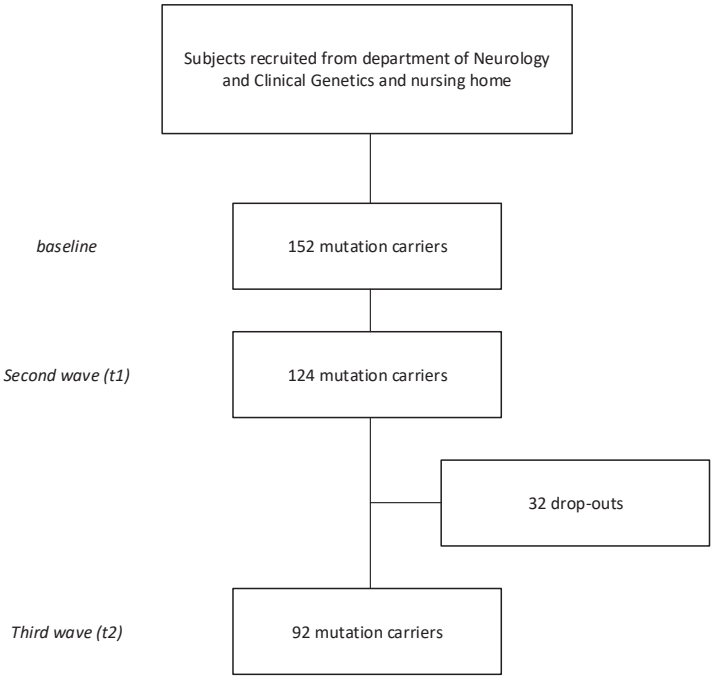


Figure 1

Specimen collection and storage

EDTA samples were collected and centrifuged for 15 minutes at 3000 g within 30 minutes of collection. Blood samples were collected between 9 am and 3 pm, but time of withdrawal was not ascertained for each blood sampling. EDTA-plasmas were aliquoted in 500 µL fractions in cryo vials from Sarstedt and stored at -80 °C until analysis took place. EDTA-plasmas sampled at t1 had median (min-max) storage times of 328 (246-361) weeks before cytokine analysis; t2 samples were stored up to 170 (77-203) weeks before analysis. Plasma aliquots used for IL1ra, IL5 and IL8 were thawed twice before analysis, whereas plasma aliquots used for high sensitivity (hs) IL-1β, hs IL6, hs IL10, hs TNF-α were thawed only once. Samples were assessed in duplicates, taking the average of both measurements. To explore the possibility of IL-6 degradation due to prolonged storage in the freezer, we performed an analysis correlating levels of IL-6 with levels of C-reactive protein (CRP) which are known to be stable during storage. In this analysis, we found a correlation of 0.49 which is comparable to the correlation of 0.49 found in a large meta-analysis that comprised 19,053 participants [25]. In our sample of 90 HD patients with assessments at both time points, correlation coefficients varied between 0.483 and 0.728 ($P < 0.001$).

Cytokine Reagents

Cytokine analyses were done using traditional uniplex ELISA's, the assay in principle being based on quantitative sandwich enzyme immunoassay technology. Standardization is done according to purified NIBSC/WHO recombinant standards. Analytical selectivity is guaranteed by means of pre-coated monoclonal antibodies, specific for each cytokine under investigation. Detection took place using a Reader SpectraMAX250 (Molecular Devices) and Softmax Pro calculation software (version 5.4). The cytokines IL6, IL10, TNFα and IL1β were measured with Quantikine® hs Human Immunoassay kits from R&D Systems. These hs ELISA's for IL1b, IL6, IL10 and TNFα are optimized for measuring cytokines in the low plasma range with inter-assay coefficients of variation (CVs) below 10%. Minimum detectable levels, defined as two standard deviations above the mean of twenty zero standard replicates, are 0.057; 0.039; 0.09 and 0.106 pg/mL for IL1b, IL6, IL10 and TNFα respectively. The cytokines IL8, IL1-ra and IL5 were analysed with Quantikine® ELISA Human Immunoassay from R&D Systems, which is the non-high sensitivity version as the high sensitivity version is not available. These ELISA's are optimized for cytokines in the pathophysiological range. Minimum detectable levels are 6.26; 0.29 and 3.5 pg/mL for IL1ra, IL5 and IL8.

Assessment of neuropsychiatric symptoms

Depressive mood was assessed using the depression subscale of the Dutch translation of the Problem Behaviours Assessment (NL-PBA) [24]. This scale has a range from 0 to 80. Apathy was assessed using the Apathy Scale[26], a 14-item questionnaire with a sum score range between 0 and 42. Caregivers' information and the interviewers' judgment were included in rating the Apathy Scale, since patients with apathy may lack adequate insight into their own symptoms.

Irritability was assessed using the irritability scale[27], a 14-item questionnaire with a sum score range between 0 and 42.

Assessment of cognition

Global cognitive functioning was assessed using The Mini-Mental State Examination (MMSE) [28]. Executive cognitive functioning was assessed using the cognitive scales of the Unified Huntington's Disease Rating Scale (UHDRS), including the Verbal Fluency Test (VFT)[29] the Symbol Digit Modalities Test (SDMT)[30], and the three Stroop tests [31]. For all cognitive scales, lower scores indicate worse executive functioning. Because of substantial co-linearity between the executive cognitive functioning scales (Pearson's $r > 0.80$), executive cognitive functioning was summarised in the ExCog variable, a composite variable obtained by averaging the standardized z -scores of the five executive cognitive scales (i.e. VFT, SDMT and Stroop tests).

Assessment of clinical characteristics

Information on sociodemographic and clinical characteristics including alcohol consumption, use of psychotropic medication and information about the household was collected in a standardized manner. Global daily functioning was assessed using the Total Functional Capacity (TFC) scale of the UHDRS[32]. Trained neurologists assessed motor symptoms using the motor section of the UHDRS (UHDRS-m). Mutation carriers with an UHDRS-m confidence level >1 were considered motor symptomatic. Disease stage was classified according to functional status (TFC) in five categories with scores on the TFC of 11 to 13, 7 to 10, 3 to 6, 1 to 2 and 0 corresponding to disease stages I, II, III, IV and V, respectively. Stages I – III were defined as early disease and stages IV-V as late disease.

Statistical analysis

Data are presented as n (%), mean ($\pm$ standard deviation [SD]), mean (95% CI), or median (inter-quartile range [IQR]), when appropriate. Because of skewed distributions of values and residuals in our regression analyses, scores on the irritability scale, apathy scale and PBA depression subscale were naturally $\log_e$ -transformed. After $\log_e$ -transformation, residuals displayed approximately normal distributions. For the ExCog score, no such transformation was necessary. Multilevel regression models (i.e., Mixed Models) were used to analyse the association between cytokine levels (i.e. independent variable) and neuropsychiatric symptoms (i.e. dependent variable) using a two-level structure, with plasma cytokine levels at the two time points as the lower level and patients as the upper levels. To increase power, we used data from both time points, taking into account the covariance between both measurements within patients. In multivariable models, we adjusted for sex, age, body mass index (BMI), smoking and the use of antipsychotics. We used a compound symmetry covariance matrix to ensure that data from the same individual at two different time-points are not treated as independent observations. We chose not to adjust for disease stage as we hypothesized that inflammation (reflected in serum cytokine levels) is

part of the causal pathway between disease progression and neuropsychiatric symptoms, and therefore adding disease stage as a confounding variable would lead to over-adjustment. To ascertain whether the associations between cytokine levels and outcome variables were stronger in late versus early disease stages (defined as Stages IV–V vs. I–III according to TFC), appropriate interaction terms were added to the statistical models. To adjust for the potential confounding effect of prevalent infections, a sensitivity analysis was carried out in which we excluded 18 observations with hsCRP levels > 10 mmol/mL. Also, a partial correlation coefficient was calculated for the similarity of levels at second measurement and third measurement and for the correlation with hsCRP as a measure of validity. The SPSS 21.0 software package was used for statistical analyses. A two-tailed p-value <0.05 was considered to denote statistical significance.

RESULTS

Population characteristics

Data from 124 observations at t1 and 90 observations from t2 were available and used in the present analysis. (Table 1) On average, mutation carriers were 50.7 years old, 57% was male, 71% was motor symptomatic and the average TFC score was 7.7. Twenty percent of the mutation carriers were in a late stage. Cytokine levels could not be obtained in 5 mutation carriers. In addition, data was missing for the apathy scale, irritability scale, MMSE and ExCog in respectively 1, 2, 2 and 4 mutation carriers out of the 214 observations.

Associations between cytokine levels and neuropsychiatric symptoms

No associations were found between cytokine levels and the PBA depression subscale score, neither in univariate nor in multivariate models. The apathy scale score was positively associated with IL-1 β , IL-6 and IL-8, but after adjustment for sex, age, BMI, smoking status and the use of antipsychotics, these associations were no longer statistically significant. No associations were found between cytokine levels and irritability. The score on the MMSE was inversely associated with IL-1 β and IL-1ra, but again only in crude but not in adjusted models.

The ExCog score was inversely associated with IL-6 and IL-1ra in both crude and adjusted models (β = -0.114 and β = -0.110, respectively; Table 2 and Figure 2). Interaction terms between disease stage and cytokine level for the relationship with ExCog were not statistically significant. When excluding a total of 18 observations with hsCRP levels > 10 mmol/mL, effect sizes for the adjusted association between ExCog on the one hand and both IL-6 and IL-1ra on the other hand remained significant (β = -0.192, p <0.001 and β = -0.125, p =0.02, respectively). Also, after adjustment for hsCRP levels, the effect sizes for the adjusted association remained similar and significant (β = -0.146, p =0.003 and β = -0.108, p =0.04, respectively).

Table 1 Characteristics of mutation carriers at t1 and t2

	t1 n=124	t2 n=90
<i>Demographic characteristics</i>		
Age	49.3 ± 11.7	52.6 ± 11.5
Sex (male)	69 (56%)	53 (59%)
BMI	25.6 ± 5.1	25.9 ± 5.7
Smoking	31 (25%)	25 (28%)
<i>Clinical characteristics</i>		
CAG repeats	44.1 ± 3.2	43.9 ± 3.0
UHDRS-m score	30.2 ± 28.6	35.6 ± 31.3
Motor symptomatic n	88 (71%)	63 (70%)
TFC score	7.7 ± 4.9	7.6 ± 4.7
Late stage	26 (21%)	17 (19%)
Use of antipsychotics	28 (23%)	31 (34%)
<i>Neuropsychiatric characteristics</i>		
PBA depression score	9.7 ± 14.3	9.9 ± 13.0
Apathy scale score	8.6 ± 8.7	12.1 ± 9.3
Irritability scale score	10.2 ± 8.3	11.7 ± 7.5
MMSE score	26.2 ± 4.7	25.3 ± 5.7
ExCog score	0.03 ± 0.94	-0.04 ± 1.08

Data are presented as mean (±standard deviations) or number (percentage) where appropriate. BMI denoted Body Mass Index in kg/m², UHDRS-m denotes Unified Huntington's Disease Rating Scale motor section, TFC denotes Total Functioning Capacity.

Table 2. Mixed model analysis of the association between plasma cytokine levels and neuropsychiatric symptoms in HD mutation carriers

	PBA depression subscale			Apathy Scale		Irritability Scale		MMSE		ExCog	
	209 observations		P-value	208 observations		207 observations		207 observations		205 observations	
	β	P-value		β	P-value	β	P-value	β	P-value	β	P-value
IL-1 β											
	crude	0.055	0.47	0.093	0.02	0.062	0.42	-0.145	0.03	-0.039	0.25
	adjusted	0.012	0.87	0.005	0.94	0.041	0.61	-0.047	0.43	-0.013	0.72
IL-6											
	crude	-0.031	0.71	0.196	0.01	0.090	0.23	-0.213	0.00	-0.114	0.002
	adjusted	-0.057	0.49	0.110	0.15	0.105	0.21	-0.114	0.08	-0.114	0.003
IL-8											
	crude	0.108	0.19	0.167	0.03	0.132	0.08	-0.112	0.11	-0.023	0.50
	adjusted	0.087	0.25	0.116	0.10	0.124	0.11	-0.079	0.19	-0.025	0.49
TNF- α											
	crude	-0.039	0.65	-0.041	0.60	-0.055	0.47	-0.048	0.52	-0.029	0.46
	adjusted	-0.015	0.85	-0.062	0.39	-0.079	0.31	-0.074	0.27	-0.036	0.38
IL-1ra											
	crude	0.045	0.59	0.106	0.16	0.127	0.09	-0.145	0.05	-0.097	0.02
	adjusted	0.109	0.24	0.087	0.31	0.180	0.06	-0.036	0.65	-0.110	0.02
IL-5											
	crude	0.029	0.73	-0.006	0.94	0.146	0.06	0.033	0.66	0.003	0.94
	adjusted	0.008	0.92	-0.044	0.53	0.130	0.09	0.055	0.41	0.031	0.46
IL-10											
	crude	-0.011	0.88	-0.045	0.53	0.039	0.58	0.062	0.34	0.013	0.67
	adjusted	0.016	0.83	-0.043	0.52	0.028	0.97	0.038	0.51	0.019	0.57

The adjusted model was adjusted for age, sex, BMI, smoking status and the use of antipsychotics. PBA denotes Problem Behaviours Assessment, MMSE denotes Mini Mental State Examination, ExCog is an aggregate variable derived from the standardized scores of the five executive cognitive functioning scales; Verbal Fluency Test, Symbol Digit Modality Test and the Stroop tests.

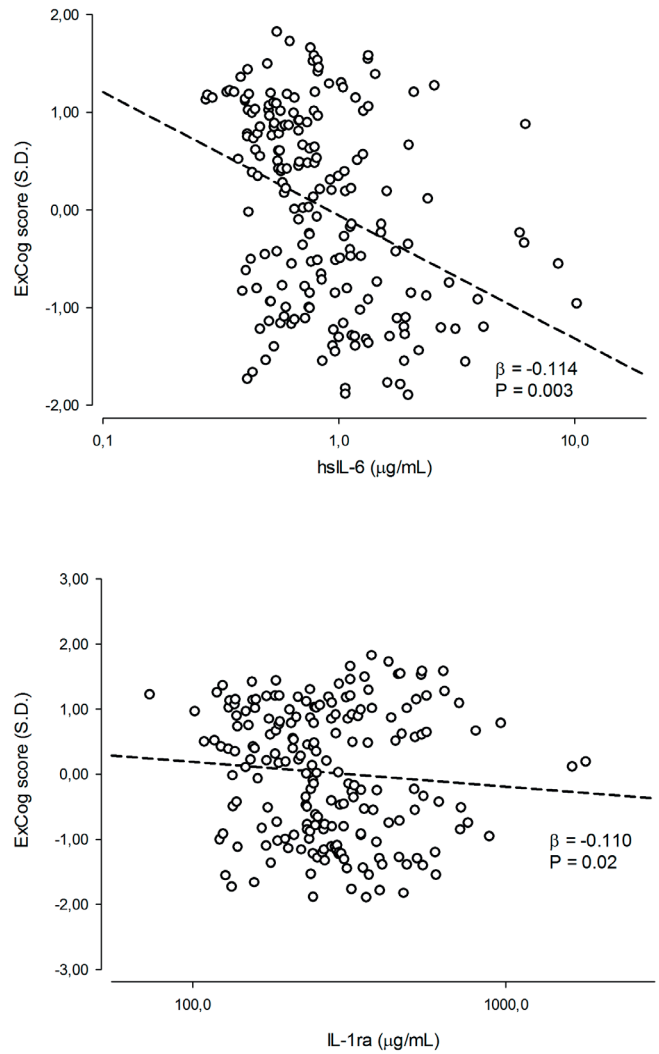


Figure 2

DISCUSSION

We found that higher plasma levels of unstimulated IL-6 and IL-1ra showed a weak association with more cognitive executive dysfunction. We found no support for our hypothesis that these associations would be stronger in moderate and late stages of HD as compared to early stages. Also, contrary to our hypothesis, levels of cytokines were not associated with depression, apathy and.

Our findings that levels of IL-6 and IL-1ra in plasma were associated with cognitive dysfunction have not yet been reported in HD. In non-HD populations, levels of cytokines in relation to cognitive dysfunction have been studied most extensively in patients with Alzheimer's disease (AD), showing that particularly IL-6 and TNF- α levels were associated with cognitive dysfunction in some, but not all studies among patients with AD [20]. Both IL-6 and IL-1ra play an important role in the acute-phase response. Upon disturbance of the homeostasis by infection or tissue injury, cells of the innate immune system become activated and release pro-inflammatory cytokines [33]. One of the major pro-inflammatory cytokines is IL-6 [34]. IL-6 initiates the acute-phase response by induction of a systemic reaction comprising hepatic release of C-reactive protein (CRP) [35], the release of other pro-inflammatory cytokines and, activation of the hypothalamic-pituitary-adrenal axis [36]. Another effect of IL-6 is the induction of IL-1ra [37]. IL-1ra is an inhibitor of the pro-inflammatory cytokine IL-1 β [38]. Thus, IL-1ra is regulator of the acute phase response by providing a feedback mechanism for the systemic effects of pro-inflammatory cytokines.

Given the cross-sectional nature of our investigation, the association between the cytokines IL-6 and IL-1ra with cognitive dysfunction may be explained by either causal effects, reverse causation, or confounding by another process. For a causal effect to become likely, IL-6 and IL-1ra in the peripheral plasma should be able to influence the CNS in some way. Two direct pathways between the peripheral plasma and the CNS exist [39]. First, cytokines can be transported by active passage through the blood brain barrier [40]. Second, cytokines can diffuse passively into the CNS in the choroid plexus [41]. Furthermore, IL-6 can increase the permeability of the blood-brain barrier [42], thereby allowing toxins to enter the brain that can interfere with brain functions such as cognition. Indirectly, the CNS can be affected by stimulation of the vagal nerve by intra-peritoneal cytokines [43]. Also, under certain conditions, microglia and endothelial cells in the brain produce inflammatory factors [43]. Once in the brain, cytokines can activate other microglia which in turn secrete their own pro-inflammatory cytokines [44]. This activation of microglia has indeed been demonstrated by several studies, in both PET scans [9] and in post-mortem brain tissue of HD patients [8]. Long-term exposure to cytokines, together with the presence of activated microglia may lead to neurodegeneration that causes cognitive dysfunction [39, 45]. In earlier studies, it has further been demonstrated that exposure to pro-inflammatory cytokines such as IL-6 impairs neurogenesis [39, 46, 47]. Also, due to its atherothrombotic potential, IL-6 may induce vascular pathology that contributes to neuronal dysfunction [48]. On the other hand, tissue damage due to neurodegeneration in HD caused by other pathophysiological mechanisms may be a common cause of both elevated plasma cytokine levels and cognitive dysfunction and may thus be a confounder for the relationship we found.

We were not able to demonstrate associations between cytokines and the neuropsychiatric symptoms depression, apathy and irritability. This finding contrasts with previous studies in non-HD populations demonstrating associations of cytokine levels with several neuropsychiatric symptoms [15-23]. Depressive symptoms in relation to plasma cytokines have been extensively

investigated. In a meta-analysis of over 60 studies measuring depressive symptom severity, both IL-1 and IL-6 levels were increased in patients with a depressive disorder versus controls (Cohen's $d=0.35$ and $d=0.25$, respectively) [49, 50]. Another meta-analysis of 24 studies among patients with major depressive disorder (MDD) showed that TNF- α and IL-6 levels were significantly higher in 492 patients compared to 400 controls [51]. No significant differences for IL-1 β , IL-8, and IL-10 levels were found. Moreover, trials in cancer and hepatitis C patients also showed the increased risk of depression when treated with interferon (IFN)-gamma [15, 19]. Apathy and loss of interest have been studied less often in relation to inflammation; levels of IL-6 have been associated with these neuropsychiatric symptoms in 48 stroke patients [50]. The association between plasma cytokines and the hostility and anger seen in irritable subjects has been investigated both in healthy populations and patient groups [16-18, 21-23]. Observational studies showed that IL-1, IL-6, IL-8 and TNF- α were associated with higher levels of anger in populations of healthy couples [16], and healthy men [23] and women [22]. Intervention studies in hepatitis C patients showed increased anger and hostility upon treatment with IFN-gamma [17, 18]. However, these previous studies did not include HD patients, and our contrasting findings may be explained by disease specific factors of HD. Neurodegenerative changes in frontal-striatal circuits in HD may account for several neuropsychiatric symptoms, thereby obscuring the subtle associations with plasma cytokines, that tend to vary considerably over time.

The strengths of this study are the relatively large cohort of HD mutation carriers assessed at two time points, and the use of robust high sensitivity ELISA assays for the assessment of cytokine levels. Also some limitations warrant discussion. First, the average storage time of plasma samples was 3 years and it has been demonstrated that levels of particularly IL-6 may degrade significantly over longer storage times [52]. In addition, we did not ascertain time of withdrawal of our blood samples. As a consequence, this may have resulted in a measurement error, resulting in an underestimation of the association with cognitive dysfunction. We found however, that cytokine plasma levels were rather stable and that measurement error was therefore likely within acceptable limits. As a limited number of HD mutation carriers completed both the second and third measurement ($n=90$), we could not perform longitudinal analyses because of the low statistical power as relatively few HD patients had new-onset neuropsychiatric symptoms during the 2 years of follow-up. Therefore, given the cross-sectional nature of our study, we cannot infer causal relationships.

In conclusion, levels of IL-6 and IL-1 α were positively associated with executive cognitive dysfunction in our cross-sectional study among HD mutation carriers. A causal relation may exist between pro-inflammatory cytokines and executive cognitive dysfunction; however the association may also be confounded by the neurodegenerative process or by environmental factors. No associations were found between cytokine levels and other neuropsychiatric symptoms. Precise measurement of both cytokine levels as well as of neuropsychiatric symptoms is difficult and may introduce errors. Our results should therefore be interpreted with caution. Future studies should have a longitudinal design to assess possible causal or alternative relations and should be designed to further reduce measurement errors.

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CHAPTER 7

General discussion and summary

GENERAL DISCUSSION

In this thesis, we investigated the activity of the innate immune system in HD as reflected in several inflammatory immune parameters. Also, we aimed to gain further insight into the neuropsychiatric symptoms in HD, in particular irritability. In addition, we investigated the association between inflammatory immune parameters on the one hand and measures of disease progression (including neuropsychiatric symptoms and cognitive decline) on the other. In aging, and in neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, (neuro) inflammation is associated with the loss of normal functioning. Investigation of inflammatory immune parameters of inflammation in relation to markers of disease progression may provide further insight into the development of symptoms of HD. Also, inflammatory immune parameters that reflect disease severity in HD may be used as biomarkers in research and clinical care. In HD, such biomarkers are important because they may help to detect whether or not a potential therapeutic intervention is beneficial [1, 2]. We expected to find a correlation between increased levels of pro-inflammatory cytokines and positive acute-phase proteins on the one hand and markers of disease progression on the other; we also expected that, with progressing disease, levels of regulatory anti-inflammatory cytokines would decrease. Finally, we also expected to find an association between levels of immune parameters and neuropsychiatric symptoms.

INTEGRATION OF THE STUDY FINDINGS INTO CURRENT KNOWLEDGE

Irritability

We assessed the psychometric properties of the Irritability Scale (IS) and established that a cut-off point of 14 provides a valid and reliable assessment of irritability in HD gene expansion carriers.

Using the IS, in our HD population the prevalence of irritability in the past two weeks was 35%, whereas in other studies the prevalence ranged from 33-73% [3]. These differences in reported prevalence can probably be attributed to the wide range of instruments used to assess irritability, the different time periods of the studies, as well as the sociodemographic and clinical differences in the HD populations investigated. Currently, the IS the only symptom-specific scale that is recommended for the assessment of irritability in HD [4]. Although several scales are available that assess irritability as one of the behavioral domains (such as the UHDRS behavioral subscale), these subscales have either not been extensively validated[5] or no data are available on their reliability or other psychometric properties in HD [6-8].

In our study, the incidence of irritability was found to be 23% over a two-year period. The rate of remittance (for which we were unable to find any predictors) was comparable to the incidence of irritability over time. Increasing irritability was associated with increased apathy and the continuous use of antipsychotics. However, we found no correlation between irritability and measures of disease progression, such as cognitive decline, motor abnormalities and functional

incapacity. Also, at baseline, none of these parameters could predict the incidence of irritability over a two-year period. Earlier studies showed that irritability may be a characteristic neuropsychiatric symptom of pre-manifest and early HD[9-11]. This is in line with findings of more recent imaging studies that demonstrated functional changes in the brain of gene expansion carriers, specifically for the early stages of HD, that correlated with irritability[12, 13]. Possible explanations for the development of irritability include overcompensation of damaged circuits early in the disease[13] and cognitive overload caused by early cognitive decline[12]. Also, the construct of irritability as assimilated in the IS may lose validity in later stages of HD because of the interference of severe motor dysfunction, cognitive decline and neuropsychiatric symptoms.

Immune parameters

We found that activation of the acute-phase response, as reflected in levels of both positive and negative acute-phase proteins, was associated with impaired daily functioning, apathy and cognitive impairment. However, these associations were confounded by the use of antipsychotics. Gene expansion carriers that continuously used antipsychotics had higher levels of hsCRP and lower levels of albumin than gene expansion carriers who did not use antipsychotics during the study period. In other HD cohorts, CRP levels were significantly increased in advanced disease stages when compared to healthy controls; to our knowledge, in these cohorts, no adjustment was made for the use of antipsychotics.

Levels of TNF- α and IL-10 increased over the two-year study period. Pre-motor symptomatic gene expansion carriers had lower levels of IL-5 and IL-6 than symptomatic gene expansion carriers. IL-6 and IL-8 were inversely associated with daily functioning. Also, increased levels of both IL-1ra and IL-6 were independently associated with impaired cognitive functioning. Previous cross-sectional studies with a limited number of mutation carriers in disease stages from premanifest to moderately advanced disease stage, presented evidence for increased immune activation in the plasma of HD mutation carriers[14-18]. Most consistently, levels of IL-6 were higher in motor symptomatic HD mutation carriers than in matched controls, whereas increased plasma levels of sIL-2R, sTNF- α R[18], IL-8, and TNF- α [14] were also found. The study that assessed IL-4, IL-5, and IL-10, found positive correlations with a more advanced disease stage, although levels of IL-4 and IL-5 were increased only in a moderately advanced disease stage, compared with controls[14].

The triggering of innate immune activation is also emerging in other neurodegenerative diseases[19]. However, the relationship between immune activation and disease progression is complex. In Alzheimer's disease, inflammatory pathways are activated as reflected by increased levels of chemokines and cytokines in both the brain and cerebrospinal fluid of patients, and neuroinflammation has been demonstrated by PET scans[20]. On the other hand, inflammatory processes are also a risk factor for Alzheimer's disease. Studies on patients with Alzheimer's disease have demonstrated the negative effects of severe infection[21], sepsis[22] and periodontitis[23] on cognition.

By chance, we found evidence for activation of the acute-phase response in gene-expansion carriers who used antipsychotics. This was found when adjusting for the use of antipsychotics in the relationship between acute-phase proteins on the one hand, and daily functioning, cognitive impairment and apathy on the other. After this adjustment, effect sizes for these relations were considerably reduced and no longer significant. Furthermore, the group of gene-expansion carriers that continuously used antipsychotics had higher levels of CRP and lower levels of albumin than those who did not use antipsychotics. To our knowledge, this phenomenon has not earlier been described.

Antipsychotics are frequently prescribed in late-stage HD for the symptomatic treatment of movement disorder. Therefore, the idea that increased acute-phase response activity is at least partly due to the use of antipsychotics is in line with a study reporting that premanifest and early-HD gene-expansion carriers had similar lower CRP levels compared to controls[24]. In non-HD populations, two pathways for activation of the acute-phase response by antipsychotics have been described. First, low-grade inflammation may be the result of obesity[25], dyslipidemia[26] and insulin resistance[27] which are elements of the metabolic syndrome, a well-known side-effect of second-generation antipsychotics. Second, both classical antipsychotics[28] and second-generation antipsychotics[29] may directly induce low-grade inflammation through their hepatic impact.

Strengths and limitations

The strengths of this study are the cohort study design and the well-characterized study population with HD. Furthermore, we included a control group consisting of first-degree relatives without the HD gene expansion which had been exposed to a similar environment as the gene expansion carriers. Also, the group of gene-expansion carriers was heterogeneous, comprising a range of pre-manifest individuals many years from clinical onset to institutionalized patients with end-stage disease. In addition, the same individuals were interviewed up to three times, with a two-year interval in between. Specifically, we used valid and reliable measurement tools in a standardized setting. Dropout rates between measurements were moderate and did not exceed 20%. Cytokine levels were also measured in a standardized and state-of-the-art manner. Where possible, we used high-sensitivity enzyme-linked-immuno-sorbent assays (ELISA); due to their conceptual design, ELISAs are less prone to altered levels of circulating proteins and inhibitors than multiplexed immunoassays[30]. Also, the number of freeze-thaw cycles was limited to one (for IL1-b, IL-6, IL-10 and TNF- α) or two (for IL-1ra, IL-5 and IL-8). Third, the methodological design eliminated any plate-to-plate and/or lot-to-lot variation and/or operator variability, as paired samples from HD gene expansion carriers were measured simultaneously on the same plate for the longitudinal analyses. Fourth, significant partial correlations were found for all cytokines between paired HD samples, ranging from 0.483 to 0.728 ($p < 0.001$).

Some limitations also need to be addressed. An important drawback is the limited validity of the measurement of behavioral symptoms in HD. Validated systems, such as the Diagnostic

and Statistical Manual of Mental Disorders (DSM), are used for the classification of psychiatric disorders. However, their usefulness in HD is limited because they were not designed to assess symptoms and do not assess the severity of symptoms in dimensional scales. Next, cytokine levels in the plasma may not accurately reflect the highly complex state of the immune system. Although blood and its subsequent handling was done carefully, we were not able to take into account the effect of diurnal and seasonal changes in cytokine levels, which can be significant[31]. Another drawback is that we could not adjust for all possible confounding variables. Medication is frequently prescribed for movement disorder and behavioral symptoms; these medications include antipsychotics and benzodiazepines, which are known to have a wide range of biological adverse effects. Given the observational nature of our study, we could not determine whether the associations found were explained by causative mechanisms, confounding, or confounding by indication. Given the low incidence of HD, collecting a sufficiently large sample of HD gene expansion carriers remains a challenge. In particular, for analyses within subgroups with a sizable number of covariates, sample sizes were too low to reach adequate statistical power; because this may have caused type-I and type-II errors, our findings need to be replicated in independent samples of HD gene-expansion carriers.

Implications for clinical practice

No specific treatment for irritability in HD is available. Current treatment guidelines are largely based on case series and expert opinion, and on findings from studies in patients with psychiatric or other neurodegenerative disorders[32-34]. Our study showed that, in a significant number of patients, irritability may remit over a two-year study period; however, we were unable to predict which patients had the highest chance to remit from irritability. Nonetheless, we found that the use of benzodiazepines and antipsychotics was associated with the prevalence and incidence of irritability, respectively. Both types of drugs can be used in the treatment of irritability; however, especially in patients who receive these drugs for other indications, clinicians should be aware that irritability might be a possible side-effect.

Although evidence for increased levels of (low-grade) systemic inflammation was found in our population, the cause of this systemic inflammation could not be identified; the inflammatory state most likely reflects a complex system of mechanisms. In other populations, the effects of systemic inflammation on mood[35-37] and cognition[38-40] are detrimental. Studies have also indicated negative effects on metabolic disease and cardiovascular health[41-43]. Several studies have investigated the positive effects of anti-inflammatory drugs in HD. However, until now, agents like minocycline[44, 45], cannabinoids[46, 47] and non-steroidal anti-inflammatory drugs[48, 49] have not proven effective in humans, although some studies may have been underpowered. Further studies are needed to assess whether a more specific tweaking of neuroinflammation may benefit the many ailments of HD gene expansion carriers. In HD, treatable causes of systemic inflammation, such as infectious processes, should be managed properly. Also, iatrogenic damage, caused by the possible adverse effects of prescribed drugs, should be prevented.

Although our findings offer additional insight into the role of the innate immune system in HD, they also indicate that the rather crude immune parameters that we investigated were not of clinical use as biomarkers for the individual HD gene expansion carrier. It is important that a biomarker is measurable in a cost-effective manner, changes with the disease state over time, and is able to discriminate between gene expansion carriers in different disease states.

Directions for future research

The studies in this thesis confirm that inflammatory processes play an important role in HD. Immune parameters, indicating activation of the innate immune system, correlated with neuropsychiatric and motor symptoms, as well as with functional decline. However, given the observational nature of our investigation, we cannot infer any causal relations. Most likely, the immune system plays both a pathogenic and homeostatic role; however, more longitudinal studies are required to unravel its precise function in HD. Importantly, our findings also demonstrated that iatrogenic factors, such as the use of (psychotropic) medication, were related to both immune system parameters and outcome measures and, thus, need to be taken into account.

Although we have provided a thorough assessment of the psychometric properties of the IS, these findings still need to be replicated in larger studies. The validity and comparability of the scale, and its sum score in different stages of the disease, should be studied further using more sophisticated analysis techniques that include confirmatory factor analysis to assess measurement invariance. This might help establish whether factor loadings, intercepts and residual variances are equivalent in a factor model that measures a latent variable irritability. If so, comparisons of irritability scores will be valid across disease stages and across time within a HD gene expansion carrier.

Our findings concerning the associations between immune system parameters on the one hand and neuropsychiatric symptoms and disease progression on the other, yielded far more modest effect sizes than other studies of a comparable size. Therefore, larger studies are needed to replicate these findings. Moreover, these studies should be able to adequately assess cytokine levels, taking into account possible confounding factors such as diurnal and seasonal variations in the levels of cytokines and the use of medication.

In observational studies, no firm conclusions regarding causality can be drawn. In our study, the confounding effect of the use of antipsychotics on immune system parameters and neuropsychiatric symptoms, warrants investigating whether this is explained by confounding by indication or by a direct effect of these drugs. Therefore, the results of previous studies on antipsychotics cannot be generalized. The effectiveness of antipsychotics should be assessed critically in well-designed and sufficiently powered studies and be weighed against the possible negative consequences, preferably in randomized controlled trials (RCTs) in a HD population. In addition to evaluation of the safety of antipsychotics, RCTs can shed more light on the treatment of various neuropsychiatric symptoms which, so far, relies mostly on case reports and expert opinion.

SUMMARY

In a cohort of HD gene expansion carriers, we assessed neuropsychiatric symptoms and several inflammatory immune system parameters. The aim was to investigate neuropsychiatric symptoms, in particular irritability, in more detail and gain further insight into the activation of the innate immune system in HD, as well as its role in disease progression.

The irritability scale (IS) was used to assess irritability. This scale proved to be a reliable and valid instrument for the assessment of irritability in HD. Using a cut-off point of 14, the prevalence of irritability was 35% in our population of HD gene expansion carriers. Gene expansion carriers tended to underestimate their level of irritability when compared to reports of their caregivers or spouses. Being married/living together and a longer CAG expansion in the Htt-gene were independent correlates of self-reported irritability. The incidence of new-onset irritability over a two-year study period was 23% and coincided with an increase of apathy; during this period, irritability remitted in 30% of previously irritable gene expansion carriers.

Levels of the pro-inflammatory cytokines TNF- α and IL-10 increased over the two-year study period. Also, pre-motor symptomatic gene expansion carriers had lower levels of IL-5 and IL-6 than motor symptomatic gene expansion carriers. Additionally, levels of IL-6 and IL-8 were inversely associated with the level of daily functioning.

Activation of the acute-phase response, as reflected by higher levels of CRP and lower levels of albumin, was associated with impaired daily functioning, apathy and cognitive impairment. However, these associations were confounded by the use of antipsychotics.

A weak association was found between increased levels of both IL-1ra and IL-6 on the one hand and cognitive decline on the other. No associations were found between cytokine levels and apathy, irritability and depressive mood.

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Nederlandse samenvatting

In dit proefschrift worden neuropsychiatrische symptomen bij de ziekte van Huntington (ZvH) beschreven. In het bijzonder wordt stilgestaan bij het vóórkomen en het beloop van prikkelbaarheid. Tevens wordt de rol van het immuunsysteem onderzocht. Hierbij wordt nagegaan of er toegenomen activiteit van met name het pro-inflammatoire gedeelte van het niet-adaptieve immuunsysteem optreedt en of deze activiteit van invloed is op het optreden van neuropsychiatrische symptomen.

Prikkelbaarheid is een tot nu toe niet-gevalideerd construct bij de ziekte van Huntington. In **Hoofdstuk 2** worden de psychometrische eigenschappen van de prikkelbaarheidsschaal (PS) beschreven en wordt aangetoond dat de PS een betrouwbaar en valide meetinstrument is voor het vaststellen van prikkelbaar. Een afkapwaarde van 14 punten op deze schaal blijkt hiervoor het meest betrouwbaar. Gebruik makend van die afkapwaarde wordt de prevalentie van irritabiliteit in ons cohort geschat op 35%. Genexpansiedragers onderschatten de mate van hun eigen prikkelbaarheid als deze wordt vergeleken met de rapportage van zorgverleners of partners. Getrouwd zijn en samenwonen, alsmede drager zijn van een langere genexpansie, zijn onafhankelijke correlaten van toegenomen prikkelbaarheid. In **Hoofdstuk 3** wordt vastgesteld dat de incidentie van prikkelbaarheid gedurende 2 jaar 23% is en samengaat met een toename van apathie. In diezelfde periode is er sprake van een remissie van 30% van bij baseline vastgestelde prikkelbaarheid.

In **Hoofdstuk 4** wordt de relatie onderzocht tussen de acuut-fase eiwitten C-reactief proteïne (CRP) en albumine enerzijds, en het voorkomen van neuropsychiatrische symptomen anderzijds. Activatie van de acuut-fase respons blijkt geassocieerd met verminderd algemeen functioneren, toegenomen apathie en meer cognitieve achteruitgang. Ook blijkt dat het gebruik van antipsychotica dit verband geheel verklaart en dit gebruik dus mogelijk een oorzaak van beide is.

In **Hoofdstuk 5** worden de spiegels van zowel pro-inflammatoire als anti-inflammatoire cytokinen onderzocht in niet-motor symptomatische genexpansiedragers vergeleken met symptomatische genexpansiedragers. De hoogte van deze spiegels wordt gemeten op baseline, en bij follow-up 2 jaar later. Daarbij blijkt dat de cytokinen TNF- α en IL-10 gedurende deze periode toenemen. Niet-motor symptomatische genexpansiedragers hebben lagere spiegels van IL-5 en IL-6 dan symptomatische genexpansiedragers. Ook zijn hogere spiegels van IL-6 en IL-8 geassocieerd met verminderd algemeen functioneren. Hogere spiegels van IL-1ra en IL-6 zijn geassocieerd met cognitieve achteruitgang zoals beschreven in **Hoofdstuk 6**.

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Rotterdam, mei 2018

Curriculum vitae

Jos Bouwens werd op 15 oktober 1985 in Rotterdam geboren. Zijn middelbare school volgde hij aan het R.K. Alfrink college in Zoetermeer. Na het behalen van het VWO diploma in 2003, begon hij met zijn studie geneeskunde in Leiden.

Tijdens zijn studie volbracht hij zijn semi-arts stage en zijn wetenschapsstage op de afdeling psychiatrie van het Leids Universitair Medisch Centrum (LUMC). Gedurende deze wetenschapsstage verzamelde hij gegevens waarop dit proefschrift is gebaseerd en schreef hij mee aan een destijds gepubliceerd artikel. Zijn artsensbul behaalde hij in december 2009.

In januari 2010 begon hij als arts-assistent op de high care van de GGZ Haagstreek te Leidschendam. In oktober van dat jaar begon hij met zijn opleiding tot psychiater in het LUMC. Gedurende zijn opleiding werkte hij in het LUMC, op verschillende afdelingen van GGZ Rivierduinen en bij de Brijder verslavingszorg. Tijdens en naast zijn opleiding heeft hij ook de gegevens verzameld voor zijn proefschriftonderzoek en waarop de inmiddels gepubliceerde artikelen zijn gebaseerd.

Sinds 2015 is hij geregistreerd als psychiater. Hij werkte sindsdien in het Erasmus Medisch Centrum, binnen de verslavingszorg en op dit moment binnen de forensische psychiatrische zorg.

Publicaties

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