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SOCIETY OF  
NUCLEAR MEDICINE  
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# Neuroinflammation in Patients with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: An $^{11}\text{C}$ -(*R*)-PK11195 PET Study

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Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a disease characterized by chronic, profound, disabling, and unexplained fatigue. Although it is hypothesized that brain inflammation is involved in the pathophysiology of CFS/ME, there is no direct evidence of neuroinflammation in patients with CFS/ME. Activation of microglia or astrocytes is related to neuroinflammation.  $^{11}\text{C}$ -(*R*)-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline-carboxamide ( $^{11}\text{C}$ -(*R*)-PK11195) is a ligand of PET for a translocator protein that is expressed by activated microglia or astrocytes. We used  $^{11}\text{C}$ -(*R*)-PK11195 and PET to investigate the existence of neuroinflammation in CFS/ME patients. **Methods:** Nine CFS/ME patients and 10 healthy controls underwent  $^{11}\text{C}$ -(*R*)-PK11195 PET and completed questionnaires about fatigue, fatigue sensation, cognitive impairments, pain, and depression. To measure the density of translocator protein, non-displaceable binding potential ( $\text{BP}_{\text{ND}}$ ) values were determined using linear graphical analysis with the cerebellum as a reference region. **Results:** The  $\text{BP}_{\text{ND}}$  values of  $^{11}\text{C}$ -(*R*)-PK11195 in the cingulate cortex, hippocampus, amygdala, thalamus, midbrain, and pons were 45%–199% higher in CFS/ME patients than in healthy controls. In CFS/ME patients, the  $\text{BP}_{\text{ND}}$  values of  $^{11}\text{C}$ -(*R*)-PK11195 in the amygdala, thalamus, and midbrain positively correlated with cognitive impairment score, the  $\text{BP}_{\text{ND}}$  values in the cingulate cortex and thalamus positively correlated with pain score, and the  $\text{BP}_{\text{ND}}$  value in the hippocampus positively correlated with depression score. **Conclusion:** Neuroinflammation is present in widespread brain areas in CFS/ME patients and was associated with the severity of neuropsychologic symptoms. Evaluation of neuroinflammation in CFS/ME patients may be essential for understanding the core pathophysiology and for developing objective diagnostic criteria and effective medical treatments.

**Key Words:** neuroinflammation; chronic fatigue syndrome (CFS); myalgic encephalomyelitis (ME);  $^{11}\text{C}$ -(*R*)-PK11195; positron emission tomography (PET)

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**C**hronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a disease characterized by chronic, profound, disabling, and unexplained fatigue (*1*). CFS/ME patients experience neuropsychologic symptoms, including cognitive impairment (thinking difficulty, decreased alertness, and impaired memory and concentration), chronic widespread pain (muscle pain, pain in multiple joints, headaches, and sore throat), and depressive symptoms (*1*). To date, no specific biomarkers for diagnosing and evaluating the severity of CFS/ME have been established.

The neuropsychologic symptoms in CFS/ME suggest that the central nervous system is involved in the pathophysiology, and several studies including ours have shown central nervous system abnormalities in CFS/ME patients. Our previous study (*2*) with PET showed hypoperfusion and reduction of biosynthesis of neurotransmitters such as glutamate, aspartate, and  $\gamma$ -aminobutyric acid through acetylcarnitine in the frontal, cingulate, temporal, and occipital cortices; basal ganglia; and hippocampus in CFS/ME patients. CFS/ME patients also had a decrease in serotonin transporter densities in the rostral sector of the anterior cingulate cortex, and the serotonin transporter density in the middle sector of the cingulate cortex was negatively correlated with pain score (*3*). Furthermore, our voxel-based morphometry studies demonstrated volume reduction of the bilateral prefrontal cortices in CFS/ME patients, and the volume reduction level in the right prefrontal cortex was associated with the severity of fatigue (*4*).

Other fatigue-related neurologic diseases such as multiple sclerosis, Parkinson disease, and postpolio fatigue syndrome are also thought to arise from dysfunction of the central nervous system (*5,6*), and it has been suggested that neuroinflammation is involved in the pathogenesis or progression of these diseases (*7*). There are also related reports that the levels of proinflammatory cytokines in the peripheral blood and cerebral spinal fluid, which might be indicative of neuroinflammation, are higher in CFS/ME patients than in healthy controls (*8,9*), suggesting that neuroinflammation may also be related to the pathophysiology of CFS/ME (*10*). However, to prove the existence of neuroinflammation and its possible contribution to the pathophysiology of CFS/ME, it is necessary to directly evaluate

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neuroinflammation using a neuroimaging technique such as PET and to investigate the relations between neuroinflammation and symptom severity.

Neuroinflammation is evidenced by activation of microglia or astrocytes, and activated glial cells exhibit an increase in expression of the 18-kDa translocator protein (TSPO).  $^{11}\text{C}$ -(*R*)-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline-carboxamide ( $^{11}\text{C}$ -(*R*)-PK11195) is a ligand of PET for the TSPO that is expressed in activated microglia or astrocytes and is widely used to assess neuroinflammation in neurologic diseases (7). Recently, it has been reported that although TSPO polymorphisms affect the affinities of second-generation TSPO radioligands,  $^{11}\text{C}$ -(*R*)-PK11195 binds to a different site on TSPO with no apparent difference in affinity (11). Therefore, in the present study, we used  $^{11}\text{C}$ -(*R*)-PK11195 and PET to assess neuroinflammation in patients with CFS/ME. As far as we know, this is the first study to investigate neuroinflammation in CFS/ME patients. Evidence of neuroinflammation in this population would contribute to the clarification of CFS/ME pathophysiology and to the development of objective diagnostic criteria, criteria for evaluation of disease severity, and effective medical treatments.

## MATERIALS AND METHODS

### Participants

Nine patients who fulfilled the international diagnostic criteria for CFS (12) and ME (13) were recruited from the Fatigue Clinical Center at Osaka City University Hospital, Osaka, Japan. Patients taking medications known to affect autonomic nerve function, including  $\beta$ -blockers, benzodiazepines, corticosteroids, and medications known to affect the central nervous system (e.g., methylphenidate, dexamphetamine, antidepressants, and antipsychotic drugs), were also excluded. Ten age- and sex-matched healthy controls who had no symptoms related to fatigue and no problems in their daily activities were also recruited. Individuals with neuropsychiatric disorders were identified by doctors of general medicine, neurology, and psychiatry at the Osaka City University Hospital and excluded. Demographic and clinical characteristics of the CFS/ME and healthy control groups are shown in

[Table 1] Table 1. All participants were right-handed.

**TABLE 1**  
Demographic and Clinical Characteristics of All Participants

Characteristic	CFS/ME ( <i>n</i> = 9)	Healthy control ( <i>n</i> = 10)	<i>P</i>
Age (y)	38.4 ± 5.1	39.1 ± 6.0	0.8030
Sex (F/M)	6/3	7/3	0.8843
Disease duration (y)	5.2 ± 7.3		
VAS of fatigue sensation (score)	60.4 ± 24.2	27.3 ± 23.2	0.0074
Chalder fatigue scale (score)	21.9 ± 7.1	10.9 ± 5.0	0.0011
Cognitive impairment (score)	9.6 ± 3.1	4.0 ± 3.0	0.0013
Pain (score)	9.1 ± 4.0	2.2 ± 1.8	0.0001
CES-D (score)	17.6 ± 6.5	7.5 ± 5.6	0.0020

Data are mean ± SD or number/number. Student *t* tests or Fisher exact test was conducted.

The severity of neuropsychologic symptoms was measured using the following questionnaires: a visual analog scale (VAS) for fatigue sensation (14), the Chalder fatigue scale, the Center for Epidemiological Studies depression scale (CES-D), cognitive impairment and pain scores, and duration of disease. The VAS for fatigue sensation ranges from 0 (no fatigue) to 100 (complete exhaustion). The Chalder fatigue scale (15) consists of 11 questions rated on a 4-point Likert scale. The total score ranges from 0 to 33, with higher scores indicating a greater degree of daily fatigue. The CES-D consists of 20 questions rated on a 4-point Likert scale. The total score ranges from 0 to 60, with higher scores indicating a greater degree of depression (16). The cognitive impairment score was assessed using 4 questions on thinking difficulty, inability to concentrate, impairment in memory, and absence of alertness. The pain score was assessed using 4 questions on headache, sore throat, myalgia, and arthralgia. All questions were rated on a 4-point Likert scale, and the total scores range from 0 to 16, with higher scores indicating a greater degree of cognitive impairment and pain (3).

The Ethics Committee of Osaka City University Graduate School of Medicine approved the protocol, and all participants provided written informed consent for participation in the study. This study is registered with the University Hospital Medical Information Network, number UMIN000005469.

### Cytokine Assay

Whole-blood samples were collected just before the  $^{11}\text{C}$ -(*R*)-PK11195 PET scan. After collection, blood samples were coagulated at room temperature for more than 30 min and centrifuged at 3,000 rpm for 5 min to obtain sera. Sera were stored at  $-80^{\circ}\text{C}$  until measurements. Measurements of serum tumor necrosis factor- $\alpha$ , interferon- $\gamma$ , interleukin-1 $\beta$ , and interleukin-6 were performed by the Mitsubishi Chemical Medience Corp. The lower detection limit was 0.550 pg/mL for tumor necrosis factor- $\alpha$ , 1.560 pg/mL for interferon- $\gamma$ , 0.125 pg/mL for interleukin-1 $\beta$ , and 0.300 pg/mL for interleukin-6. Values less than the detection limit were regarded as zero.

### MR Imaging

Structural brain images were obtained using a 1.5-T Signa Horizon LX scanner (GE Healthcare) and used to identify the brain regions of interest. Three-dimensional spoiled gradient recalled acquisition was used in the steady-state sequence with the following parameters: repetition time, 17 ms; echo time, 2 ms; flip angle,  $40^{\circ}$ ; and slice thickness, 1.5 mm.

### PET Data Acquisition

PET experiments were conducted using an Eminence-B PET scanner (Shimadzu).  $^{11}\text{C}$ -(*R*)-PK11195 was injected during 30 s after the start of the PET scan. The tracer dose was  $218.0 \pm 31.5$  MBq (mean ± SD), with a specific activity of  $55.4 \pm 22.9$  GBq/ $\mu\text{mol}$ . Dynamic data were collected over 60 min as 23 temporal frames. Before the dynamic scan, attenuation correction factors were measured with a 10-min transmission scan using an external source of  $^{137}\text{Cs}$ . Images were reconstructed with segmented attenuation correction, using Fourier rebinning followed by 2-dimensional filtered back-projection applying a ramp filter cutoff at the Nyquist frequency. The intrinsic spatial resolution was 4 mm in full width at half maximum for the in-slice direction and 6 mm in full width at half maximum for the axial direction. A 3-dimensional gaussian kernel of 6 mm in full width at half maximum was applied for postprocessing. No arterial blood sampling was performed.

### Image Data Processing

Structural MR images were coregistered to PET images (summation PET images from 15 to 45 min and dynamic PET images) by using normalized mutual function based on a simple multiresolution hill-climbing algorithm (17) on the PMOD Fusion Tool, version 3.2

(PMOD Technologies). Each MR image was spatially normalized to the Montréal Neurologic Institute stereotactic brain, and the corresponding PET images were normalized using the same parameters. Thus, the PET and MR images of each participant were anatomically standardized to the Montréal Neurologic Institute template. Because there is no brain region devoid of TSPO (although a cerebellum reference may add stability to quantitative analyses (18)), and because there was no difference between mean time-activity curves of standardized uptake value in CFS/ME patients and healthy controls (Fig. 1C), we generated parametric images of regional  $^{11}\text{C}$ -(R)-PK11195 nondisplaceable binding potential ( $\text{BP}_{\text{ND}}$ ) using linear graphical analysis according to Logan (19), with the cerebellum as a reference region and corresponding to the linear part of the plot covering the last 40–60 min of measurement (20).

### Image Data Analysis

All  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  images were normalized to the Montréal Neurologic Institute space, smoothed with an isotropic 8-mm gaussian kernel, and analyzed by Statistical Parametric Mapping 5 software (SPM5; Wellcome Department of Cognitive Neurology) using a categorical design. The between-group comparison (CFS/ME patients vs. healthy controls) of  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  and correlation analysis of  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  values and clinical scores were performed on a voxel-by-voxel basis using *t* statistics with the statistical threshold set at a *P* value of less than 0.005 at the voxel level and a *P* value of less than 0.05 with a correction for multiple comparisons at the cluster level for the entire brain (familywise error). The regions of interest for the cingulate cortex, hippocampus, amygdala, thalamus, midbrain, and pons were defined from the Wake Forest University PickAtlas (21) and applied to the smoothed images.

### Statistical Analyses

Values are shown as mean  $\pm$  SD unless stated otherwise. Symptom severity, blood-cytokine data, and regional  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  values were compared across the groups using Student *t* tests. Cate-

goric variables were compared across the groups using Fisher exact tests. The relation between symptom severity scores and  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  values was analyzed using Pearson correlation in the CFS/ME group. All *P* values were 2-tailed, and values of less than 0.05 were considered to be statistically significant. Statistical analyses were performed using SPSS 20.0 software (SPSS).

## RESULTS

### Clinical Scores and Cytokine Data

The VAS score of fatigue sensation, Chalder fatigue scale score, cognitive impairment score, pain score, and CES-D score were higher in CFS/ME patients than in healthy controls (Table 1). There were some missing cytokine values in the CFS/ME group because of insufficient blood samples (tumor necrosis factor- $\alpha$  missing for 2 participants, and interferon- $\gamma$  and interleukin-6 missing for 1 participant). In the remaining participants, the concentration of interferon- $\gamma$  tended to be higher in CFS/ME patients than in healthy controls ( $1.66 \pm 2.77$  vs.  $0.00 \pm 0.00$  pg/mL, *P* = 0.0740). The concentration of tumor necrosis factor- $\alpha$  ( $0.80 \pm 0.63$  vs.  $0.45 \pm 0.80$  pg/mL, *P* = 0.3735), interleukin-1 $\beta$  ( $1.02 \pm 0.99$  vs.  $0.40 \pm 0.60$  pg/mL, *P* = 0.1129), and interleukin-6 ( $0.60 \pm 0.56$  vs.  $0.75 \pm 1.10$  pg/mL, *P* = 0.7277) was similar between the groups.

### $^{11}\text{C}$ -(R)-PK11195 $\text{BP}_{\text{ND}}$ and Clinical Correlations

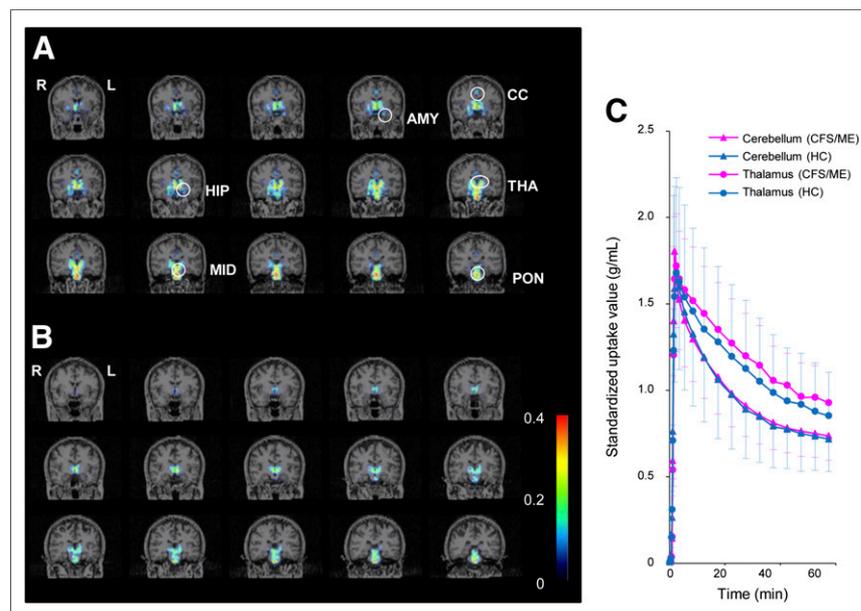
Representative maps of  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  are presented in Figure 1.  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  values for CFS/ME patients (Fig. 1A) were higher than those for healthy controls (Fig. 1B) in widespread brain regions. Region-of-interest analysis revealed that  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  values in CFS/ME patients were significantly higher than those in healthy controls in the cingulate, hippocampus, thalamus, midbrain, and pons and tended to be higher in the amygdala (Table 2).

[Table 2]

The SPM analysis also revealed significantly higher  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  in CFS/ME patients than in healthy controls in the left thalamus, midbrain, and pons (Fig. 2). The Montréal Neurologic Institute coordinates of peak  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  (highest *t* score on SPM5) in the CFS/ME group corresponded to the intralaminar nucleus of the left thalamus ( $-6, -22, -4$ ) and midbrain ( $-4, -30, -14$ ). In the CFS/ME group, the peak value of  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  in the left thalamic intralaminar nucleus ( $-6, -22, -4$ ) was positively correlated with the cognitive impairment score ( $r = 0.86$ ; *P* = 0.0028) and tended to be positively correlated with the VAS score for fatigue sensation ( $r = 0.63$ ; *P* = 0.0683), and the peak value of  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  in the midbrain ( $-4, -30, -14$ ) was positively correlated with the cognitive impairment score ( $r = 0.72$ , *P* = 0.0293).

In the CFS/ME group, the SPM correlation analysis revealed that  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  in the amygdala was positively correlated with cognitive impairment score (Fig. 3A),  $^{11}\text{C}$ -(R)-PK11195  $\text{BP}_{\text{ND}}$  in the hippocampus was positively correlated with

RGB



**FIGURE 1.** (A and B) Representative parametric PET images of  $^{11}\text{C}$ -(R)-PK11195 binding in CFS/ME patient (A) and healthy control (B). Anatomic locations were mapped on coronal MR images. (C) Mean ( $\pm$ SD) regional tissue time-activity curves of  $^{11}\text{C}$ -(R)-PK11195 for region of interest in cerebellum and thalamus in CFS/ME group and HC group. Scale indicates  $\text{BP}_{\text{ND}}$ . AMY = amygdala; CC = cingulate cortex; HC = healthy control; HIP = hippocampus; MID = midbrain; THA = thalamus; and PON = pons.

[Fig. 2]

[Fig. 3]

**TABLE 2**  
Regional  $^{11}\text{C}$ -(*R*)-PK11195  $\text{BP}_{\text{ND}}$  in CFS/ME Patients and Healthy Controls

Region	CFS/ME	Healthy control	<i>P</i>	Increase (%)
Midbrain	0.181 ± 0.027	0.123 ± 0.024	0.0001	47
Pons	0.155 ± 0.030	0.107 ± 0.028	0.0021	45
Thalamus	0.097 ± 0.021	0.058 ± 0.023	0.0013	66
Cingulate	0.010 ± 0.008	0.003 ± 0.003	0.0353	199
Amygdala	0.057 ± 0.031	0.031 ± 0.025	0.0586	85
Hippocampus	0.053 ± 0.023	0.029 ± 0.017	0.0212	81

Data are mean ± SD. Student *t* tests or Fisher exact test was conducted.

CES-D score (Fig. 3B), and  $^{11}\text{C}$ -(*R*)-PK11195  $\text{BP}_{\text{ND}}$  in the thalamus tended to be correlated positively with pain score (voxel level,  $P < 0.005$ ; cluster level,  $P > 0.05$ ; Fig. 3C). There were no other correlations between  $^{11}\text{C}$ -(*R*)-PK11195  $\text{BP}_{\text{ND}}$  in any brain regions and other parameters (age, duration of disease, VAS score of fatigue sensation, score of Chalder fatigue scale, or cytokine concentration).

## DISCUSSION

The present PET study demonstrated a higher  $^{11}\text{C}$ -(*R*)-PK11195  $\text{BP}_{\text{ND}}$  in patients with CFS/ME than in healthy controls in widespread brain regions.  $^{11}\text{C}$ -(*R*)-PK11195  $\text{BP}_{\text{ND}}$  in the CFS/ME group was closely related to the severity of neuropsychologic symptoms including fatigue sensation, cognitive impairment, pain, and depression. To our knowledge, this was the first study to provide evidence of neuroinflammation in CFS/ME patients and to demonstrate its possible relation to the pathophysiology of CFS/ME.

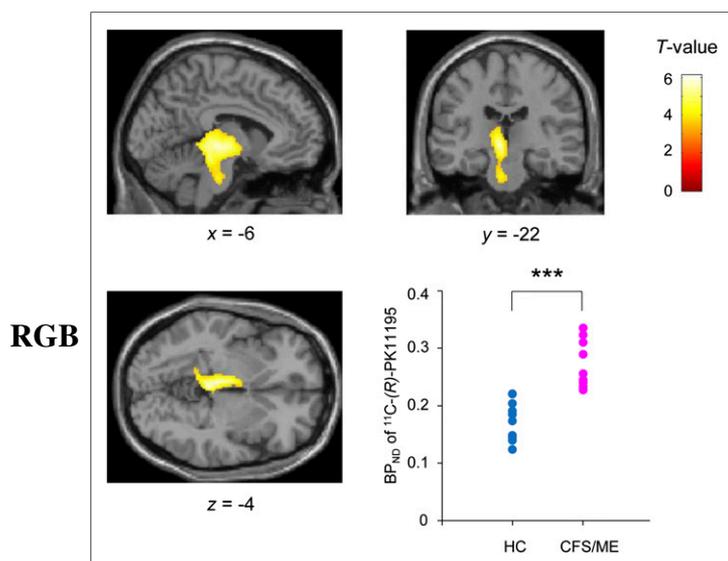
Although the mechanisms underlying neuroinflammation in CFS/ME are unclear, one plausible mechanism is overactivity of neurons. To compensate for functional loss associated with

CFS/ME, patients have to exert greater effort to perform daily activities, resulting in enhanced neural activation (22). Overactivation of *N*-methyl-D-aspartate receptors caused by enhanced neural activation results in production of proinflammatory cytokines, reactive oxygen species, and nitrogen species that cause inflammation (23). Another plausible mechanism is the immunologic response to the initial infectious process (24), which can also induce the production of the proinflammatory cytokines, reaction oxygen species, and nitrogen species that cause neuroinflammation (10,25).

Inflammation of the thalamus, particularly of the left intralaminar nucleus, was higher in CFS/ME patients than in healthy controls as shown in Figure 2. Previous studies have discussed the lateralization of  $^{11}\text{C}$ -(*R*)-PK11195  $\text{BP}_{\text{ND}}$  that might have resulted from selection bias assessment of predominantly right-handed subjects (26,27). In addition, the level of inflammation in these brain regions was positively associated with cognitive impairment score and fatigue sensation in CFS/ME patients. The intralaminar nucleus of the thalamus and midbrain are involved in the reticular activating system (28), which is considered to play a role in arousal and awareness states. In a previous pharmacologic study of CFS/ME, treatment with clonidine, which controls arousal level through the postsynaptic actions at  $\alpha_2$  adrenoreceptors, improved cognitive impairment (29). Inflammation of the thalamus and midbrain may induce cognitive impairment and severe fatigue sensation by perturbing the arousal and awareness states.

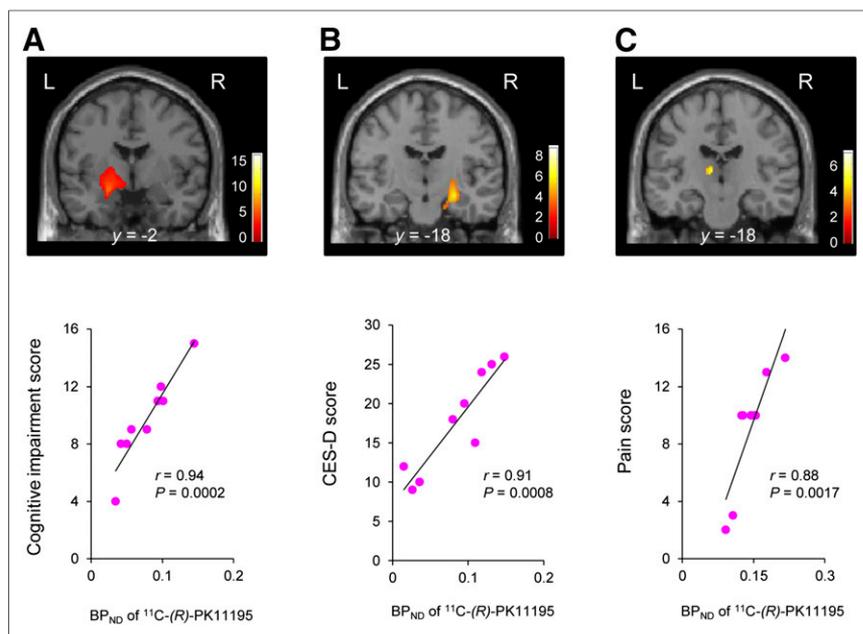
Inflammation of the amygdala was also related to the cognitive impairment score in CFS/ME patients. The amygdala receives direct projection from the thalamus, which monitors the sensory information and mediates the facilitation of attention (30). Neuroinflammation in this brain region may reduce cognitive activity through the deterioration of attentional function (31). Although our voxel-based morphometry studies demonstrated volume reduction of bilateral prefrontal cortices in CFS/ME patients (4), we did not focus on neuroinflammation in the prefrontal cortex and did not apply partial-volume correction since the  $\text{BP}_{\text{ND}}$  range of  $^{11}\text{C}$ -(*R*)-PK11195 in the prefrontal cortex was extremely small (0–0.0004).

In the present study, although CFS/ME patients did not meet diagnostic criteria for major depression or other psychiatric disorders, their depressive scores were higher than those of healthy controls. This finding supports a previous report that depressive symptoms are present in CFS/ME (32). Interestingly, we observed a relation between the severity of depressive symptoms and the level of inflammation in the hippocampus in CFS/ME patients. Neuroinflammation in several brain regions, including



**FIGURE 2.** Statistical parametric maps of  $\text{BP}_{\text{ND}}$  of  $^{11}\text{C}$ -(*R*)-PK11195 in CFS/ME patients and healthy controls. Anatomic locations were mapped on template brains. Peak coordinates (–6, –22, –4) correspond to intralaminar nucleus of left thalamus.  $\text{BP}_{\text{ND}}$  of this coordinate for each individual in CFS/ME and HC groups is plotted in bottom right panel. \*\*\* $P < 0.0001$  (Student *t* test). HC = healthy control.

RGB



**FIGURE 3.** Relationships between <sup>11</sup>C-(R)-PK11195 BP<sub>ND</sub> and neuropsychologic symptoms in CFS/ME patients. Top row: Statistical parametric maps of correlations between <sup>11</sup>C-(R)-PK11195 BP<sub>ND</sub> in amygdala (A), hippocampus (B), and thalamus (C) and cognitive impairment, depression, and pain scores, respectively. Anatomic locations were mapped on template coronal brains. Bottom row: Scatterplots of <sup>11</sup>C-(R)-PK11195 BP<sub>ND</sub> in amygdala (–22, –2, 14) and cognitive impairment score (A), <sup>11</sup>C-(R)-PK11195 BP<sub>ND</sub> in hippocampus (26, –14, –16) and depression (CES-D) score (B), and <sup>11</sup>C-(R)-PK11195 BP<sub>ND</sub> in thalamus (–10, –18, 8) and pain score (C). Pearson coefficient value ( $r$ ) and  $P$  value are shown for each relation. Scales show  $t$  values.

the hippocampus, was observed in an animal model of depression (33), and abnormal neurogenesis in the hippocampus is considered to be related to the pathophysiology of mood disorder (34). These results suggest that neuroinflammation of the hippocampus is associated with the severity of depressive symptoms in CFS/ME patients.

An association between inflammation of the thalamus and pain tends to be observed in CFS/ME patients using SPM-based correlation analysis. In addition, region-of-interest–based correlation analysis revealed that pain score was positively correlated with averaged BP<sub>ND</sub> values of the cingulate cortex ( $r = 0.73$ ,  $P = 0.0254$ ) and thalamus ( $r = 0.78$ ,  $P = 0.0126$ ). Functional interaction between the anterior part of the cingulate cortex and thalamus has been reported to suppress pain (35); therefore, inflammation in the cingulate cortex and thalamus may decrease pain suppression in CFS/ME patients. Our previous PET study showed that impaired serotonin dynamics in the anterior cingulate cortex were associated with the severity of pain in CFS/ME patients (3), suggesting that serotonergic dysfunction is also related to pain in these patients. Future combined PET studies with <sup>11</sup>C-(R)-PK11195 and a PET ligand for serotonin transporters would clarify inflammatory and serotonergic mechanisms and their interactions associated with pain in CFS/ME patients. We have already started this type of combined PET study on the same patients.

Further studies are needed for confirmation of this finding of the existence of neuroinflammation, and its correlation in some way with deterioration, in CFS/ME patients. Also, the relation between neuroinflammation and peripheral proinflammatory cytokines remains unclear. It is difficult to accurately measure the levels

of serum cytokines because of their short half-lives and the degradation of molecules that may occur during the storage of blood samples (36). This suggests that neuroinflammation should be assessed using PET rather than by measuring peripheral proinflammatory cytokines. However, analysis for the BP<sub>ND</sub> of <sup>11</sup>C-(R)-PK11195 had several limitations. Although we showed no change in the identical standardized uptake value curves of the reference region (cerebellum) between groups (Fig. 1C), these curves are not proof of no changes in the reference region, as there could be between-group input function differences; arterial input functions might have confirmed this issue more authoritatively. The BP<sub>ND</sub> values of <sup>11</sup>C-(R)-PK11195 were low. This result is highly dependent on there being no differences in the reference region. <sup>11</sup>C-(R)-PK11195 is known to offer a poorer signal-to-noise ratio than the second-generation radioligands for TSPO such as <sup>11</sup>C-PBR28 (37). Therefore, we are currently performing the next-phase international collaboration study using <sup>11</sup>C-PBR28 with arterial input function to evaluate neuroinflammation of CFS/ME patients in relation to other neurotransmitter dysfunctions. Furthermore, we hope that more specific

radioligands for TSPO or glial cells, such as <sup>11</sup>C-(S)-ketoprofen-methyl ester, which is a good radiotracer for cyclooxygenase-1 imaging in brain microglia activation (38), will provide more information on neuroinflammation in CFS patients.

## CONCLUSION

Our results provide evidence of neuroinflammation in CFS/ME patients, as well as evidence of the possible contribution of neuroinflammation to the pathophysiology of CFS/ME. Furthermore, our results demonstrate the usefulness of PET imaging for the development of objective diagnostic criteria, evaluation of disease severity, and effective medical treatment strategies using antiinflammatory agents in CFS/ME.

## DISCLOSURE

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## REFERENCES

- Afari N, Buchwald D. Chronic fatigue syndrome: a review. *Am J Psychiatry*. 2003;160:221–236.
- Kuratsune H, Yamaguti K, Lindh G, et al. Brain regions involved in fatigue sensation: reduced acetylcarnitine uptake into the brain. *Neuroimage*. 2002;17:1256–1265.
- Yamamoto S, Ouchi Y, Onoe H, et al. Reduction of serotonin transporters of patients with chronic fatigue syndrome. *Neuroreport*. 2004;15:2571–2574.
- Okada T, Tanaka M, Kuratsune H, Watanabe Y, Sadato N. Mechanisms underlying fatigue: a voxel-based morphometric study of chronic fatigue syndrome. *BMC Neurol*. 2004;4:14.
- Chaudhuri A, Behan PO. Fatigue in neurological disorders. *Lancet*. 2004;363:978–988.
- Pavese N, Metta V, Bose SK, Chaudhuri KR, Brooks DJ. Fatigue in Parkinson's disease is linked to striatal and limbic serotonergic dysfunction. *Brain*. 2010;133:3434–3443.
- Chen MK, Guilarte TR. Translocator protein 18 kDa (TSPO): molecular sensor of brain injury and repair. *Pharmacol Ther*. 2008;118:1–17.
- Natelson BH, Haghighi MH, Ponzio NM. Evidence for the presence of immune dysfunction in chronic fatigue syndrome. *Clin Diagn Lab Immunol*. 2002;9:747–752.
- Natelson BH, Weaver SA, Tseng CL, Ottenweller JE. Spinal fluid abnormalities in patients with chronic fatigue syndrome. *Clin Diagn Lab Immunol*. 2005;12:52–55.
- Morris G, Maes M. A neuro-immune model of myalgic encephalomyelitis/chronic fatigue syndrome. *Metab Brain Dis*. 2013;28:523–540.
- Owen DR, Yeo AJ, Gunn RN, et al. An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J Cereb Blood Flow Metab*. 2012;32:1–5.
- Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med*. 1994;121:953–959.
- Carruthers BM, van de Sande MI, De Meirleir KL, et al. Myalgic encephalomyelitis: international consensus criteria. *J Intern Med*. 2011;270:327–338.
- Lee KA, Hicks G, Nino-Murcia G. Validity and reliability of a scale to assess fatigue. *Psychiatry Res*. 1991;36:291–298.
- Chalder T, Berelowitz G, Pawlikowska T, et al. Development of a fatigue scale. *J Psychosom Res*. 1993;37:147–153.
- Radloff L. The CES-D scale: a self-report depression scale for research in the general population. *Appl Psychol Meas*. 1977;1:385–401.
- Studholme C, Hill DLG, Hawkes DJ. An overlap invariant entropy measure of 3D medical image alignment. *Patt Recog*. 1999;32:71–86.
- Kropholler MA, Boellaard R, van Berckel BN, et al. Evaluation of reference regions for (R)-[<sup>11</sup>C]-PK11195 studies in Alzheimer's disease and mild cognitive impairment. *J Cereb Blood Flow Metab*. 2007;27:1965–1974.
- Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL. Distribution volume ratios without blood sampling from graphical analysis of PET data. *J Cereb Blood Flow Metab*. 1996;16:834–840.
- Vas A, Shchukin Y, Karrenbauer VD, et al. Functional neuroimaging in multiple sclerosis with radiolabelled gliia markers: preliminary comparative PET studies with <sup>11</sup>C-vinopocetine and <sup>11</sup>C-PK11195 in patients. *J Neurol Sci*. 2008;264:9–17.
- Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage*. 2003;19:1233–1239.
- de Lange FP, Kalkman JS, Bleijenberg G, et al. Neural correlates of the chronic fatigue syndrome: an fMRI study. *Brain*. 2004;127:1948–1957.
- Lipton P. Ischemic cell death in brain neurons. *Physiol Rev*. 1999;79:1431–1568.
- Pall ML. Elevated, sustained peroxynitrite levels as the cause of chronic fatigue syndrome. *Med Hypotheses*. 2000;54:115–125.
- Basu S. F2-isoprostanes in human health and diseases: from molecular mechanisms to clinical implications. *Antioxid Redox Signal*. 2008;10:1405–1434.
- Cagnin A, Brooks DJ, Kennedy AM, et al. In-vivo measurement of activated microglia in dementia. *Lancet*. 2001;358:461–467.
- Yokokura M, Mori N, Yagi S, et al. In vivo changes in microglial activation and amyloid deposits in brain regions with hypometabolism in Alzheimer's disease. *Eur J Nucl Med Mol Imaging*. 2011;38:343–351.
- Van der Werf YD, Witter MP, Groenewegen HJ. The intralaminar and midline nuclei of the thalamus: anatomical and functional evidence for participation in processes of arousal and awareness. *Brain Res Brain Res Rev*. 2002;39:107–140.
- Morris RK, Robson MJ, Deakin JF. Neuropsychological performance and nor-adrenaline function in chronic fatigue syndrome under conditions of high arousal. *Psychopharmacology (Berl)*. 2002;163:166–173.
- Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron*. 2005;48:175–187.
- Gupta A. Unconscious amygdalar fear conditioning in a subset of chronic fatigue syndrome patients. *Med Hypotheses*. 2002;59:727–735.
- Van Houdenhove B, Kempke S, Luyten P. Psychiatric aspects of chronic fatigue syndrome and fibromyalgia. *Curr Psychiatry Rep*. 2010;12:208–214.
- Farooq RK, Isingrini E, Tanti A, et al. Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation? *Behav Brain Res*. 2012;231:130–137.
- Ekdahl CT, Kokaia Z, Lindvall O. Brain inflammation and adult neurogenesis: the dual role of microglia. *Neuroscience*. 2009;158:1021–1029.
- Harte SE, Spuz CA, Borszcz GS. Functional interaction between medial thalamus and rostral anterior cingulate cortex in the suppression of pain affect. *Neuroscience*. 2011;172:460–473.
- Lyll M, Peakman M, Wessely S. A systematic review and critical evaluation of the immunology of chronic fatigue syndrome. *J Psychosom Res*. 2003;55:79–90.
- Briard E, Zoghbi SS, Imaizumi M, et al. Synthesis and evaluation in monkey of two sensitive <sup>11</sup>C-labeled aryloxyanilide ligands for imaging brain peripheral benzodiazepine receptors in vivo. *J Med Chem*. 2008;51:17–30.
- Shukuri M, Takashima-Hirano M, Tokuda K, et al. In vivo expression of cyclooxygenase-1 in activated microglia/macrophages during neuroinflammation visualized by PET with <sup>11</sup>C-ketoprofen-methyl ester. *J Nucl Med*. 2011;52:1094–1101.