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

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Chapter 6

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-1β, 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 (β =–0.114; p=0.01) and between IL-1ra and ExCog score (β=–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)-αR, 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 $\geq 36$ 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](image_url)
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 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, TNFa and IL1β were measured with Quantikine® hs Human Immunoassay kits from R&D Systems. These hs ELISA's for IL1b, IL6, IL10 and TNFa 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 TNFa 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 (± 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-1β, 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-1β 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

<table>
<thead>
<tr>
<th></th>
<th>t1</th>
<th>t2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=124</td>
<td>n=90</td>
<td></td>
</tr>
</tbody>
</table>

#### Demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>t1 ± SD</th>
<th>t2 ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>49.3 ± 11.7</td>
<td>52.6 ± 11.5</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>69 (56%)</td>
<td>53 (59%)</td>
</tr>
<tr>
<td>BMI</td>
<td>25.6 ± 5.1</td>
<td>25.9 ± 5.7</td>
</tr>
<tr>
<td>Smoking</td>
<td>31 (25%)</td>
<td>25 (28%)</td>
</tr>
</tbody>
</table>

#### Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>t1 ± SD</th>
<th>t2 ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAG repeats</td>
<td>44.1 ± 3.2</td>
<td>43.9 ± 3.0</td>
</tr>
<tr>
<td>UHDRS-m score</td>
<td>30.2 ± 28.6</td>
<td>35.6 ± 31.3</td>
</tr>
<tr>
<td>Motor symptomatic n</td>
<td>88 (71%)</td>
<td>63 (70%)</td>
</tr>
<tr>
<td>TFC score</td>
<td>7.7 ± 4.9</td>
<td>7.6 ± 4.7</td>
</tr>
<tr>
<td>Late stage</td>
<td>26 (21%)</td>
<td>17 (19%)</td>
</tr>
<tr>
<td>Use of antipsychotics</td>
<td>28 (23%)</td>
<td>31 (34%)</td>
</tr>
</tbody>
</table>

#### Neuropsychiatric characteristics

<table>
<thead>
<tr>
<th></th>
<th>t1 ± SD</th>
<th>t2 ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBA depression score</td>
<td>9.7 ± 14.3</td>
<td>9.9 ± 13.0</td>
</tr>
<tr>
<td>Apathy scale score</td>
<td>8.6 ± 8.7</td>
<td>12.1 ± 9.3</td>
</tr>
<tr>
<td>Irritability scale score</td>
<td>10.2 ± 8.3</td>
<td>11.7 ± 7.5</td>
</tr>
<tr>
<td>MMSE score</td>
<td>26.2 ± 4.7</td>
<td>25.3 ± 5.7</td>
</tr>
<tr>
<td>ExCog score</td>
<td>0.03 ± 0.94</td>
<td>-0.04 ± 1.08</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>PBA depression subscale</th>
<th>Apathy Scale</th>
<th>Irritability Scale</th>
<th>MMSE</th>
<th>ExCog</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β P-value</td>
<td>β P-value</td>
<td>β P-value</td>
<td>β P-value</td>
<td>β P-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>crude</td>
<td>0.055 0.47</td>
<td>0.093 0.02</td>
<td>0.062 0.42</td>
<td>-0.145 0.03</td>
</tr>
<tr>
<td></td>
<td>adjusted</td>
<td>0.012 0.87</td>
<td>0.005 0.94</td>
<td>0.041 0.61</td>
<td>-0.047 0.45</td>
</tr>
<tr>
<td>IL-6</td>
<td>crude</td>
<td>-0.031 0.71</td>
<td>0.196 0.01</td>
<td>0.090 0.23</td>
<td>-0.213 0.00</td>
</tr>
<tr>
<td></td>
<td>adjusted</td>
<td>-0.057 0.49</td>
<td>0.110 0.15</td>
<td>0.105 0.21</td>
<td>-0.114 0.08</td>
</tr>
<tr>
<td>IL-8</td>
<td>crude</td>
<td>0.108 0.19</td>
<td>0.167 0.03</td>
<td>0.132 0.08</td>
<td>-0.112 0.11</td>
</tr>
<tr>
<td></td>
<td>adjusted</td>
<td>0.087 0.25</td>
<td>0.116 0.10</td>
<td>0.124 0.11</td>
<td>-0.079 0.19</td>
</tr>
<tr>
<td>TNF-α</td>
<td>crude</td>
<td>-0.039 0.65</td>
<td>-0.041 0.60</td>
<td>-0.055 0.47</td>
<td>-0.048 0.52</td>
</tr>
<tr>
<td></td>
<td>adjusted</td>
<td>-0.015 0.85</td>
<td>-0.062 0.39</td>
<td>-0.079 0.31</td>
<td>-0.074 0.27</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>crude</td>
<td>0.045 0.59</td>
<td>0.106 0.16</td>
<td>0.127 0.09</td>
<td>-0.145 0.05</td>
</tr>
<tr>
<td></td>
<td>adjusted</td>
<td>0.109 0.24</td>
<td>0.087 0.31</td>
<td>0.180 0.06</td>
<td>-0.036 0.65</td>
</tr>
<tr>
<td>IL-5</td>
<td>crude</td>
<td>0.029 0.73</td>
<td>-0.006 0.94</td>
<td>0.146 0.06</td>
<td>0.033 0.66</td>
</tr>
<tr>
<td></td>
<td>adjusted</td>
<td>0.008 0.92</td>
<td>-0.044 0.53</td>
<td>0.130 0.09</td>
<td>0.055 0.41</td>
</tr>
<tr>
<td>IL-10</td>
<td>crude</td>
<td>-0.011 0.88</td>
<td>-0.045 0.53</td>
<td>0.039 0.58</td>
<td>0.062 0.34</td>
</tr>
<tr>
<td></td>
<td>adjusted</td>
<td>0.016 0.83</td>
<td>-0.043 0.52</td>
<td>0.028 0.97</td>
<td>0.038 0.51</td>
</tr>
</tbody>
</table>

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.
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-1beta [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 though 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] an 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-analyses 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-1β, 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 studied 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-1ra 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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REFERENCES


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