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Association of CRP genetic variation with symptomatology, cognitive function, and circulating proinflammatory markers in civilian women with PTSD

（PTSD 女性患者における CRP 遺伝的多型と症候学、認知機能、末梢血炎症マーカーとの関連）

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Association of *CRP* genetic variation with symptomatology, cognitive function, and circulating proinflammatory markers in civilian women with PTSD

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Abstract

Background: Posttraumatic stress disorder (PTSD) has been associated with increased inflammation. C-reactive protein (CRP) is a marker of systemic inflammation, and recently, single nucleotide polymorphisms (SNPs) in the *CRP* gene have been associated with increased blood CRP protein levels and illness severity in PTSD patients. However, the mechanism by which the *CRP* SNPs are involved in PTSD remains unclear. Here we investigated the association of *CRP* genetic variation with blood proinflammatory protein levels, symptomatology, and cognitive function, and further explored the moderating effect of childhood maltreatment history, in adult patients with PTSD.

Methods: Fifty-seven Japanese civilian women with PTSD and 73 healthy control women were enrolled. Three SNPs in the *CRP* gene, rs2794520, rs1130864, and rs3093059, were genotyped, and analyses focused on rs2794520 (T/C). Serum levels of high-sensitivity CRP (hsCRP), high-sensitivity tumor necrosis factor-α (hsTNF-α), and interleukin-6 were measured. PTSD symptoms were evaluated by the Posttraumatic Diagnostic Scale. Cognitive function was assessed by the Repeatable Battery for the Assessment of Neuropsychological Status. Childhood maltreatment history was assessed by the Childhood Trauma Questionnaire.

Results: Patients with the rs2794520 CC/CT genotype, compared to those with TT genotype, showed significantly higher levels of hsCRP ($p=0.009$) and hsTNF-α ($p=0.001$), more severe PTSD symptoms ($p=0.036$), and poorer cognitive function ($p=0.018$). A two-way analysis of variance revealed a significant genotype-by-maltreatment interaction for more severe PTSD avoidance symptom ($p=0.012$).

Limitations: The relatively small sample size limited our findings.

Conclusions: These findings may provide an insight into the etiology of PTSD from the inflammatory perspective.

Keywords: Posttraumatic stress disorder (PTSD); Inflammation; *CRP* gene; Cognitive function; Symptomatology; Childhood maltreatment.

1. Introduction

Posttraumatic stress disorder (PTSD) is a serious psychiatric condition that develops in a subset of individuals following exposure to traumatic events. The lifetime prevalence of PTSD is estimated at around 3.9% worldwide (Koenen et al., 2017). The disorder is characterized by intrusion, avoidance, hyperarousal, and negative alterations in cognitions and mood.

Evidence shows that PTSD is associated with alterations in the immune/inflammatory system (Hori and Kim, 2019; Michopoulos et al., 2017). In line with this, studies have demonstrated that patients with PTSD exhibit elevated blood levels of proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) compared to healthy controls (Passos et al., 2015). Recent studies have also reported increased levels of C-reactive protein (CRP), a peripheral marker of inflammation, in PTSD patients (Lindqvist et al., 2017; Miller et al., 2018; O'Donovan et al., 2017). However, the evidence for elevated CRP levels in this disorder is somewhat mixed; for example, a meta-analysis did not observe significant differences between PTSD patients and healthy controls (Passos et al., 2015). It is postulated that such variability in findings can be partly attributable to genetic variation in inflammation-related genes, including the *CRP* gene (Zass et al., 2017).

Both PTSD (Stein et al., 2002; True et al., 1993) and baseline variations in CRP (de Maat et al., 2004; Pankow et al., 2001) are reported to be 30-50% heritable. Studies have shown that specific single nucleotide polymorphisms (SNPs), including tag-SNPs (rs1205, rs1130864, rs1800947, rs2794520, and rs3093059), of the *CRP* gene account for substantial individual differences in circulating CRP protein levels (Crawford et al., 2006; Kathiresan et al., 2006; Michopoulos et al., 2015; Miller et al., 2005; Miller et al., 2018). Moreover, a longitudinal study in male veterans demonstrated that elevated CRP levels were

prospectively associated with PTSD symptom emergence (Eraly et al., 2014). These findings together suggest that the *CRP* gene polymorphisms can contribute to the development of PTSD by affecting inflammatory activity as indexed by peripheral CRP levels. Indeed, a study conducted in predominantly African-American subjects (Michopoulos et al., 2015) and another study conducted in mostly Caucasian subjects (Miller et al., 2018) have consistently shown that SNPs in the CRP gene are associated with both blood CRP levels and PTSD symptom severity. However, the mechanism by which the *CRP* SNPs are involved in the development and symptoms of PTSD is not fully understood.

CRP is a sensitive inflammatory molecule synthesized primarily in liver hepatocytes. While its role in the brain is not clear, recent evidence suggests that peripheral CRP can affect the central nervous system through the blood-brain barrier (Elwood et al., 2017). In agreement with this, it is shown that higher circulating CRP levels are associated with worse cognitive function in the general population (Wersching et al., 2010; Yang et al., 2015). Patients with PTSD may have inflammation in the brain as well as in the periphery; for example, they show dysfunction in a wide range of cognitive domains such as memory, attention, working memory, and executive function (Narita-Ohtaki et al., 2018; Scott et al., 2015). Concordantly, we observed that higher high-sensitivity CRP (hsCRP) levels, in addition to IL-6 and high-sensitivity TNF-α (hsTNF-α) levels, were significantly negatively correlated with attention in PTSD patients (Imai et al., 2018). To our knowledge, however, no studies have examined the relationship between *CRP* polymorphisms and cognitive function in PTSD patients.

It is well documented that a variety of environmental factors as well as the genetic background, and the resultant complex gene-environment interactions, can increase the risk for developing PTSD (Koenen et al., 2008). Among these environmental components,

childhood maltreatment has been reliably associated with an increased likelihood of this disorder (Agorastos et al., 2014b; Scott et al., 2010). Moreover, polymorphisms of several candidate genes for PTSD, including *FKBP5* (Binder et al., 2008; Xie et al., 2010) and *BDNF* (Jin et al., 2019), have been shown to interact with childhood maltreatment to influence the development and severity of PTSD. Further, studies have shown that childhood maltreatment history is associated with increased inflammation (Baumeister et al., 2016; Slopen et al., 2013). It can therefore be expected that the *CRP* genetic variation may interact with childhood maltreatment to affect PTSD severity; however, we are not aware of any studies that have investigated this interaction in individuals with PTSD.

This study aimed to investigate the relationships of *CRP* genetic variation with circulating CRP protein levels, PTSD symptomatology, and cognitive function in a sample of Japanese women. We focused on three SNPs within the *CRP* gene, namely rs2794520, rs1130864, and rs3093059, based on evidence for their association with PTSD (Michopoulos et al., 2015; Miller et al., 2018) and with CRP levels (Liu et al., 2016; Michopoulos et al., 2015; Miller et al., 2005; Miller et al., 2018). Protein levels of IL-6 and TNF-α, in addition to the CRP levels, were also measured, considering the close interactions between CRP and these proinflammatory cytokines; IL-6 and TNF-α induce the production of CRP (Calabro et al., 2003; Zhang et al., 1996) whereas CRP induces TNF-α (Sproston and Ashworth, 2018) and IL-6 (Hattori et al., 2003). Furthermore, we explored the interaction between *CRP* genetic variation and childhood maltreatment history for present PTSD severity. We hypothesized that patients carrying the *CRP* risk alleles would show higher levels of inflammatory markers and greater PTSD symptoms and cognitive dysfunction and that childhood maltreatment would interact with the risk allele to cause more severe PTSD symptoms. This study was conducted as part of an ongoing larger project, and the reasons for sole inclusion of females in this study were that the vast majority of the sample included in our entire

project were females and that sex differences have been shown in the pathology of PTSD (Ney et al., 2019) and inflammatory responses (Lakoski et al., 2006).

2. Methods

Detailed methods are described in Supplementary Methods.

2.1. Participants

This study was approved by the ethics committees of the institutes involved, including National Center of Neurology and Psychiatry, Tokyo Women's Medical University, and Nagoya City University, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants after they had received a detailed explanation of the study.

A total of 57 civilian patients with PTSD (age range: 23–59 years) participated in this study. All patients had already been diagnosed as having PTSD by their attending clinicians. The experience of traumatic events and diagnosis of PTSD were confirmed by the Posttraumatic Diagnostic Scale (PDS) (Foa, 1995). The Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) was also administered to identify any other Axis-I disorders as well as PTSD.

In addition, 73 non-trauma-exposed healthy control subjects (20–64 years) were recruited through advertisements and by word of mouth. The PDS and MINI were also administered to healthy controls in order to ascertain the absence of traumatic experiences or any Axis-I disorders (if present, they were excluded from this study).

All participants were Japanese women who resided in metropolitan areas in Japan, including Tokyo and Nagoya. There were no subjects, including both patients and controls, who presented clinically apparent signs/symptoms of acute infection or had severe physical illness or apparent intellectual disability.

2.2. Psychological assessment

Psychological and clinical characteristics of participants were assessed by using three self-report questionnaires, including the PDS (Foa, 1995), the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003), and the Athens Insomnia Scale (AIS) (Soldatos et al., 2000). Cognitive functions were measured using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph et al., 1998).

The PDS was created in accordance with the diagnostic criteria of PTSD in DSM-IV. This scale comprises four parts that evaluate traumatic experiences (Parts 1 & 2), PTSD severity during the past month (Part 3), and the associated functional impairments (Part 4). The assessment of PTSD severity in Part 3 consists of 17 items, each scored on a 4-point scale of symptom frequency, with higher scores indicating greater symptoms. In the present study, we used the validated Japanese version of PDS (Nagae et al., 2007), and administered Parts 1 & 2 to all participants and Parts 3 & 4 to only patients with PTSD.

The CTQ (Bernstein et al., 2003) assesses five types of childhood maltreatment, including emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. The 28-item version of CTQ, which was translated from the original English version into Japanese by one author, was used in the present study. All items are rated on a 5-point scale and can be summed to generate the five subscale scores as well as the total score, with higher

scores indicating more severe maltreatment. We classified those subjects with the total CTQ score of 51 or lower into "lower CTQ" group and those with the score of 52 or higher into "higher CTQ" group. This cut-off score of 51 was created by summing up the upper limit scores for "slight to moderate" maltreatment for each CTQ subscale across the five subscales, according to the cut-off scores defined in the manual of CTQ (Bernstein and Fink, 1998).

The AIS is an 8-item questionnaire for assessing insomnia symptoms (Soldatos et al., 2000). Each item is scored on a 4-point scale, with higher scores indicating more severe symptoms. In the present study, the validated Japanese version (Okajima et al., 2013) of the AIS was used.

The RBANS is a neuropsychological test battery that assesses five main cognitive domains, including immediate memory, visuospatial construction, language, attention, and delayed memory (Randolph et al., 1998). Index scores of these five domains can be combined to generate the total score, which provides a global measure of neuropsychological performance. These age-corrected scaled scores are standardized such that the population mean is 100 and the standard deviation (SD) is 15. In the present study, the validated Japanese version (Matsui et al., 2010) of the RBANS was used.

2.3. Measurement of inflammatory markers

Blood samples were collected from each participant around noon before lunch (between 11:30 AM and 12:30 PM) for the measurement of three proinflammatory markers, including hsCRP, hsTNF-α, and IL-6. Serum concentrations of these markers were measured at a clinical laboratory (SRL Inc. Tokyo, Japan); hsCRP was measured by nephelometry, hsTNF-

α was measured by enzyme-linked immunosorbent assay, and IL-6 was measured by chemiluminescent enzyme immunoassay. Intra- and inter-assay coefficients of variation for hsCRP at 356.0 and 2071.4 ng/ml were all less than 2.1%. Intra- and inter-assay coefficients of variation for hsTNF-α at 0.73 and 6.3 pg/ml ranged between 2.9% and 6.0%. Intra- and inter-assay coefficients of variation for IL-6 at 59.3, 170.8, and 523.0 pg/ml were all less than 2.6%. There were no subjects who showed hsCRP levels > 10,000 ng/ml (i.e., 10 mg/l), an objective feature of acute infection (Chu et al., 2019).

2.4. *CRP* genotyping and analysis

The human *CRP* gene comprises two exons separated by one intron, and a long 3' untranslated region. As mentioned earlier, the three tag-SNPs, i.e., rs2794520, rs1130864, and rs3093059, were selected in this study; while the other two tag-SNPs of the *CRP* gene that are also shown to influence CRP protein levels, i.e., rs1205 and rs1800947, were excluded, given that rs1205 is in a perfect linkage disequilibrium with rs2794520 (both D' and r^2 are 1.0) and that the minor allele frequency of rs1800947 is less than 0.05, in a Japanese population (Machiela and Chanock, 2018; Yamaguchi-Kabata et al., 2015).

Genomic DNA was extracted using the Maxwell 16 Blood DNA Purification Kit (Promega, Madison, WI, USA) from buffy coat smears as part of centrifuged venous blood. The three SNPs, rs2794520 (assay ID: C____177486_10), rs1130864 (C___7479332_10), and rs3093059 (custom assay ID: ANGZMNP; forward primer, 5'-TTTGGTTTTTTGCATGGACACA-3'; reverse primer, 5'-TGTCAGGGCCGTCATTTAGTG-3'; probe 1, 5'-VIC-TCTCAGCCGATTGAGTA-MGB-NFQ-3'; probe 2, 5'-FAM-CTCAGCCAATTGAGTACA-MGB-NFQ-3'), were genotyped using the TaqMan SNP Genotyping Assays. The polymerase chain reaction was carried out

using GeneAce Probe qPCR Mixα (Nippon Gene, Toyama City, JPN) under following conditions: 1 cycle at 95°C for 10 min followed by 45 cycles of 95°C for 15 sec and 60°C for 1 min. The allele-specific fluorescence was measured with ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA). All samples had a genotyping call rate of 97% or greater. Linkage disequilibrium was calculated using Haploview version 4.2 software (Barrett et al., 2005).

2.5. Statistical analysis

Averages are reported as "mean ± SD", or "median (25–75th percentile)" when appropriate. To compare averages between two groups, the t-test or Mann-Whitney U test was used; this selection was based on the nature and distribution of the data. Categorical variables were compared using the χ^2 test, or Fisher's exact test when expected cell frequencies were less than five. Correlations among serum inflammatory marker levels were calculated by the Spearman's rank order correlation (rho).

The main data analyses were conducted through the following steps. First, we compared genotype/allele frequencies of the three SNPs between patients and controls. Then, PTSD symptom severity, cognitive function, and inflammatory marker levels were compared between the genotype groups in each diagnostic group, except that PTSD severity was compared only in patients. Lastly, a two-way analysis of variance (ANOVA) was performed to assess the main effect of genotype and of maltreatment (i.e., the lower CTQ group vs. higher CTQ group), as well as their interactive effect, on PTSD severity.

All statistical analyses were performed using the Statistical Package for the Social Sciences version 25.0 (IBM Corp., Tokyo, Japan). Statistical significance was set at two-

tailed $p < 0.05$ unless otherwise specified. For the three subscales of PDS and five domains of RBANS, the Bonferroni-corrected p values were adopted for the significance threshold to correct for multiple comparisons (this correction was not applied to the total score of PDS and that of RBANS). Specifically, the significance was set at $p < 0.017$ (= 0.05/3) for the three PDS subscales and at $p < 0.01$ (= 0.05/5) for the five RBANS domains; while the original threshold of $p < 0.05$ (and greater than the Bonferroni-corrected p values) was considered as statistical trend.

3. Results

3.1. Sample characteristics

Demographic characteristics, clinical/psychological variables, and inflammatory marker levels in patients with PTSD and healthy controls are summarized in Table 1.

Mean age of patients and that of controls were both mid-to-late 30s, but they were significantly different ($p = 0.03$). Patients and controls did not significantly differ in education level, smoking status, or body mass index (calculated as weight in kilograms divided by height in meters squared) (all $p > 0.1$). Compared to controls, patients reported significantly more childhood experiences of maltreatment (assessed with the CTQ) and poorer sleep quality (assessed with the AIS) (all $p < 0.001$). Numbers of "lower CTQ" subjects and "higher CTQ" subjects were 21 and 35 for patients and 70 and 3 for controls, respectively, which was significantly different ($p < 0.001$ by Fisher's exact test).

Concerning clinical characteristics, most patients developed PTSD after experiencing interpersonal violence such as physical and/or sexual violence during adulthood, and had

been ill for more than six months at the time of study participation. Many of the patients had psychiatric comorbidity, and were receiving psychotropic medications. The patients were, on average, moderately severely ill, as indexed by the mean total score of PDS.

As for comorbid physical illness and use of medications that can influence peripheral inflammation, three subjects (patients) had diabetes mellitus, two subjects (one subject and one control) had hypertension, two subjects (patients) had dyslipidemia, 13 subjects (11 patients and two controls) were taking non-steroidal anti-inflammatory drugs, and one subject (patient) was taking statin.

Compared to controls, patients showed significantly higher serum IL-6 levels ($p = 0.026$) while there were no significant differences in hsCRP or hsTNF-α levels between groups (both $p > 0.1$). Patients demonstrated significantly poorer performance on all the RBANS subscales, including immediate memory ($p < 0.001$), visuospatial construction ($p = 0.006$), language ($p = 0.001$), attention ($p < 0.001$), and delayed memory ($p < 0.001$), as well as the total score ($p < 0.001$). These results on inflammatory markers and cognitive function all confirmed those of our previous study (Imai et al., 2018).

Serum hsCRP levels were significantly, albeit not strongly, correlated with hsTNF-α (rho = 0.30, $p < 0.001$) and IL-6 (rho = 0.37, $p < 0.001$) levels in the total sample ($n = 129$).

3.2. *CRP* gene polymorphisms

Genotype and allele distributions of the three *CRP* polymorphisms in patients and controls are summarized in Table 2. As shown in this table, genotype frequencies did not deviate from Hardy-Weinberg equilibrium in controls or in patients for any of the three SNPs genotyped.

There were no significant case-control differences in genotype or allele frequencies for any of the three SNPs (Table 2).

Minor allele frequencies of rs2794520 (T/C), rs1130864 (G/A), and rs3093059 (A/G) in our total sample (i.e., patients and controls combined) were 0.29, 0.06, and 0.14, respectively; these frequencies closely matched those reported in a representative genome variation database of Japanese individuals, i.e., 0.32, 0.07, and 0.14, respectively (Machiela and Chanock, 2018; Yamaguchi-Kabata et al., 2015).

Linkage disequilibrium was calculated using the total sample, which revealed that rs2794520 was in strong linkage disequilibrium with both rs1130864 ($D' = 1.0$, $r^2 = 0.15$) and rs3093059 ($D' = 1.0$, $r^2 = 0.40$) although r^2 values were low. Given this result, together with the low frequency of the minor allele for rs1130864 and rs3093059 (minor allele frequency in the patient group was less than 0.10 for these two SNPs), we decided to focus only on rs2794520 in all subsequent analyses. Furthermore, given the small number of the CC genotype of rs2794520 (three subjects in the PTSD group and seven in the control group), we combined the CC genotype with the CT genotype into a single CC/CT genotype group.

3.3. Relationships of *CRP* rs2794520 with circulating proinflammatory marker levels, symptomatology, and cognitive function

Demographic/clinical/psychological variables and inflammatory marker levels in PTSD patients and healthy controls, stratified by the rs2794520 genotype groups, are shown in Supplementary Table S1.

As presented in Fig. 1 and Supplementary Table S1, PTSD patients with the CC/CT genotype of rs2794520 showed significantly higher levels of hsCRP ($p = 0.009$) and hsTNF-α ($p = 0.001$) than those with the TT genotype; for IL-6, a trend-level difference was observed ($p = 0.055$). There was no significant difference in level of hsCRP between PTSD patients with the CC/CT allele and healthy controls with the CC/CT allele.

Fig. 2 and Supplementary Table S1 present the relationships of rs2794520 genotypes with PTSD symptom severity and cognitive function in patients. Compared to patients with the TT genotype, those with the CC/CT genotype had significantly more symptoms as indexed by PDS total scores ($p = 0.036$), with the difference in intrusion subscale scores being at a trend level ($p = 0.027$); in contrast, no significant differences were seen in avoidance or hyperarousal subscale scores. Compared to patients with the TT genotype, those with the CC/CT genotype demonstrated significantly lower RBANS total scores ($p = 0.018$), with the differences in language ($p = 0.035$), attention ($p = 0.039$), and delayed memory ($p = 0.033$) performance being at a trend level; no significant differences were found in immediate memory or visuospatial construction.

In healthy controls, no significant differences were observed between those with the CC/CT genotype and those with the TT genotype for any proinflammatory markers or cognitive functions (Supplementary Table S1).

3.4. Interaction between the *CRP* polymorphism and childhood maltreatment for PTSD symptoms

The two-way ANOVA for evaluating the effects of rs2794520 genotype, childhood maltreatment, and their interaction on PTSD symptom severity revealed that there were no

significant main effects of genotype, maltreatment, or genotype-by-maltreatment interaction for PDS total scores, intrusion subscale scores, or hyperarousal subscale scores (all $p > 0.05$). For avoidance subscale scores, however, this analysis revealed significant genotype-by-maltreatment interaction [$F(1,52) = 6.83$, $p = 0.012$], with no significant main effect of genotype [$F(1,52) = 1.02$, $p = 0.32$] or maltreatment [$F(1,52) = 1.56$, $p = 0.22$] (Fig. 3). This result indicated that the rs2794520 C-allele interacts with childhood maltreatment to increase PTSD avoidance symptoms.

4. Discussion

In this study we investigated effects of the *CRP* rs2794520 polymorphism on peripheral proinflammatory markers, symptomatology, and cognition in Japanese civilian women with PTSD. We also examined interaction between this SNP and childhood maltreatment history for PTSD symptoms. The main findings can be summarized as follows. Compared to patients with the TT genotype, those with the CC/CT genotype exhibited significantly higher hsCRP and hsTNF-α levels, more severe PTSD symptoms, and greater cognitive impairment. Furthermore, significant interaction was observed between this polymorphism and childhood maltreatment for avoidance symptom severity.

Our finding of the association of *CRP* genetic variation with higher blood CRP levels and more severe symptoms in Japanese female patients mostly triggered by interpersonal trauma is consistent with previous studies of PTSD patients among urban African American men and women (Michopoulos et al., 2015) and veterans with predominantly white men (Miller et al., 2018). These results suggest that *CRP* genetic variation may lead to more severe phenotype of PTSD by causing increased inflammation. We also found that the *CRP*

rs2794520 polymorphism is associated with significantly higher hsTNF-α levels. This observation may be accounted for by the bidirectional interaction between CRP and hsTNF-α (Calabro et al., 2003; Sproston and Ashworth, 2018), and suggests that the *CRP* genetic variation may result in an increase in multiple proinflammatory markers including cytokines as well as CRP. It should be noted, however, that patients and controls did not significantly differ in the rs2794520 genotype frequency or blood levels of CRP and hsTNF-α. Taken together, it may be that this polymorphism defines more severe subgroup of PTSD in terms of inflammation and symptomatology rather than confers vulnerability for this disorder in general. Alternatively, given the results of significantly higher IL-6 levels in patients than in controls and of the trend-level association between rs2794520 polymorphism and IL-6 levels in patients, it is possible that this polymorphism may cause increased inflammation in PTSD patients as a group via the mechanism of interaction between CRP and IL-6 (Calabro et al., 2003; Hattori et al., 2003; Zhang et al., 1996). In this regard, further studies with larger sample sizes are needed to draw any conclusion.

As mentioned earlier, PTSD is associated with wide-ranging cognitive impairments (Narita-Ohtaki et al., 2018; Scott et al., 2015). It is also shown that PTSD patients have hippocampal morphologic abnormalities (Logue et al., 2018) and increased risk of developing dementia (Yaffe et al., 2010). Neurogenesis in the hippocampus is associated with cognitive function (Alam et al., 2018), and inflammation in the brain adversely affects neurogenesis and cognition (Muhie et al., 2017; Ryan and Nolan, 2016). The present study is the first, to our knowledge, to show the association between *CRP* genetic variation and cognitive impairment in patients with PTSD. CRP was originally thought to be a peripheral proinflammatory marker, but it has recently been suggested that CRP may influence the central nervous system (Elwood et al., 2017; Strang et al., 2012). Therefore, the *CRP* genetic variation can cause increased levels of proinflammatory molecules, thereby leading to

neuroinflammation and cognitive dysfunction. Besides cognitive impairment, the more severe PTSD symptoms associated with the *CRP* polymorphism might also be caused by inflammation in the brain, given the evidence that inflammation can be causally involved in the emergence and maintenance of such symptoms. For instance, animal studies have shown that elevated inflammation impairs extinction of fear memory (Young et al., 2018; Yu et al., 2017). In line with this, human studies have demonstrated that increased inflammation is associated with enhanced amygdala activation in response to threatening stimuli (Inagaki et al., 2012; Swartz et al., 2017).

Our findings indicate that the C-allele, relative to the T-allele, of *CRP* rs2794520 is associated with elevated inflammation and more severe phenotype of PTSD. In line with our results, previous studies have identified that the C-allele of rs2794520 was associated with higher blood CRP levels (Chu et al., 2012; Li et al., 2016). On the other hand, frequencies of rs2794520 C-allele compared to T-allele vary substantially across different ethnic populations. The frequency of C-allele was approximately 0.30 in our sample, being consistent with that described in a database of Japanese individuals (Yamaguchi-Kabata et al., 2015). In contrast, the frequency of this allele is reported to be around 0.65 among many other populations such as Europeans, according to the Genome Aggregation Database (gnomAD). Thus, the minor allele of rs2794520 is the C-allele in Japanese populations whereas globally it is the T-allele. These together indicate that the C-allele of rs2794520 is associated with increased inflammation (and related phenotypes) across ethnicities, irrespective of its relative frequency in the population.

Effects of environmental factors can be modified by genetic factors, and it is widely accepted that complex gene-environment interactions are involved in the pathogenesis of PTSD (Koenen et al., 2008). Among a wide variety of environmental factors, childhood

maltreatment can be particularly relevant here, as it has been associated with both increased inflammation (Baumeister et al., 2016; Slopen et al., 2013) and risk for PTSD (Agorastos et al., 2014b; Scott et al., 2010). Supporting this, we observed the significant interaction between rs2794520 genotype and childhood maltreatment severity for PTSD avoidance symptoms, demonstrating an example of gene-environment interaction involved in this disorder. While we are not aware of any previous studies reporting the interaction between *CRP* genetic variation and childhood maltreatment in PTSD, there are studies that show such interactions for SNPs of other key genes, such as *FKBP5* (Binder et al., 2008; Xie et al., 2010) and *BDNF* (Jin et al., 2019). The reason for the specific association with avoidance symptom observed here is not clear, but this may suggest that childhood maltreatment might magnify the *CRP* genetic effect on this aspect of symptomatology.

There were several limitations to this study. First, the sample size may have been not large enough for some statistical comparisons, in which type II errors might have occurred. Moreover, the limited sample size did not allow us to include the two genotyped SNPs with relatively low minor allele frequencies, i.e., rs1130864 and rs3093059, in the analyses. Second, this study was conducted only with Asian women, and therefore our findings cannot be readily extrapolated to other populations such as men and other ethnicities. For example, the risk C-allele frequencies of *CRP* rs2794520 are known to vary widely across ethnicities, as mentioned earlier. In addition, prevalence of PTSD has been consistently shown to be higher in women compared to men (Kessler et al., 1995). The heritability of PTSD can also differ between men and women, such that this heritability is generally estimated at around 30-40% (Stein et al., 2002) whereas it can be as high as 72% when the sample was restricted to females (Sartor et al., 2011). Still, the observed significant association of the *CRP* polymorphism with symptom severity and blood CRP levels in PTSD patients was consistent with the findings from previous studies targeting male (and both sex) African-

Americans/Caucasians (Michopoulos et al., 2015; Miller et al., 2018), which suggests that this association can be observed across different populations. Third, as the proinflammatory marker levels were examined using peripheral blood, the inflammatory status in the brain is not known. For instance, several studies have shown that plasma and cerebrospinal fluid IL-6 levels in PTSD patients do not correspond to each other (Agorastos et al., 2019; Agorastos et al., 2014a). Fourth, although there were no significant differences in any comorbid psychiatric disorders between the CC/CT patients and TT patients (Supplementary Table S1), potentially confounding effects of these comorbidities such as depression and alcohol/substance abuse/dependence cannot be totally excluded. Finally, we used a retrospective measure to assess childhood maltreatment, which may have caused certain biases such as recall bias.

In conclusion, this study shows significant relationships of the *CRP* genetic variation with circulating proinflammatory markers, symptom severity, and cognitive dysfunction in a sample of Japanese women with PTSD. We further demonstrate the significant interaction between the *CRP* polymorphism and childhood maltreatment for more severe PTSD avoidance symptom. These findings may provide an insight into understanding the etiology of PTSD from the inflammatory perspective. Future studies with different populations and larger sample sizes are required to replicate our findings and verify the generalizability.

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Declaration of interest

None.

References

Agorastos, A., Hauger, R.L., Barkauskas, D.A., Lerman, I.R., Moeller-Bertram, T., Snijders, C., Haji, U., Patel, P.M., Geracioti, T.D., Chrousos, G.P., Baker, D.G., 2019. Relations of combat stress and posttraumatic stress disorder to 24-h plasma and cerebrospinal fluid interleukin-6 levels and circadian rhythmicity. Psychoneuroendocrinology. 100, 237-245.

Agorastos, A., Hauger, R.L., Barkauskas, D.A., Moeller-Bertram, T., Clopton, P.L., Haji, U., Lohr, J.B., Geracioti, T.D., Jr., Patel, P.M., Chrousos, G.P., Baker, D.G., 2014a. Circadian rhythmicity, variability and correlation of interleukin-6 levels in plasma and cerebrospinal fluid of healthy men. Psychoneuroendocrinology. 44, 71-82.

Agorastos, A., Pittman, J.O., Angkaw, A.C., Nievergelt, C.M., Hansen, C.J., Aversa, L.H., Parisi, S.A., Barkauskas, D.A., Baker, D.G., 2014b. The cumulative effect of different childhood trauma types on self-reported symptoms of adult male depression and PTSD, substance abuse and health-related quality of life in a large active-duty military cohort. J. Psychiatr. Res. 58, 46-54.

Alam, M.J., Kitamura, T., Saitoh, Y., Ohkawa, N., Kondo, T., Inokuchi, K., 2018. Adult Neurogenesis Conserves Hippocampal Memory Capacity. J. Neurosci. 38, 6854-6863.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 21, 263-265.

Baumeister, D., Akhtar, R., Ciufolini, S., Pariante, C.M., Mondelli, V., 2016. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-alpha. Mol. Psychiatry. 21, 642-649.

Bernstein, D.P., Fink, L., 1998. Childhood Trauma Questionnaire: A retrospective self-report manual. The Psychological Corporation, San Antonio, TX.

Bernstein, D.P., Stein, J.A., Newcomb, M.D., Walker, E., Pogge, D., Ahluvalia, T., Stokes, J., Handelsman, L., Medrano, M., Desmond, D., Zule, W., 2003. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. Child Abuse Negl. 27, 169-190.

Binder, E.B., Bradley, R.G., Liu, W., Epstein, M.P., Deveau, T.C., Mercer, K.B., Tang, Y., Gillespie, C.F., Heim, C.M., Nemeroff, C.B., Schwartz, A.C., Cubells, J.F., Ressler, K.J., 2008. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA. 299, 1291-1305.

Calabro, P., Willerson, J.T., Yeh, E.T., 2003. Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. Circulation. 108, 1930-1932.

Chu, A.L., Stochl, J., Lewis, G., Zammit, S., Jones, P.B., Khandaker, G.M., 2019. Longitudinal association between inflammatory markers and specific symptoms of depression in a prospective birth cohort. Brain. Behav. Immun. 76, 74-81.

Chu, A.Y., Guilianini, F., Barratt, B.J., Nyberg, F., Chasman, D.I., Ridker, P.M., 2012.

Pharmacogenetic determinants of statin-induced reductions in C-reactive protein. Circ. Cardiovasc. Genet. 5, 58-65.

Crawford, D.C., Sanders, C.L., Qin, X., Smith, J.D., Shephard, C., Wong, M., Witrak, L., Rieder, M.J., Nickerson, D.A., 2006. Genetic variation is associated with C-reactive protein levels in the Third National Health and Nutrition Examination Survey. Circulation. 114, 2458-2465.

de Maat, M.P., Bladbjerg, E.M., Hjelmborg, J., Bathum, L., Jespersen, J., Christensen, K., 2004. Genetic influence on inflammation variables in the elderly. Arterioscler. Thromb. Vasc. Biol. 24, 2168-2173.

Elwood, E., Lim, Z., Naveed, H., Galea, I., 2017. The effect of systemic inflammation on human brain barrier function. Brain. Behav. Immun. 62, 35-40.

Eraly, S.A., Nievergelt, C.M., Maihofer, A.X., Barkauskas, D.A., Biswas, N., Agorastos, A., O'Connor, D.T., Baker, D.G., Team, f.t.M.R.S., 2014. Assessment of Plasma C-Reactive Protein as a Biomarker of Posttraumatic Stress Disorder Risk. JAMA Psychiatry. 71, 423-431.

Foa, E.B., 1995. The posttraumatic diagnostic scale (PDS) manual. National Computer Systems, Minneapolis, MN.

Hattori, Y., Matsumura, M., Kasai, K., 2003. Vascular smooth muscle cell activation by C-reactive protein. Cardiovasc. Res. 58, 186-195.

Hori, H., Kim, Y., 2019. Inflammation and post-traumatic stress disorder. Psychiatry Clin. Neurosci. 73, 143-153.

Imai, R., Hori, H., Itoh, M., Lin, M., Niwa, M., Ino, K., Ogawa, S., Ishida, M., Sekiguchi, A., Matsui, M., Kunugi, H., Akechi, T., Kamo, T., Kim, Y., 2018. Inflammatory markers and their possible effects on cognitive function in women with posttraumatic stress disorder. J. Psychiatr. Res. 102, 192-200.

Inagaki, T.K., Muscatell, K.A., Irwin, M.R., Cole, S.W., Eisenberger, N.I., 2012. Inflammation selectively enhances amygdala activity to socially threatening images. Neuroimage. 59, 3222-3226.

Jin, M.J., Jeon, H., Hyun, M.H., Lee, S.H., 2019. Influence of childhood trauma and brain-derived neurotrophic factor Val66Met polymorphism on posttraumatic stress symptoms and cortical thickness. Sci. Rep. 9, 6028.

Kathiresan, S., Larson, M.G., Vasan, R.S., Guo, C.Y., Gona, P., Keaney, J.F., Jr., Wilson, P.W., Newton-Cheh, C., Musone, S.L., Camargo, A.L., Drake, J.A., Levy, D., O'Donnell, C.J., Hirschhorn, J.N., Benjamin, E.J., 2006. Contribution of clinical correlates and 13 C-reactive protein gene polymorphisms to interindividual variability in serum C-reactive protein level. Circulation. 113, 1415-1423.

Kessler, R.C., Sonnega, A., Bromet, E., Hughes, M., Nelson, C.B., 1995. Posttraumatic stress disorder in the National Comorbidity Survey. Arch. Gen. Psychiatry. 52, 1048-1060.

Koenen, K.C., Nugent, N.R., Amstadter, A.B., 2008. Gene-environment interaction in posttraumatic stress disorder: review, strategy and new directions for future research. Eur. Arch. Psychiatry Clin. Neurosci. 258, 82-96.

Koenen, K.C., Ratanatharathorn, A., Ng, L., McLaughlin, K.A., Bromet, E.J., Stein, D.J., Karam, E.G., Meron Ruscio, A., Benjet, C., Scott, K., Atwoli, L., Petukhova, M., Lim, C.C.W., Aguilar-Gaxiola, S., Al-Hamzawi, A., Alonso, J., Bunting, B., Ciutan, M., de Girolamo, G., Degenhardt, L., Gureje, O., Haro, J.M., Huang, Y., Kawakami, N., Lee, S., Navarro-Mateu, F., Pennell, B.E., Piazza, M., Sampson, N., Ten Have, M., Torres, Y., Viana, M.C., Williams, D., Xavier, M., Kessler, R.C., 2017. Posttraumatic stress disorder in the World Mental Health Surveys. Psychol. Med. 47, 2260-2274.

Lakoski, S.G., Cushman, M., Criqui, M., Rundek, T., Blumenthal, R.S., D'Agostino, R.B., Jr., Herrington, D.M., 2006. Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. Am. Heart J. 152, 593-598.

Li, C.I., Li, T.C., Liao, L.N., Liu, C.S., Yang, C.W., Lin, C.H., Hsiao, J.H., Meng, N.H., Lin, W.Y., Wu, F.Y., Lin, C.C., 2016. Joint effect of gene-physical activity and the interactions among CRP, TNF-alpha, and LTA polymorphisms on serum CRP, TNF-alpha levels, and handgrip strength in community-dwelling elders in Taiwan - TCHS-E. Age (Dordr). 38, 46.

Lindqvist, D., Dhabhar, F.S., Mellon, S.H., Yehuda, R., Grenon, S.M., Flory, J.D., Bierer, L.M., Abu-Amara, D., Coy, M., Makotkine, I., Reus, V.I., Bersani, F.S., Marmar, C.R., Wolkowitz, O.M., 2017. Increased pro-inflammatory milieu in combat related PTSD - A new cohort replication study. Brain. Behav. Immun. 59, 260-264.

Liu, Z.Y., Wang, Z.D., Li, L.Z., Chu, X.F., Zhu, Y.S., Shi, J.M., Xie, X.J., Jin, L., Wang, Y., Wang, X.F., 2016. Association of CRP gene polymorphisms with CRP levels, frailty and co-morbidity in an elderly Chinese population: results from RuLAS. Age Ageing. 45, 360-365.

Logue, M.W., van Rooij, S.J.H., Dennis, E.L., Davis, S.L., Hayes, J.P., Stevens, J.S., Densmore, M., Haswell, C.C., Ipser, J., Koch, S.B.J., Korgaonkar, M., Lebois, L.A.M., Peverill, M., Baker, J.T., Boedhoe, P.S.W., Frijling, J.L., Gruber, S.A., Harpaz-Rotem, I., Jahanshad, N., Koopowitz, S., Levy, I., Nawijn, L., O'Connor, L., Olff, M., Salat, D.H., Sheridan, M.A., Spielberg, J.M., van Zuiden, M., Winternitz, S.R., Wolff, J.D., Wolf, E.J., Wang, X., Wrocklage, K., Abdallah, C.G., Bryant, R.A., Geuze, E., Jovanovic, T., Kaufman, M.L., King, A.P., Krystal, J.H., Lagopoulos, J., Bennett, M., Lanius, R., Liberzon, I., McGlinchey, R.E., McLaughlin, K.A., Milberg, W.P., Miller, M.W., Ressler, K.J., Veltman, D.J., Stein, D.J., Thomaes, K., Thompson, P.M., Morey, R.A., 2018. Smaller Hippocampal Volume in Posttraumatic Stress Disorder: A Multisite ENIGMA-PGC Study: Subcortical Volumetry Results From Posttraumatic Stress Disorder Consortia. Biol. Psychiatry. 83, 244-253.

Machiela, M.J., Chanock, S.J., 2018. LDassoc: an online tool for interactively exploring genome-

wide association study results and prioritizing variants for functional investigation. Bioinformatics. 34, 887-889.

Matsui, M., Kasai, Y., Nagasaki, M., 2010. Reliability and validity of the Japanese version of the Repeatable Battery for the Assessment of Neuropsychological Status. Toyama Med. J. 21, 31-36.

Michopoulos, V., Powers, A., Gillespie, C.F., Ressler, K.J., Jovanovic, T., 2017. Inflammation in Fear- and Anxiety-Based Disorders: PTSD, GAD, and Beyond. Neuropsychopharmacology. 42, 254-270.

Michopoulos, V., Rothbaum, A.O., Jovanovic, T., Almli, L.M., Bradley, B., Rothbaum, B.O., Gillespie, C.F., Ressler, K.J., 2015. Association of CRP genetic variation and CRP level with elevated PTSD symptoms and physiological responses in a civilian population with high levels of trauma. Am. J. Psychiatry. 172, 353-362.

Miller, D.T., Zee, R.Y., Suk Danik, J., Kozlowski, P., Chasman, D.I., Lazarus, R., Cook, N.R., Ridker, P.M., Kwiatkowski, D.J., 2005. Association of common CRP gene variants with CRP levels and cardiovascular events. Ann. Hum. Genet. 69, 623-638.

Miller, M.W., Maniates, H., Wolf, E.J., Logue, M.W., Schichman, S.A., Stone, A., Milberg, W., McGlinchey, R., 2018. CRP polymorphisms and DNA methylation of the AIM2 gene influence associations between trauma exposure, PTSD, and C-reactive protein. Brain. Behav. Immun. 67, 194-202.

Muhie, S., Gautam, A., Chakraborty, N., Hoke, A., Meyerhoff, J., Hammamieh, R., Jett, M., 2017. Molecular indicators of stress-induced neuroinflammation in a mouse model simulating features of post-traumatic stress disorder. Transl Psychiatry. 7, e1135.

Nagae, N., Hirohata, S., Shimura, Y., Yamada, S., Foa, E., Nedate, K., Kim, Y., 2007. Development of the Japanese version of the Posttraumatic Diagnostic Scale: ascertaining its reliability and validity among university students. Japanese J. Trauma. Stress. 5, 51-56.

Narita-Ohtaki, R., Hori, H., Itoh, M., Lin, M., Niwa, M., Ino, K., Imai, R., Ogawa, S., Sekiguchi, A., Matsui, M., Kunugi, H., Kamo, T., Kim, Y., 2018. Cognitive function in Japanese women with posttraumatic stress disorder: Association with exercise habits. J. Affect. Disord. 236, 306-312.

Ney, L.J., Gogos, A., Ken Hsu, C.M., Felmingham, K.L., 2019. An alternative theory for hormone effects on sex differences in PTSD: The role of heightened sex hormones during trauma. Psychoneuroendocrinology. 109, 104416.

O'Donovan, A., Ahmadian, A.J., Neylan, T.C., Pacult, M.A., Edmondson, D., Cohen, B.E., 2017. Current posttraumatic stress disorder and exaggerated threat sensitivity associated with elevated inflammation in the Mind Your Heart Study. Brain. Behav. Immun. 60, 198-205.

Okajima, I., Nakajima, S., Kobayashi, M., Inoue, Y., 2013. Development and validation of the Japanese version of the Athens Insomnia Scale. Psychiatry Clin. Neurosci. 67, 420-425.

Pankow, J.S., Folsom, A.R., Cushman, M., Borecki, I.B., Hopkins, P.N., Eckfeldt, J.H., Tracy, R.P.,

2001. Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. Atherosclerosis. 154, 681-689.

Passos, I.C., Vasconcelos-Moreno, M.P., Costa, L.G., Kunz, M., Brietzke, E., Quevedo, J., Salum, G., Magalhaes, P.V., Kapczinski, F., Kauer-Sant'Anna, M., 2015. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. Lancet Psychiatry. 2, 1002-1012.

Randolph, C., Tierney, M.C., Mohr, E., Chase, T.N., 1998. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): preliminary clinical validity. J. Clin. Exp. Neuropsychol. 20, 310-319.

Ryan, S.M., Nolan, Y.M., 2016. Neuroinflammation negatively affects adult hippocampal neurogenesis and cognition: can exercise compensate? Neurosci. Biobehav. Rev. 61, 121-131.

Sartor, C.E., McCutcheon, V.V., Pommer, N.E., Nelson, E.C., Grant, J.D., Duncan, A.E., Waldron, M., Bucholz, K.K., Madden, P.A., Heath, A.C., 2011. Common genetic and environmental contributions to post-traumatic stress disorder and alcohol dependence in young women. Psychol. Med. 41, 1497-1505.

Scott, J.C., Matt, G.E., Wrocklage, K.M., Crnich, C., Jordan, J., Southwick, S.M., Krystal, J.H., Schweinsburg, B.C., 2015. A quantitative meta-analysis of neurocognitive functioning in posttraumatic stress disorder. Psychol. Bull. 141, 105-140.

Scott, K.M., Smith, D.R., Ellis, P.M., 2010. Prospectively ascertained child maltreatment and its association with DSM-IV mental disorders in young adults. Arch. Gen. Psychiatry. 67, 712-719.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry. 59 Suppl 20, 22-33;quiz 34-57.

Slopen, N., Kubzansky, L.D., McLaughlin, K.A., Koenen, K.C., 2013. Childhood adversity and inflammatory processes in youth: a prospective study. Psychoneuroendocrinology. 38, 188-200.

Soldatos, C.R., Dikeos, D.G., Paparrigopoulos, T.J., 2000. Athens Insomnia Scale: validation of an instrument based on ICD-10 criteria. J. Psychosom. Res. 48, 555-560.

Sproston, N.R., Ashworth, J.J., 2018. Role of C-Reactive Protein at Sites of Inflammation and Infection. Front. Immunol. 9, 754.

Stein, M.B., Jang, K.L., Taylor, S., Vernon, P.A., Livesley, W.J., 2002. Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms: a twin study. Am. J. Psychiatry. 159, 1675-1681.

Strang, F., Scheichl, A., Chen, Y.C., Wang, X., Htun, N.M., Bassler, N., Eisenhardt, S.U., Habersberger, J., Peter, K., 2012. Amyloid plaques dissociate pentameric to monomeric C-reactive protein: a novel pathomechanism driving cortical inflammation in Alzheimer's disease?

Brain Pathol. 22, 337-346.

Swartz, J.R., Prather, A.A., Hariri, A.R., 2017. Threat-related amygdala activity is associated with peripheral CRP concentrations in men but not women. Psychoneuroendocrinology. 78, 93-96.

True, W.R., Rice, J., Eisen, S.A., Heath, A.C., Goldberg, J., Lyons, M.J., Nowak, J., 1993. A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. Arch. Gen. Psychiatry. 50, 257-264.

Wersching, H., Duning, T., Lohmann, H., Mohammadi, S., Stehling, C., Fobker, M., Conty, M., Minnerup, J., Ringelstein, E.B., Berger, K., Deppe, M., Knecht, S., 2010. Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function. Neurology. 74, 1022-1029.

Xie, P., Kranzler, H.R., Poling, J., Stein, M.B., Anton, R.F., Farrer, L.A., Gelernter, J., 2010. Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder. Neuropsychopharmacology. 35, 1684-1692.

Yaffe, K., Vittinghoff, E., Lindquist, K., Barnes, D., Covinsky, K.E., Neylan, T., Kluse, M., Marmar, C., 2010. Posttraumatic stress disorder and risk of dementia among US veterans. Arch. Gen. Psychiatry. 67, 608-613.

Yamaguchi-Kabata, Y., Nariai, N., Kawai, Y., Sato, Y., Kojima, K., Tateno, M., Katsuoka, F., Yasuda, J., Yamamoto, M., Nagasaki, M., 2015. iJGVD: an integrative Japanese genome variation database based on whole-genome sequencing. Hum Genome Var. 2, 15050.

Yang, J., Fan, C., Pan, L., Xie, M., He, Q., Li, D., Wang, S., 2015. C-reactive protein plays a marginal role in cognitive decline: a systematic review and meta-analysis. Int. J. Geriatr. Psychiatry. 30, 156-165.

Young, M.B., Howell, L.L., Hopkins, L., Moshfegh, C., Yu, Z., Clubb, L., Seidenberg, J., Park, J., Swiercz, A.P., Marvar, P.J., 2018. A peripheral immune response to remembering trauma contributes to the maintenance of fear memory in mice. Psychoneuroendocrinology. 94, 143-151.

Yu, Z., Fukushima, H., Ono, C., Sakai, M., Kasahara, Y., Kikuchi, Y., Gunawansa, N., Takahashi, Y., Matsuoka, H., Kida, S., Tomita, H., 2017. Microglial production of TNF-alpha is a key element of sustained fear memory. Brain. Behav. Immun. 59, 313-321.

Zass, L.J., Hart, S.A., Seedat, S., Hemmings, S.M., Malan-Muller, S., 2017. Neuroinflammatory genes associated with post-traumatic stress disorder: implications for comorbidity. Psychiatr. Genet. 27, 1-16.

Zhang, D., Sun, M., Samols, D., Kushner, I., 1996. STAT3 participates in transcriptional activation of the C-reactive protein gene by interleukin-6. J. Biol. Chem. 271, 9503-9509.

Figure legends

Fig. 1. Comparisons of blood proinflammatory marker levels between PTSD patients with the *CRP* rs2794520 CC/CT genotype (n = 28) and those with TT genotype (n = 28).

Combined dot- and box-plot shows serum concentrations of (a) hsCRP, (b) hsTNF-α, and (c) IL-6 for PTSD patients with the CC/CT genotype and those with the TT genotype. **: $p < 0.01$; ***: $p = 0.001$; †: $p < 0.1$ (by Mann-Whitney U test). *Abbreviations:* hsCRP, high-sensitivity C-reactive protein; hsTNF-α, high-sensitivity tumor necrosis factor-α; IL-6, interleukin-6.

Fig. 2. Comparisons of symptom severity and cognitive function between PTSD patients with the *CRP* rs2794520 CC/CT genotype (n = 28) and those with TT genotype (n = 29).

(a) PTSD severity was assessed by the PDS. (b) Cognitive function was assessed by the RBANS. Error bars indicate SEM. *: $p < 0.05$ for total scores; †: $p < 0.05$ for subscale scores (by t-test). *Abbreviations:* PDS, Posttraumatic Diagnostic Scale; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status.

Fig. 3. Interaction between *CRP* rs2794520 genotype and childhood maltreatment severity for PTSD avoidance symptom severity in patients.

Patients were classified into "lower CTQ" (n = 21) and "higher CTQ" (n = 35) groups using the cut-off of total CTQ score (i.e., 51/52). Avoidance symptom severity was assessed by the PDS. Error bars indicate SEM. *: interaction $p < 0.017$ (by two-way analysis of variance). *Abbreviations:* PDS, Posttraumatic Diagnostic Scale; CTQ, Childhood Trauma Questionnaire.

Table 1. Demographic/clinical/psychological variables and inflammatory markers in PTSD patients and healthy controls

	PTSD patients (n = 57)	Healthy controls (n = 73)	Analysis		
			Statistic	d.f.	p
Age, year: mean ± SD	39.7 ± 9.3	35.1 ± 13.8	t = 2.3[d]	125.4	**0.03**
Education level[a]: median (25-75th percentile)	3 (3-4)	3 (3-4)	Mann-Whitney U = 1885.0		0.32
Smoking: yes, n (%)	8 (14.0)	5 (6.8)	χ^2 = 1.8	1	0.18
Body mass index: mean ± SD	21.5 ± 3.3	20.7 ± 2.6	t = 1.6 [d]	103.7	0.12
Outpatients/inpatients: n/n	56/1	N.A			
Duration of illness, less than 6 months/6 months or more: n/n	3/54	N.A			
Type of index trauma					
Interpersonal violence: yes, n (%)	47 (82.5)	0 (0.0)			
Accident: yes, n (%)	3 (5.3)	0 (0.0)			
Disaster: yes, n (%)	1 (1.8)	0 (0.0)			
Other: yes, n (%)	6 (10.5)	0 (0.0)			
Comorbid psychiatric disorder, any: yes, n (%)	54 (94.7)	0 (0.0)			
Major depressive disorder: yes, n (%)	33 (57.9)	0 (0.0)			
Bipolar disorder: yes, n (%)	4 (7.0)	0 (0.0)			
Anxiety disorder: yes, n (%)	30 (52.6)	0 (0.0)			
Alcohol/substance abuse or dependence: yes, n (%)	9 (15.8)	0 (0.0)			
Medication, any: yes, n (%)	46 (80.7)	0 (0.0)			
Antidepressant: yes, n (%)	30 (52.6)	0 (0.0)			
Anxiolytic: yes, n (%)	30 (52.6)	0 (0.0)			
Hypnotic: yes, n (%)	21 (36.8)	0 (0.0)			
Antipsychotic: yes, n (%)	14 (24.6)	0 (0.0)			
Mood stabilizer: yes, n (%)	7 (12.3)	0 (0.0)			
CTQ[b], total score: mean ± SD	60.1 ± 20.8	36.0 ± 8.4	t = 8.2 [d]	68.9	**<0.001**
Emotional abuse[b]: mean ± SD	14.9 ± 6.8	7.2 ± 3.2	t = 7.9 [d]	73.2	**<0.001**
Physical abuse[b]: mean ± SD	8.8 ± 4.6	5.1 ± 0.38	t = 6.0 [d]	55.7	**<0.001**
Sexual abuse[b]: mean ± SD	8.1 ± 5.4	5.3 ± 1.0	t = 3.9 [d]	58.0	**<0.001**
Emotional neglect[b]: mean ± SD	18.1 ± 5.5	11.9 ± 5.0	t = 6.7	127	**<0.001**
Physical neglect[b]: mean ± SD	10.1 ± 4.2	6.5 ± 1.8	t = 6.1 [d]	70.6	**<0.001**
PDS, total score: mean ± SD	31.2 ± 9.9	N.A			
Intrusion: mean ± SD	8.2 ± 3.4	N.A			
Avoidance: mean ± SD	13.6 ± 4.7	N.A			
Hyperarousal: mean ± SD	9.4 ± 3.5	N.A			
AIS[b] : mean ± SD	10.7 ± 4.9	3.8 ± 3.0	t = 9.3[d]	85.7	**<0.001**
RBANS, total score[c]: mean ± SD	87.5 ± 21.9	105.5 ± 13.3	t = 5.5[d]	87.6	**<0.001**
Immediate memory[c]: mean ± SD	84.4 ± 20.0	98.3 ± 13.8	t = 4.5[d]	95.3	**<0.001**
Visuospatial construction: mean ± SD	94.9 ± 13.9	101.0 ± 10.3	t = 2.8[d]	99.6	**0.006**
Language: mean ± SD	98.7 ± 19.3	108.9 ± 14.0	t = 3.5	128	**0.001**
Attention: mean ± SD	94.0 ± 17.2	107.1 ± 14.4	t = 4.7	128	**<0.001**
Delayed memory[c]: mean ± SD	90.9 ± 17.6	101.8 ± 13.2	t = 3.9[d]	101.3	**<0.001**
hsCRP (ng/ml)[b]: median (25-75th percentile)	167.0 (92.0-443.0)	200.0 (128.0-377.0)	Mann-Whitney U = 1880.5		0.44
hsTNF-α (pg/ml)[b]: median (25-75th percentile)	0.78 (0.59-0.95)	0.70 (0.60-0.88)	Mann-Whitney U = 1852.0		0.36
IL-6 (pg/ml)[b]: median (25-75th percentile)	0.90 (0.70-1.25)	0.80 (0.60-1.10)	Mann-Whitney U = 1579.0		**0.026**

Abbreviations: PTSD, posttraumatic stress disorder; SD, standard deviation; d.f., degree of freedom; CTQ, Childhood Trauma Questionnaire; PDS, Posttraumatic Diagnostic Scale; AIS, Athens Insomnia Scale; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; hsCRP, high-sensitivity C-reactive protein; hsTNF-α, high-sensitivity tumor necrosis factor-α; IL-6, interleukin-6; N.A., not applicable.

Notes: Bold p values represent significant results.

[a]Coded as follows: 1, junior high school graduate; 2, high school graduate; 3, some college graduate / partial university; 4, university graduate; 5, graduate school graduate.

[b]n = 56 for PTSD patients

[c]n = 72 for healthy controls

[d]Assumption of homogeneity of variance was not satisfied.

Table 2. Genotype and allele distributions of the *CRP* gene polymorphisms in PTSD patients and healthy controls

SNP	Chr	Location	Allele (M/m)	Genotype counts (frequency)							Minor allele frequency				HWE p-value (df = 1)	
				PTSD patients (n = 57)			Control subjects (n = 73)			χ^2 test/ Fisher's exact test p-value	Patients	Controls	χ^2 test p-value (df = 1)	OR (95%CI)	Patients	Controls
				MM	Mm	mm	MM	Mm	mm							
rs2794520	1	3' flanking region	T/C	29 (0.509)	25 (0.439)	3 (0.053)	35 (0.479)	31 (0.425)	7 (0.096)	0.71[a]	0.272	0.308	0.52	0.84 (0.49-1.44)	0.42	0.97
rs1130864	1	3' untranslated region	G/A	51 (0.895)	6 (0.105)	0 (0.000)	64 (0.877)	9 (0.123)	0 (0.000)	0.75	0.053	0.062	0.76	0.85 (0.29-2.45)	0.67	0.57
rs3093059	1	5' flanking region	A/G	47 (0.825)	9 (0.158)	1 (0.018)	50 (0.685)	20 (0.274)	3 (0.041)	0.20[a]	0.096	0.178	0.06	0.49 (0.23-1.05)	0.48	0.58

Abbreviations: PTSD, posttraumatic stress disorder; Chr, chromosome; SNP, single nucleotide polymorphism; M, major allele (in Japanese); m, minor allele (in Japanese); CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; df, degree of freedom.

[a:] Fisher's exact test.

Fig. 1

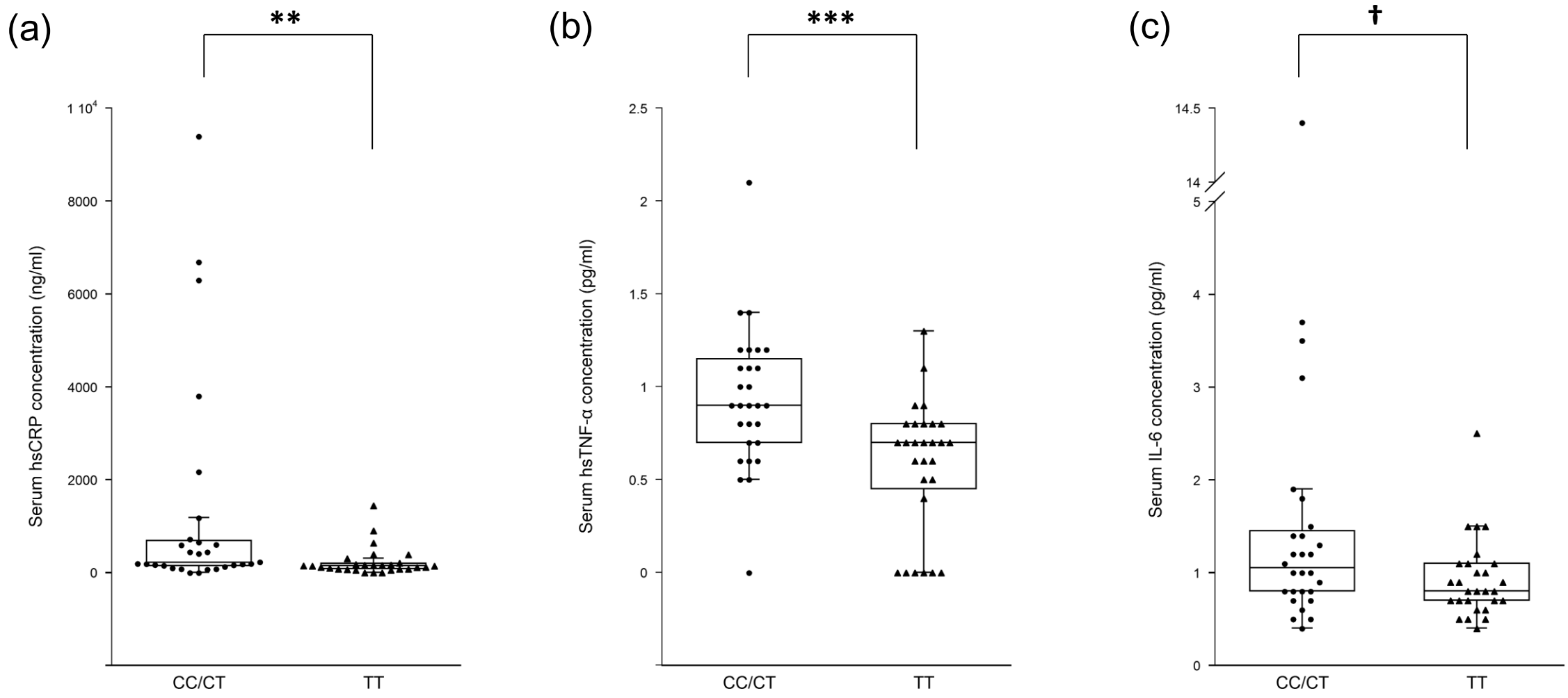

(a)
**
1 10^4
8000
6000
4000
2000
0
Serum hsCRP concentration (ng/ml)
CC/CT
TT
(b)
***
2.5
2
1.5
1
0.5
0
Serum hsTNF-α concentration (pg/ml)
CC/CT
TT
(c)
†
14.5
14
5
4
3
2
1
0
Serum IL-6 concentration (pg/ml)
CC/CT
TT

Fig. 2

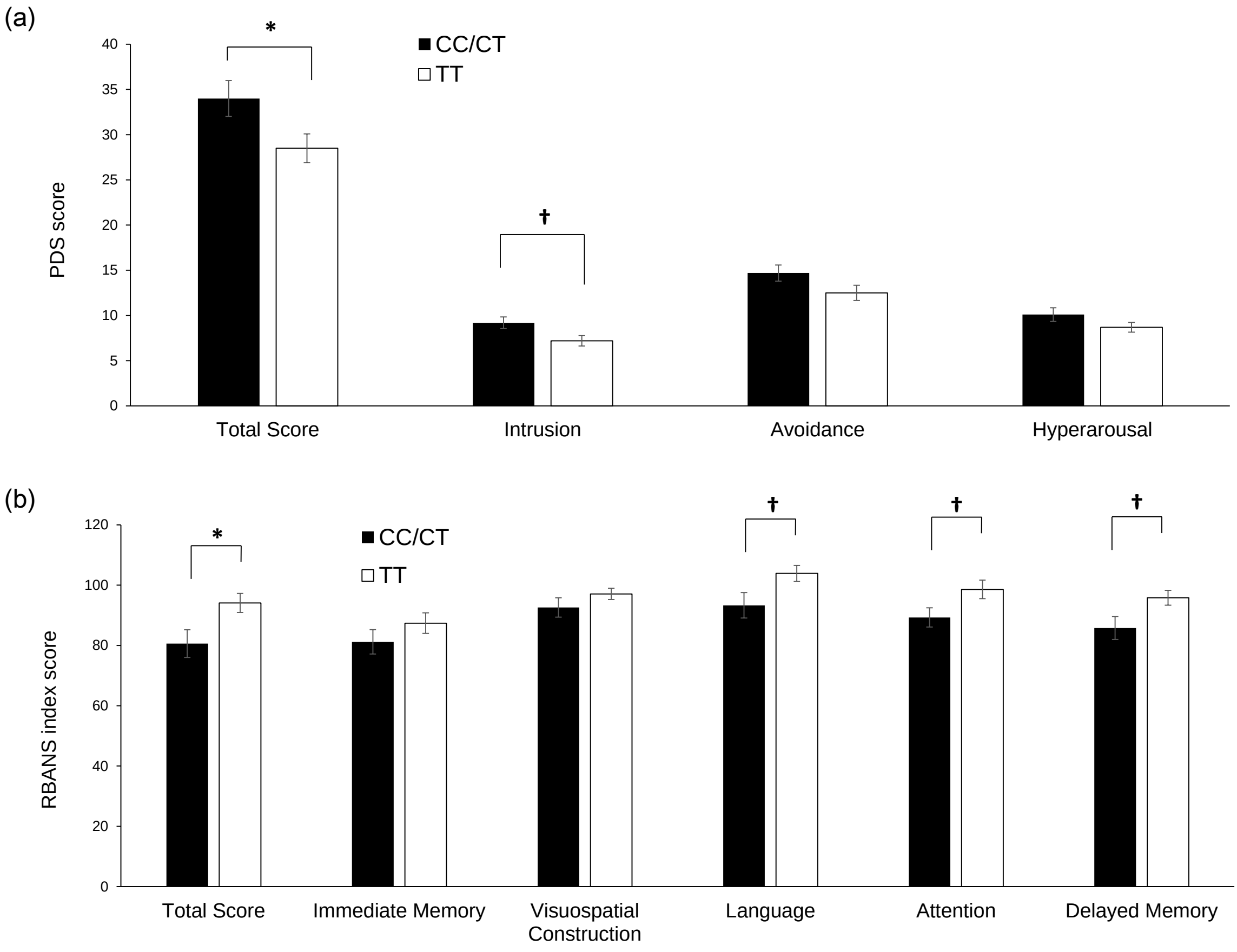
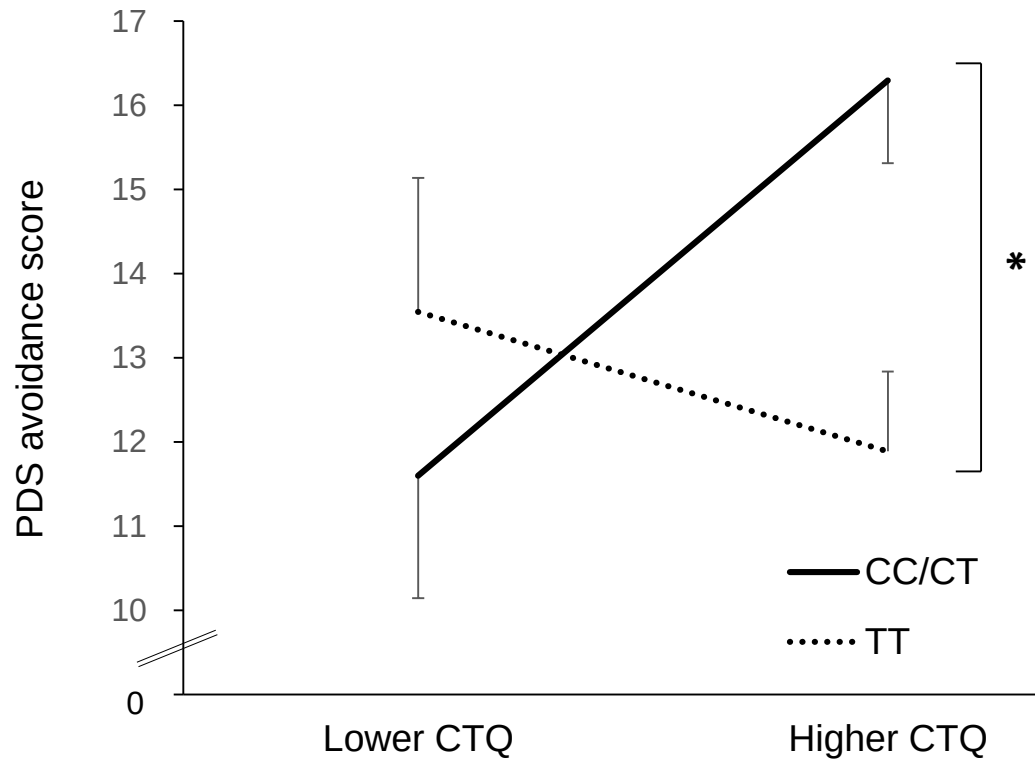

(a)
PDS score
CC/CT
TT
*
†
Total Score
Intrusion
Avoidance
Hyperarousal
(b)
RBANS index score
CC/CT
TT
*
†
†
†
Total Score
Immediate Memory
Visuospatial Construction
Language
Attention
Delayed Memory

Fig. 3



Childhood maltreatment severity

Supplementary Table S1. Demographic/clinical/psychological variables and inflammatory markers in PTSD patients and healthy controls stratified by the *CRP* rs2794520 genotype

	PTSD patients (n = 57)		Analysis			Healthy controls (n = 73)		Analysis		
	CC / CT (n = 28)	TT (n = 29)	statistics	d.f.	p	CC / CT (n = 38)	TT (n = 35)	statistics	d.f.	p
Age, year: mean ± SD	38.8 ± 9.6	40.6 ± 9.0	t = 0.72	55	0.48	33.4 ± 13.6	37.0 ± 14.0	t = 1.1	71	0.27
Education level[a]: median (25-75th percentile)	3 (3-4)	4 (3-4)	Mann-Whitney U = 334.0		0.22	3 (3-4)	3 (3-4)	Mann-Whitney U = 638.0		0.74
Smoking: yes, n(%)	7 (25.0)	1 (3.4)	Fisher's exact test		**0.025**	2 (5.3)	3 (8.6)	Fisher's exact test		0.67
Body mass index: mean ± SD	21.8 ± 3.7	21.3 ± 2.9	t = 0.48	55	0.63	20.5 ± 2.5	21.0 ± 2.7	t = 0.83	71	0.41
Outpatients/inpatients: n/n	28/0	28/1	Fisher's exact test		1.0	N.A	N.A			
Duration of illness, less than 6 months/6 months or more: n/n	2/26	1/28	Fisher's exact test		0.61	N.A	N.A			
Type of index trauma			Fisher's exact test		0.17					
Interpersonal violence: yes, n (%)	22 (78.6)	26 (89.7)				0 (0.0)	0 (0.0)			
Accident: yes, n (%)	1 (3.6)	1 (3.4)				0 (0.0)	0 (0.0)			
Disaster: yes, n (%)	0 (0.0)	1 (3.4)				0 (0.0)	0 (0.0)			
Other: yes, n (%)	5 (17.9)	1 (3.4)				0 (0.0)	0 (0.0)			
Comorbid psychiatric disorder, any: yes, n (%)	26 (92.9)	28 (96.6)				0 (0.0)	0 (0.0)			
Major depressive disorder: yes, n (%)	17 (60.7)	16 (55.2)	$\chi^2 = 0.18$	1	0.67	0 (0.0)	0 (0.0)			
Bipolar disorder: yes, n (%)	2 (7.1)	2 (6.9)	Fisher's exact test		1.0	0 (0.0)	0 (0.0)			
Anxiety disorder: yes, n (%)	14 (50.0)	16 (55.2)	$\chi^2 = 0.15$	1	0.70	0 (0.0)	0 (0.0)			
Alcohol/substance abuse or dependence: yes, n (%)	7 (25.0)	2 (6.9)	Fisher's exact test		0.079	0 (0.0)	0 (0.0)			
Medication, any: yes, n (%)	23 (82.1)	23 (79.3)				0 (0.0)	0 (0.0)			
Antidepressant: yes, n (%)	18 (64.3)	17 (58.6)	$\chi^2 = 0.19$	1	0.66	0 (0.0)	0 (0.0)			
Anxiolytic: yes, n (%)	16 (57.1)	15 (51.7)	$\chi^2 = 0.17$	1	0.68	0 (0.0)	0 (0.0)			
Hypnotic: yes, n (%)	15 (53.6)	8 (27.6)	$\chi^2 = 4.0$	1	**0.046**	0 (0.0)	0 (0.0)			
Antipsychotic: yes, n (%)	9 (32.1)	7 (24.1)	$\chi^2 = 0.45$	1	0.50	0 (0.0)	0 (0.0)			
Mood stabilizer: yes, n (%)	4 (14.3)	3 (10.3)	Fisher's exact test		0.71	0 (0.0)	0 (0.0)			
CTQ[b], total score: mean ± SD	61.8 ± 24.7	58.4 ± 16.6	t = 0.59[e]	45.1	0.56	36.6 ± 8.3	35.3 ± 8.7	t =0.67	71	0.51
Emotional abuse[b]: mean ± SD	15.2 ± 7.3	14.7 ± 6.4	t = 0.29	54	0.77	7.4 ± 3.0	7 ± 3.3	t =0.54	71	0.59
Physical abuse[b]: mean ± SD	9.2 ± 4.7	8.4 ± 4.6	t = 0.68	54	0.50	5.1 ± 0.4	5.1 ± 0.4	t =0.22	71	0.83
Sexual abuse[b]: mean ± SD	9.1 ± 6.4	7.3 ± 4.1	t = 1.2[e]	43.2	0.22	5.4 ± 1.0	5.2 ± 1.0	t =0.84	71	0.41
Emotional neglect[b]: mean ± SD	17.6 ± 6.6	18.6 ± 4.2	t = 0.67[e]	43.9	0.51	12.2 ± 5.0	11.7 ± 5.0	t =0.43	71	0.67
Physical neglect[b]: mean ± SD	10.7 ± 5.2	9.6 ± 2.9	t = 1.0[e]	40.3	0.32	6.6 ± 1.9	6.4 ± 1.7	t =0.49	71	0.62
AIS[b]: mean ± SD	11.5 ± 5.4	9.9 ± 4.3	t = 1.3	54	0.21	4.0 ± 3.4	3.6 ± 2.5	t =0.53	71	0.60
PDS, total score: mean ± SD	34.0 ± 10.5	28.5 ± 8.6	t = 2.2	55	**0.036**	N.A	N.A			
Intrusion: mean ± SD	9.2 ± 3.4	7.2 ± 3.1	t = 2.3	55	0.027	N.A	N.A			
Avoidance: mean ± SD	14.7 ± 4.7	12.5 ± 4.5	t = 1.8	55	0.083	N.A	N.A			
Hyperarousal: mean ± SD	10.1 ± 4.0	8.7 ± 2.9	t = 1.4[e]	49.0	0.16	N.A	N.A			
RBANS[c], total score: mean ± SD	80.6 ± 24.4	94.1 ± 17.0	t = 2.4	55	**0.018**	106.1 ± 13.7	104.8 ± 13.0	t =0.42	70	0.67
Immediate memory[c]: mean ± SD	81.2 ± 21.5	87.4 ± 18.3	t = 1.2	55	0.24	98.2 ± 12.3	98.4 ± 15.5	t =0.70	70	0.94
Visuospatial construction: mean ± SD	92.6 ± 16.9	97.1 ± 10.1	t = 1.2[e]	43.8	0.23	100.3 ± 10.4	101.8 ± 10.1	t =0.59	71	0.56
Language: mean ± SD	93.3 ± 22.3	103.9 ± 14.4	t = 2.2	55	0.035	110.7 ± 15.1	107.0 ± 12.7	t =1.12	71	0.27
Attention: mean ± SD	89.3 ± 16.8	98.6 ± 16.5	t = 2.1	55	0.039	106.9 ± 14.8	107.4 ± 14.1	t =0.14	71	0.89
Delayed memory[c]: mean ± SD	85.8 ± 20.2	95.8 ± 13.3	t = 2.20[e]	46.4	0.033	103.2 ± 13.0	100.3 ± 13.5	t =0.94	70	0.35
hsCRP (ng/ml)[d]: median (25-75th percentile)	212.5 (138.0-703.5)	143.5 (79.3-204.3)	Mann-Whitney U = 233.0		**0.009**	230.0 (141.5-476.0)	192.0 (108.0-325.0)	Mann-Whitney U = 570.5		0.30
hsTNF-α (pg/ml)[d]: median (25-75th percentile)	0.90 (0.70-1.175)	0.675 (0.418-0.80)	Mann-Whitney U = 183.5		**0.001**	0.775 (0.60-0.90)	0.70 (0.470-0.880)	Mann-Whitney U = 544.0		0.18
IL-6 (pg/ml)[d]: median (25-75th percentile)	1.1 (0.80-1.48)	0.80 (0.70-1.10)	Mann-Whitney U = 275.5		0.055	0.80 (0.60-1.0)	0.80 (0.50-1.10)	Mann-Whitney U = 637.5		0.76

Abbreviations: PTSD, posttraumatic stress disorder; SD, standard deviation: d.f., degree of freedom; CTQ, Childhood Trauma Questionnaire; PDS, Posttraumatic Diagnostic Scale; AIS, Athens Insomnia Scale; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; hsCRP, high-sensitivity C-reactive protein; hsTNF-α, high-sensitivity tumor necrosis factor-α; IL-6, interleukin-6; N.A., not applicable.

Notes: Bold p values represent significant results.

[a]Coded as follows: 1, junior high school graduate; 2, high school graduate; 3, some college graduate / partial university; 4, university graduate; 5, graduate school graduate.

[b]n = 56 for PTSD patients, n = 73 for controls, n = 27 for patients with CC/CT, n = 29 for patients with TT

[c]n = 57 for PTSD patients, n = 72 for healthy controls, n = 38 for controls with CC/CT, n = 34 for controls with TT

[d]n = 56 for PTSD patients, n = 73 for controls, n = 28 for patients with CC/CT, n = 28 for patients with TT

[e]Assumption of homogeneity of variance was not satisfied.

Supplementary Methods

Participants

The present study was conducted at three institutes: National Center of Neurology and Psychiatry (NCNP), Tokyo Women's Medical University, and Nagoya City University.

Patients with posttraumatic stress disorder (PTSD) were consecutively recruited at the three institutes and their affiliated hospitals/clinics in Tokyo and Nagoya (two metropolitan areas in Japan). Most patients were outpatients at these hospitals and clinics, and their attending doctors were asked to inform the researcher of all potentially eligible patients. The remaining few patients were outpatients at the nearby clinics and were recruited through advertisements on our website. All patients had already been diagnosed as having PTSD by their attending clinicians. The experience of traumatic events and diagnosis of PTSD were confirmed by the validated Japanese version (Nagae et al., 2007) of the Posttraumatic Diagnostic Scale (PDS) (Foa, 1995). In addition, the validated Japanese version (Otsubo et al., 2005) of the Mini International Neuropsychiatric Interview (M.I.N.I) (Sheehan et al., 1998) was administered by an expert clinician or clinical psychologist to identify any other Axis-I disorders as well as PTSD. Patients with comorbid schizophrenia and those with marked manic episodes of bipolar disorder were excluded from the study.

Healthy volunteers were recruited at NCNP through advertisements in free local magazines and on our website and by word of mouth. The PDS was administered to healthy controls in order to examine the presence/absence of traumatic experiences and, if present, they were excluded from this study. All healthy subjects were interviewed by a board-certified psychiatrist which included M.I.N.I and non-structured interview, and those

who demonstrated current Axis-I disorders or apparent signs of past psychiatric disorders were excluded.

A significant subset of the present participants (86 of the total 130 participants: 66.2%) had been included in our previous study on inflammatory markers in PTSD patients (Imai et al., 2018).

Psychological assessment

Psychological/clinical characteristics and cognitive functions of participants were assessed by using the following self-report questionnaires and neuropsychological test battery:

Posttraumatic Diagnostic Scale (PDS) (Foa, 1995)

The PDS comprises four parts that evaluate traumatic experiences reflecting Criteria A of the DSM-IV (Parts 1 & 2), PTSD severity during the past month reflecting Criteria B-D (Part 3), and functional impairments associated with PTSD symptoms (Part 4). In the present study, we administered Parts 1 & 2 to all participants in order to determine the presence/absence of traumatic experiences, and if present, Parts 3 & 4 were administered for the assessment of diagnosis and severity of PTSD. We used the Japanese version of PDS that has demonstrated good reliability and validity (Itoh et al., 2017; Nagae et al., 2007).

Childhood Trauma Questionnaire (CTQ; Bernstein et al., 2003)

The commonly-used 28-item version of CTQ includes five subscales that assess different types of childhood maltreatment: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect, each comprising five items, along with three items to assess response validity. In the present study, we used the CTQ after translating it from the original

English version into Japanese by one of the authors (HH), which was then back-translated into English by another Japanese researcher, and the back-translated English version was sent to and approved by the original author (Professor David Bernstein). Cronbach α coefficients of the 5 CTQ subscales, i.e., emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect, in the present sample (n = 129) were 0.92, 0.84, 0.94, 0.91, and 0.71, respectively. It was administered to both patients and control subjects; there was one subject (patient) who did not complete this questionnaire.

Athens Insomnia Scale (AIS; Soldatos et al., 2000)

The AIS is an 8-item self-report questionnaire used to assess insomnia symptoms, including both nocturnal sleep problems and daytime dysfunction (Soldatos et al., 2000). In the present study the validated Japanese version (Okajima et al., 2013) of the AIS was administered to both patients and controls. There was one subject (patient) who did not complete this questionnaire.

Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph et al., 1998)

The RBANS is a neuropsychological test battery that assesses five main cognitive domains derived from 12 subtests including immediate memory (consisting of list learning and story memory), visuospatial construction (figure copy and line orientation), language (picture naming and semantic fluency), attention (digit span and coding), and delayed memory (list recall, list recognition, story recall, and figure recall). Index scores of the 5 domains can be combined to generate a total RBANS score, which provides a global measure of neuropsychological performance. The RBANS has demonstrated good psychometric properties in both clinical and nonclinical populations (Duff et al., 2005;

Matsui et al., 2010; McKay et al., 2007; Weber, 2003). We used the Japanese version of PDS that has demonstrated good reliability and validity (Itoh et al., 2017; Nagae et al., 2007). It was administered in a quiet room on a one-on-one basis, with an average completion time of approximately 30 min. Scoring was performed in accordance with the manual guidelines (Matsui et al., 2010; Randolph et al., 1998). There was one subject (healthy control) who did not complete the memory assessment of RBANS; for this subject, only the valid part of data was included in the analysis.

Measurement of inflammatory markers

We measured serum levels of three proinflammatory markers, including high-sensitivity C-reactive protein (hsCRP), high-sensitivity tumor necrosis factor-α (hsTNF-α), and interleukin-6 (IL-6). The detection limit for hsCRP was 51 ng/ml; hsCRP levels of nine subjects (7.0 % of the total 129 subjects) were below this limit. For hsTNF-α, the detection limit was 0.6 pg/ml (except that this limit was 0.15 pg/ml for 42 subjects who were enrolled in this study after more sensitive reagent for hsTNF-α became available); hsTNF-α levels of 13 subjects (10.1%) were below this limit. For IL-6, the detection limit was 0.3 pg/ml; none of the subjects showed IL-6 levels below this limit. Values under these detection limits were treated as 0 (ng/ml or pg/ml). There was one subject (patient) who did not provide a blood sample for the measurement of these inflammatory markers.

References

Bernstein, D.P., Stein, J.A., Newcomb, M.D., Walker, E., Pogge, D., Ahluvalia, T., Stokes, J.,

Handelsman, L., Medrano, M., Desmond, D., Zule, W., 2003. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. Child Abuse Negl. 27, 169-190.

Duff, K., Beglinger, L.J., Schoenberg, M.R., Patton, D.E., Mold, J., Scott, J.G., Adams, R.L., 2005. Test-retest stability and practice effects of the RBANS in a community dwelling elderly sample. J. Clin. Exp. Neuropsychol. 27, 565-575.

Foa, E., 1995. The posttraumatic diagnostic scale (PDS) manual. Minneapolis, MN: National Computer Systems.

Imai, R., Hori, H., Itoh, M., Lin, M., Niwa, M., Ino, K., Ogawa, S., Ishida, M., Sekiguchi, A., Matsui, M., Kunugi, H., Akechi, T., Kamo, T., Kim, Y., 2018. Inflammatory markers and their possible effects on cognitive function in women with posttraumatic stress disorder. J. Psychiatr. Res. 102, 192-200.

Itoh, M., Ujiie, Y., Nagae, N., Niwa, M., Kamo, T., Lin, M., Hirohata, S., Kim, Y., 2017. The Japanese version of the Posttraumatic Diagnostic Scale: Validity in participants with and without traumatic experiences. Asian J. Psychiatr. 25, 1-5.

Matsui, M., Kasai, Y., Nagasaki, M., 2010. Reliability and validity of the Japanese version of the Repeatable Battery for the Assessment of Neuropsychological Status. Toyama Med. J. 21, 31-36.

McKay, C., Casey, J.E., Wertheimer, J., Fichtenberg, N.L., 2007. Reliability and validity of the RBANS in a traumatic brain injured sample. Arch. Clin. Neuropsychol. 22, 91-98.

Nagae, N., Hirohata, S., Shimura, Y., Yamada, S., Foa, E., Nedate, K., Kim, Y., 2007. Development of the Japanese version of the Posttraumatic Diagnostic Scale: ascertaining its reliability and validity among university students. Japanese J. Trauma. Stress. 5, 51-56.

Okajima, I., Nakajima, S., Kobayashi, M., Inoue, Y., 2013. Development and validation of the Japanese version of the Athens Insomnia Scale. Psychiatry Clin. Neurosci. 67, 420-425.

Otsubo, T., Tanaka, K., Koda, R., Shinoda, J., Sano, N., Tanaka, S., Aoyama, H., Mimura, M., Kamijima, K., 2005. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry Clin. Neurosci. 59, 517-526.

Randolph, C., Tierney, M.C., Mohr, E., Chase, T.N., 1998. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): preliminary clinical validity. J. Clin. Exp. Neuropsychol. 20, 310-319.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the

development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry. 59 Suppl 20, 22-33;quiz 34-57.

Soldatos, C.R., Dikeos, D.G., Paparrigopoulos, T.J., 2000. Athens Insomnia Scale: validation of an instrument based on ICD-10 criteria. J. Psychosom. Res. 48, 555-560.

Weber, B., 2003. RBANS has reasonable test-retest reliability in schizophrenia. Evid. Based Ment. Health. 6, 22.