Suicidality in Huntington's disease

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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, characterised by motor abnormalities, cognitive dysfunction, and neuropsychiatric symptoms[1] 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 CRP[18-20] and albumin[21-24] 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 specialised 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 ºC 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 standardised method (Cat. no. 04628918190) on a COBAS INTEGRA 800 analyser 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 standardised method (Cat. no. 11970909216) on Modular P systems from Roche Diagnostics, using a BCG-based colorimetric assay with endpoint detection. This method has been standardised 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 standardised units of alcohol a week), body weight and height, and medication use, was collected using a standardised 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 through 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 through 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 through 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 behavioural 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 PBA[38] 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 characterise 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 through 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 standardised 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 (± 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

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

	Mutation carriers (n = 122)	**Controls** (n = 42)	**p-value**[a]
Sociodemographic characteristics			
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
Biological characteristics			
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
Clinical characteristics			
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
Neuropsychiatric characteristics			
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

Table continued on the next page.

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

	Mutation carriers (n = 122)	**Controls** (n = 42)	**p-value**[a]
Cognitive characteristics			
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.

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).

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**
Clinical characteristics						
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
Neuropsychiatric characteristics						
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
Cognitive characteristics						
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 Colour 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 standardised 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. Explanations of abbreviations and symbols see legend table 3.

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**
Clinical characteristics						
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
Neuropsychiatric characteristics						
PBA suicidality	1.05	0.83*	0.92	0.83	0.80	0.86
Cognitive characteristics						
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; 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. MMSE, Mini Mental State Examination.
*$p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
[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.

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).

Associations between acute-phase proteins and clinical characteristics at t2
When analysing 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).

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.

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.

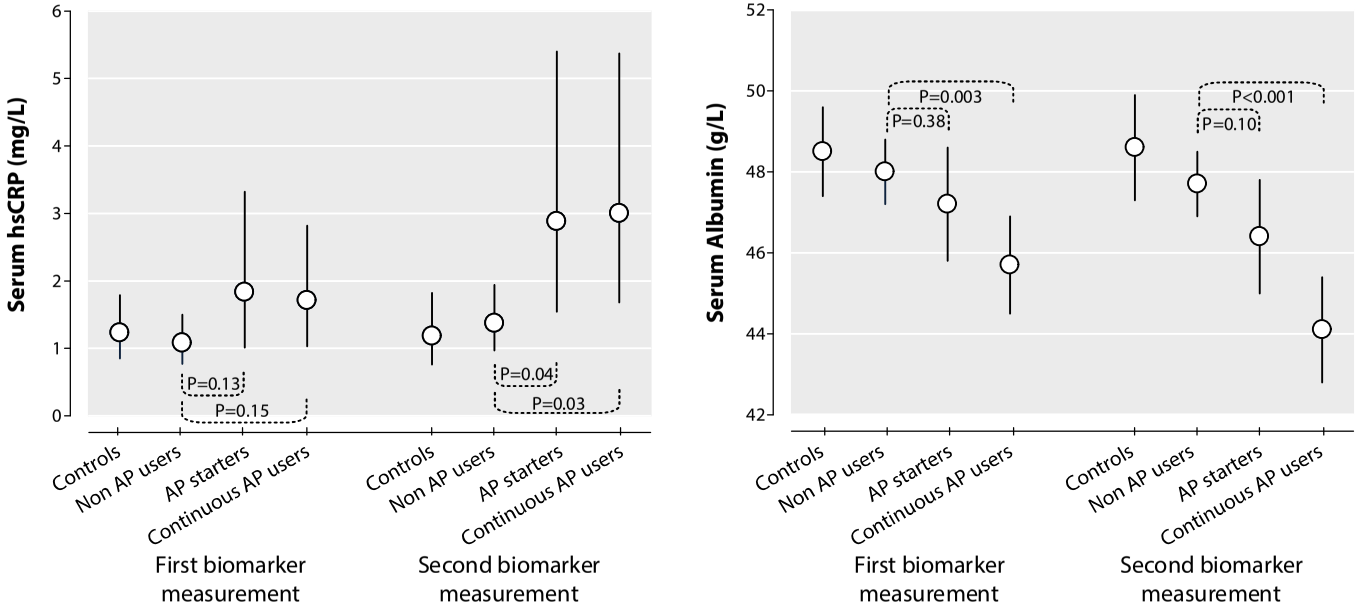

Figure 1.
High sensitivity C-reactive protein (hsCRP) and albumin levels in controls and mutation carriers. For hsCRP levels the adjusted, backtransformed geometric mean values and for albumin the adjusted mean values are presented. Error bars represent the 95% confidence intervals. Mutation carriers were categorised 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 analysed using analyses of covariance (ANCOVA) adjusting for sex, age, body mass index, smoking, and alcohol use at first biomarker measurement.

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; ß = 0.42), atypical antipsychotics (n = 26; ß = 0.25), and tiapride (n = 28; ß = 0.30). Likewise, inverse associations were found with albumin: typical antipsychotics (n = 32; ß = -0.47), atypical antipsychotic (n = 26; ß = -0.53), and tiapride (n = 28; ß = -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 characterised by abdominal obesity, dyslipidaemia, hypertension, and insulin resistance. Obesity,[45] dyslipidaemia,[31] and insulin resistance[46] 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 randomised 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 widespread[48] 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 apathy[25] 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 hypothesise 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 standardised 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 generalised 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[a]	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.
[a] 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 AP (n = 51; reference)	**Mutation carriers who started AP** (n = 15)	**p-value**	**Mutation carriers who continuously used AP** (n = 23)	**p-value**
High sensitivity C-reactive protein (mg/L)[a]					
First biomarker measurement					
- 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
Second biomarker measurement					
- 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)					
First biomarker measurement					
- 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
Second biomarker measurement					
- 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

AP denotes antipsychotics. 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.

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