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Plasma inflammatory biomarkers for Huntington's disease patients and mouse model ☆

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ABSTRACT

Huntington's disease (HD), caused by expanded CAG repeats encoding a polyglutamine tract in the huntingtin (HTT) protein, presents with a predominant degeneration of neurons in the striatum and cortex. Lines of evidence have observed neuroinflammation, particularly microglial activation, is involved in the pathogenesis of HD. Given that HTT is also expressed in peripheral inflammatory cells, it is possible that inflammatory changes detected in peripheral plasma may be biologically relevant and parallel the neuroinflammatory process of HD patients. By examining the expression levels of 13 microglia-derived inflammatory markers in the plasma of 5 PreHD carriers, 15 HD patients and 16 healthy controls, we found plasma levels of IL-6, MMP-9, VEGF and TGF-β1 were significantly increased in HD patients when compared with the controls, while plasma level of IL-18 were significantly reduced in HD patients compared with controls. Plasma level of IL-6 was reversely correlated with the UHDRS independence scale and functional capacity. To understand the temporal correlation between these inflammatory markers and HD progression, their levels were further tested in plasma from R6/2 mouse HD model at different ages. In rotarod test, R6/2 HD mice started to manifest HD phenotype at 7.5 weeks of age. Higher plasma VEGF levels of R6/2 mice than those of age-matched wild-type (WT) littermates were noted from 7 (presymptomatic stage) to 13 weeks of age (late symptomatic stage). The plasma IL-6 levels of R6/2 mice were higher than those of the WT littermates from 9 (early symptomatic stage) to 13 weeks of age. R6/2 mice demonstrated higher MMP-9 and TGF-β1 levels than their WT littermates from 11 (middle symptomatic stage) to 13 weeks of age. In contrast, the plasma IL-18 level was lower than those in WT littermates since 11 weeks of age. These altered expressions of inflammatory markers may serve as the potential biomarkers for HD onset and progression. Specific inhibition/activation of these inflammatory markers may be the targets of HD drug development.

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1. Introduction

Huntington's disease (HD) is an autosomal-dominant, progressive neurodegenerative disorder, caused by an unstable CAG trinucleotide repeat expansion encoding a polyglutamine tract in the huntingtin (HTT) protein (MacDonald et al., 1993). The polyglutamine expansion causes a conformational change in the HTT which forms aggregates in both the nucleus and/or cytoplasm of affected neurons and leads to deleterious neuronal functions (Di Prospero and Fischbeck, 2005). Impaired proteasome activity (Valera et al., 2005), transcriptional dysregulation (Cha, 2007), oxidative stress (Stack et al., 2008), mitochondrial and metabolic dysfunction (Browne, 2008), abnormal protein–protein interaction (Giorgini and Muchowski, 2005), neuroinflammation (Bjorkqvist et al., 2008; Dalrymple et al., 2007; Hsiao et al., 2013, 2014; Moller, 2010), and microglial activation (Hsiao and Chern, 2010; Pavese et al., 2006; Sapp et al., 2001; Shin et al., 2005; Tai et al., 2007) have been shown to play important roles in the pathogenesis of HD.

Lines of evidence have observed the activation of microglia during the asymptomatic stage and its correlation with disease severity in HD patients (Pavese et al., 2006; Sapp et al., 2001; Tai et al., 2007). Positron emission tomography (PET) has shown early and significant microglial activation in HD patients (Pavese et al., 2006) and presymptomatic HD gene carriers (Tai et al., 2007). Increased microglia-secreted inflammatory mediators, such as IL-6, IL-8, IL-10, matrix metallopeptidase 9 (MMP-9), and chemokine C—C motif ligand 2 (CCL2) mRNA, have been demonstrated in the brain tissue of post-mortem HD patients (Silvestroni et al., 2009). Increased levels of IL-4, IL-6, IL-8, and TNF-α have been

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detectable in plasma and cerebrospinal fluid (CSF) of HD patients (Bjorkqvist et al., 2008). Plasma levels of the chemokines eotaxin-3, macrophage inflammatory protein-1β (MIP-1β), eotaxin, monocyte chemotactic protein-1 (MCP-1) and MCP-4 are significantly elevated in HD compared with controls (Wild et al., 2011). Intranuclear aggregates have been shown in microglials in the striatum of R6/2 mice, which leads to microglial activation and subsequent inflammatory factors secretion and neuronal damage (Shin et al., 2005). Altered microglial morphology was also found recently in the YAC128 mouse model of HD (Franciosi et al., 2012). Thus activated microglia could critically regulate processes of neuronal death and survival by secreting glutamate, neurotrophic factors, and pro- and anti-inflammatory cytokines. An imbalance between neurotoxic and neuroprotective factors may ultimately be responsible for neuronal dysfunction and cell death for HD.

Although the pathology of HD is mainly in the striatum, a few studies have identified substantial biochemical deficits in peripheral tissues (Chang et al., 2012; Chen et al., 2007; Dalrymple et al., 2007; Leoni et al., 2008; Maglione et al., 2005; Nagata et al., 2004; Sawa et al., 1999; Underwood et al., 2006). Given that neuroinflammation plays a role in the pathogenesis of HD and it is practically difficult to obtain brain tissues from HD patients, we aimed to identify potential peripheral inflammatory changes by comparing the plasma levels of a panel of microglia-derived inflammatory markers between HD patients and age/gender-matched control subjects. The panel of microglia-derived inflammatory markers excluded inflammatory markers, alterations of which in HD have been previously reported in literature. In addition, we also measured plasma IL-6 that has been shown to be increased in HD patients to examine if similar result could be seen in our patients (Bjorkqvist et al., 2008). Although it is important to understand the temporal relation between alterations of these markers and the development of HD phenotypes, the limited number of HD patients and lengthy disease course make it hard to clarify this important issue in human samples. The R6/2 mouse model of HD (Mangiarini et al., 1996) carries the mutation in a fragment of the human HTT gene has been widely used to investigate the disease pathogenesis and test potential therapeutic strategies for HD (Li et al., 2005; Morton and Morton, 2013), which addresses importance of finding biomarkers of this model to test the efficacy of potential treatments in preclinical studies. Therefore, we checked the levels of these inflammatory markers in plasma from R6/2 HD mouse model at different ages in order to know if alterations of inflammatory markers can be recapitulated in presymptomatic, early, and late disease stage and if they can also be served as useful biomarkers for R6/2 mice. Our findings successfully demonstrated the potentials of these inflammatory markers as indicators for disease progression in HD patients and also in HD mice.

2. Materials and methods

2.1. Ethics statement

This study was performed under a protocol approved by the Institutional Review Boards of Chang Gung Memorial Hospital and all examinations were performed after obtaining written informed consents.

2.2. Study population and sample collection

Twenty subjects with HD, including 5 pre-symptomatic HD gene (preHD) carriers and 15 symptomatic HD patients, and 16 healthy controls were recruited in this study. Each group displayed similar gender distribution, age, body weight, body mass index (BMI) and preprandial blood sugar. Unified Huntington's Disease Rating Scale (UHDRS) were recorded for each patient (Huntington Study Group, 1996). The scale ranges (normal to most severe) of UHDRS include total motor score (0–124), independence score (100–10), and total functional capacity (13–0). None of the patients or the controls had systemic infection, autoimmune diseases, malignancies, or chronic renal, cardiac, or liver dysfunction. Plasma samples were collected from HD patients, PreHD carriers, and the controls after obtaining informed consent.

2.3. Transgenic HD mice

R6/2 mice (Mangiarini et al., 1996) and littermate controls were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and mated to female control mice (B6CBAFI/J). Offspring was identified by genotyping of tail DNA. PCR genotyping was performed using the following primers: 5′-CCG CTC AGG TTC TGC TTT TA-3′ and 5′-GGC TGA GGA AGC TGA GGA G-3′. All animals were housed at the Chang Gung Memorial Hospital, Animal Care Facility and had unlimited access to water and breeding chow (PicoLab® Rodent Diet 20, PMI® Nutrition International, St. Louis) under a 12-h light–12-h dark cycle. Body weights and blood sugar of mice were recorded once weekly. Animal experiments were performed under protocols approved by the Chang Gung Memorial Hospital, Animal Care and Utilization Committee, Taiwan. Fifteen R6/2 mice and 15 wild-type controls were used in this study.

2.4. Rotarod performance

Motor coordination of mice was assessed using a rotarod apparatus (UGO BASILE, Comerio, VA, Italy) at an accelerated speed (3–30 rpm) over a period of 6 min. The animals were pretrained for one trial at an accelerated speed (3–30 rpm) for 5 min, 2 days before the real test to allow them to become acquainted with the rotarod apparatus. Each mouse was tested for a maximum of 6 min per trial for 3 trials with an interval of 30 min between trials in a day and mean of the 3 trials was used for comparison between groups. Latency to falling was automatically recorded. The mean performance of three trials for each animal was used for the analysis.

2.5. Enzyme-linked immunosorbent assays for quantification of targeted inflammatory markers

Plasma levels of inflammatory markers, including IL-6 (R&D), IL-16 (R&D), IL-18 (R&D), MMP-3 (R&D), MMP-9 (R&D), MMP-10 (USCN Life Science), TIMP-2 (USCN Life Science), VEGF (R&D), TGF-β1 (R&D), MIP-1α (R&D), MIP-3β (USCN Life Science), VCAM-1 (R&D), and ICAM-1 (R&D), were assessed using enzyme-linked immunosorbent assay (ELISA) Kits. Each assay was performed according to the manufacturer's instruction. For each set of values, data were expressed as means ± standard error (SE). Differences between groups were evaluated by analysis of covariance (ANCOVA, adjusted for age, gender, use of dopamine antagonist, selective serotonin reuptake inhibitors (SSRI) and amantadine) with *post hoc* Bonferroni test. Correlations of UHDRS (motor scale, independence scale and functional capacity), size of expanded CAG or disease duration with plasma level of targeted markers were analyzed by Spearman correlation. All *P*-values were two-tailed. The values of $P < 0.05$ were considered significant.

3. Results

3.1. Determination of potential inflammatory cytokines in the plasma from HD patients

Microglial activation plays an important role in the neurodegeneration of HD. By examining 13 microglia-derived inflammatory

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