No signs of neuroinflammation in women with chronic fatigue syndrome or Q fever fatigue syndrome using the TSPO ligand [11C]-PK11195

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Abstract

Background

The pathophysiology of chronic fatigue syndrome (CFS) and Q fever fatigue syndrome (QFS) remains elusive. Recent data suggest a role for neuroinflammation as defined by increased expression of translocator protein (TSPO). In the present study we investigated neuroinflammation in female CFS and QFS patients compared with healthy women, using Positron Emission Tomography (PET) with the TSPO ligand [^{11}C]-PK11195.

Methods

The study population consisted of CFS patients (n = 9), QFS patients (n = 10), and healthy controls (n = 9). All subjects were women, matched for age (± 5 years) and neighbourhood, between 18 and 59 years of age, who did not use any medication other than paracetamol or oral contraceptives, and were not vaccinated in the last six months. None of the subjects reported substance abuse in the past 3 months or reported signs of underlying psychiatric disease on the Mini-International Neuropsychiatric Interview (MINI). All subjects underwent a [^{11}C]-PK11195 PET scan and the [^{11}C]-PK11195 binding potential (BP_{ND}) was calculated.

Results

No statistically significant differences in BP_{ND} were found for CFS patients or QFS patients when compared to healthy controls. BP_{ND} of [^{11}C]-PK11195 positively correlated with symptom severity scores in QFS patients, but a negative correlation was found in CFS patients.

Conclusions

In contrast to what was previously reported for CFS, we found no significant difference in BP_{ND} of [^{11}C]-PK11195 when comparing CFS or QFS patients to healthy neighbourhood controls. In this small series we were unable to find signs of neuroinflammation in patients with CFS and QFS.

Trial registration

EudraCT number: 2014-004448-37

Background

Chronic fatigue syndrome (CFS) is characterised by a debilitating fatigue without a known somatic cause that lasts for at least six months, and is often accompanied by headache, sore throat, musculoskeletal pain, and neuropsychological symptoms, mainly impairments in memory, and concentration (1). There is a strong female preponderance in CFS (~ 75%). Previous research on CFS investigating metabolism, hormones, microbes, the immune system and neuropsychology, failed to discover a unifying pathogenesis (2–6). Many patients with CFS suffered from a previous infectious disease and are considered as post-infectious fatigue syndromes (7, 8). Q fever fatigue syndrome (QFS) is such a post-infectious fatigue syndrome that is characterised by a state of prolonged fatigue following approximately 20% of acute Q fever infections (9, 10). The fatigue lasts for at least six months and usually coincides with musculoskeletal complaints, neurocognitive problems, sleeping problems, headache, respiratory tract symptoms and mood disorders (9). In many ways, complaints of QFS are similar as to those reported by CFS patients, and, like in CFS, the pathophysiology of QFS is still unclear (11).

Given the complaints that CFS patients and QFS patients have, it is conceivable that both an inflammatory and neurologic component contribute to their pathophysiology (5, 12, 13). A hypothesis that connects these pathophysiological components is that of chronic low-grade neuroinflammation (5). Within the context of this hypothesis, a peripheral inflammatory response, e.g., initiated by Q fever, ultimately extends to resident tissue macrophages, i.e., microglia, of the brain (14). Trained immunity of microglia occurs following an inflammatory or noxious stimulus. This initial stimulus elicits long-term changes that enables these cells to produce an enhanced inflammatory response upon a second, non-specific, stimulus. In this way, chronic low-grade neuroinflammation may persist following a transient single infectious insult (15). Microglia may be indirectly primed through active and passive transport of cytokines across the blood-brain-barrier (BBB) and stimulation of peripheral chemoreceptors of the vagal nerve (16).

Through investigation of inflammatory markers in cerebral spinal fluid (CSF), magnetic resonance spectroscopy (MRS), and positron emission tomography (PET), several studies point towards neuroinflammation occurring in CFS (5, 17–19). As microglial activation is its trademark characteristic, visualising neuroinflammation is best done by PET neuroimaging, using a radioligand that binds to the, increased expression of, 18kD translocator protein (TSPO) in activated microglia and astrocytes (16). In recent years, Nakatomi *et al*. showed that, compared to healthy controls, CFS patients exhibit an increased PET signal, especially at the thalamus, showing positive correlation with pain scores, when using the ^{11}C-(*R*)-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline-carbox-amide ([^{11}C]-PK11195) ligand for TSPO (5). These and other findings (20, 21) suggest that CFS patients exhibit neuroinflammation and that this phenomenon warrants further investigation in the pathophysiology of chronic fatigue syndromes. Given the overlap in symptoms with CFS patients and apparent inflammatory aetiology, we expect QFS patients to exhibit similar or more signs of neuroinflammation than CFS patients.

In this study we used the TSPO ligand [^{11}C]-PK11195 for PET neuroimaging to confirm and assess neuroinflammation in CFS and QFS patients, respectively, compared with age-, sex-, and neighbourhood-

matched healthy controls that did not use any medication, other than paracetamol or contraceptives, and were screened for psychiatric disease. We elaborate on these results by concomitant analysis of questionnaires on psychiatric and physical wellbeing.

Methods

Study population

The study population consisted of CFS patients (n = 9), QFS patients (n = 10), and healthy controls (n = 9). For reasons of homogeneity, all subjects were women and matched for age (± 5 years) and neighbourhood. All subjects were between 18 and 59 years of age and did not use any medication, other than paracetamol or oral contraceptives, and were not vaccinated, in the last six months. None of the subjects reported substance abuse in the past 3 months or showed signs of underlying psychiatric disease, i.e., depression, bipolar disorders, anxiety, schizophrenia, psychosis, or eating disorders on Mini-International Neuropsychiatric Interview (MINI).

All CFS patients were diagnosed with CFS at the Department of Internal Medicine and Expert Center for Chronic Fatigue (ECCF) of the Radboud university medical center, Nijmegen, the Netherlands, after a uniform work-up according to the Fukuda 1994 criteria for CFS (1, 22). They all had a score ≥ 40 on the subscale fatigue severity of the Checklist Individual Strength (CIS) questionnaire (23) and a score ≥ 700 on the Sickness Impact Profile-8 (SIP-8) questionnaire (24). None of them experienced an acute Q fever infection or were vaccinated against Q fever in the past. *Coxiella* PCR and IgG were not tested and no data was collected on whether or not an infection preceded CFS complaints.

All QFS patients were diagnosed at the Radboud Expert Center for Q fever, Nijmegen, the Netherlands, after a uniform work-up according to the Dutch guideline on QFS diagnosis (25). All QFS patients met the following diagnostic criteria: i. fatigue lasted ≥ 6 months; ii. sudden onset of severe fatigue (defined as a score ≥ 40 on the subscale fatigue severity of the CIS), or significant increase in fatigue, both related to a symptomatic acute Q fever infection; iii. chronic Q fever and other somatic or psychiatric causes of fatigue were excluded; and iv. fatigue resulted in significant functional impairment (defined as a total score ≥ 700 on the SIP-8 questionnaire). All QFS patients tested negative on *Coxiella* PCR and had IgG phase I or phase II titres ≥ 1:16, but IgG phase I ≤ 1:512, and none of them showed serological signs of an acute or recent Q-fever infection, reflected by IgM antibodies in absence of IgG antibodies.

Healthy controls were recruited based on age, gender, and neighbourhood that matched with both QFS and CFS patients and had a score ≤ 35 on the subscale fatigue severity of the CIS questionnaire and a score ≤ 450 on the SIP-8 questionnaire. Similar to patients, healthy controls did not use any medication, other than paracetamol or contraceptives, and were screened for psychiatric disease. None of healthy controls had experienced acute Q fever infection or were vaccinated for Q fever. *Coxiella* PCR and IgG were not tested.

Questionnaires

All subjects were asked to fill out questionnaires, used in our expert centers (23), on CFS aspects previously associated with neuroinflammation, i.e., depression, concomitant CFS complaints, and fatigue (5, 26). These are:

CIS Checklist of Individual Strength, subscale on fatigue severity, assesses the severity of fatigue, which is part of the inclusion criteria (23).

SIP-8 Sickness Impact Profile-8 assesses the influence of disease and/or health complaints on functioning in daily life, which is part of the inclusion criteria (24).

BDI-II-NL-PC Beck Depression Inventory for Primary Care (BDI-PC, shortened) assesses depressive symptoms (27).

CDC Centers for Disease Control CFS Symptom Inventory Questionnaire, subscale on active complaints, assesses concomitant CFS symptoms (28).

PET imaging

Following testing for collateral blood flow and the injection of 1% lidocaine, a cannula was inserted in the radial artery to allow for arterial blood sampling. In the other arm, a cannula was placed in the antebrachial vein for the injection of [^{11}C]-PK11195. The PET scans were performed using the Biograph mCT (Siemens Healthineers, Germany). Head movement was minimized by using a head-restraining adhesive band. After positioning in the camera, a low dose CT scan was made for attenuation and scatter correction. Hereafter, [^{11}C]-PK11195, produced under Good Manufacturing Practice conditions as described earlier (29), was injected intravenously at a speed of 0.5 mL/s (total volume 8.3 mL). The injected dose of [^{11}C]-PK11195 was 367 ± 50 MBq (healthy controls, 370 ± 53 MBq; CFS, 375 ± 37 MBq, QFS, 356 ± 55 MBq) with a molar activity of > 12,000 GBq/mmol. Simultaneously with the start of the injection, a 60-min emission scan was started during which arterial blood radioactivity was continuously measured with an automated blood sampling system (COMECER Netherlands, The Netherlands). Five manual blood samples were collected at 10, 20, 30, 45 and 60 min after [^{11}C]-PK11195 injection to determine the amount of radioactivity in blood and plasma, for calibration of the automated sampling system. The manual blood samples taken at 20, 45 and 60 min were additionally used for analysis of the percentage of intact [^{11}C]-PK11195 in plasma, according to the procedure described previously (29).

On the same day as the PET scan a T1-weighted Magnetic Resonance Imaging (MRI) scan, using a MAGNETOM Prisma (Siemens Healthineers, Germany), was made for anatomical reference.

PET data analysis

The list-mode data from the PET scans were reconstructed using the 3D OSEM algorithm (3 iterations and 24 subsets) into 24 successive frames (7*10, 2*30, 2*120, 2*180, 5*300, 2*600 seconds). Image processing and pharmacokinetic analysis was performed with PMOD software v4.1 (PMOD Technologies Ltd., Switzerland). The summed PET image (frame 1–24) was used for rigid registration of the individual

PET image to the individual MRI image. The six-tissue probability maps normalization of the individual MRI into the Montreal Neurological Institute (MNI) standard space was then performed and applied to the corresponding PET image. Predefined volumes of interest (VOIs), based on the Hammers atlas (30), were transformed back into individual PET space and time-activity curves were generated.

The two-tissue compartment model was used to obtain the non-displaceable binding potential (BP_{ND}) of [^{11}C]-PK11195, using the metabolite-corrected plasma curve as the input function. The delay and the blood volume were individually fitted. It was assumed that the distribution volume of the non-displaceable compartment (K_1/k_2) and the dissociation rate (k_4) from the specific binding site was equal for all regions. A coupled fitting was performed for cortical regions that calculated a common K_1/k_2 and k_4, which were then used to calculate an individual K_1 and k_3 for all regions. The BP_{ND} was defined as k_3/k_4.

Statistical analysis

Patient data were analysed using Graphpad Prism (Graphpad Software Inc., version 5.03). An ANOVA was used to determine differences between groups. A Pearson correlation was used to determine correlations between BP_{ND} of [^{11}C]-PK11195 in various brain regions and symptom severity scores in CFS and QFS patients. Statistical significance was attained if $P < 0.05$.

Results

Patients and controls

At the time of PET imaging, CFS patients had a significantly longer median duration of illness than QFS patients (240 months versus 84 months, $P = 0.01$). The median age of CFS patients, QFS patients, and healthy controls did not differ significantly (Table 1). Other than the fact that CFS patients were significantly more functionally impaired than QFS patients ($P = 0.02$), no significant differences in other scores were found between CFS and QFS patients (Table 1).

Table 1
Characteristics of female QFS patients, CFS patients, and HC

Characteristics	QFS (n = 10)	CFS (n = 9)	Healthy controls (n = 9)
Age, years Median (IQR)	43 (32–48)	43 (30–52)	41 (27–47)
Duration of symptoms, months [a] **Median (IQR)**	84 (74–93)	240 (84–390)	-
CIS subscale fatigue severity score mean ± SD	50 ± 4.7	49 ± 5.0	12 ± 5.1
SIP-8 total score, mean ± SD	1432 ± 362	1890 ± 395	0 ± 0
BDI-II-NL-PC score, mean ± SD	2.1 ± 1.5	3.8 ± 2.7	0.0 ± 0.0
CDC subscale on complaints score, mean ± SD	16 ± 4.1	17 ± 4.5	1 ± 1.7

BP_{ND} of [^{11}C]-PK11195 in various brain regions

The BP_{ND} of [^{11}C]-PK11195 in various brain regions for CFS and QFS patients compared to healthy controls were not significantly different (Fig. 1 and Table 2). No differences were found in BP_{ND} values comparing means of CFS and QFS patients to healthy controls for the cingulate (rostral anterior: mean difference − 0.33 [95% CI, -0.82–0.17] and − 0.30 [95% CI, -0.77–0.17] with P = 0.30 and 0.33, respectively, caudal anterior: mean difference − 0.33 [95% CI, -0.83–0.16] and − 0.20 [95% CI, -0.67–0.27] with P = 0.29 and 0.83, respectively, posterior: mean difference − 0.32 [95% CI, -0.85–0.22] and − 0.18 [95% CI, -0.69–0.33] with P = 0.43 and 1.00, respectively), hippocampus (mean difference − 0.37 [95% CI, -0.90–0.16] and − 0.23 [95% CI, -0.73–0.27] with P = 0.25 and 0.73, respectively), thalamus (mean difference − 0.32 [95% CI, -0.94–0.31] and − 0.20 [95% CI, -0.80–0.39] with P = 0.61 and 1.00, respectively), midbrain (mean difference − 0.40 [95% CI, -1.06–0.25] and − 0.33 [95% CI, -0.95–0.29] with P = 0.38 and 0.54, respectively), or pons (mean difference − 0.44 [95% CI, -1.23–0.36] and − 0.30 [95% CI, -1.05–0.45] with P = 0.51 and 0.95, respectively). In fact, as seen by the mean difference, BP_{ND} values tended to be lower rather than higher for both CFS and QFS patients compared to healthy controls.

Table 2
BPND of [11C]-PK11195 for various brain regions in QFS and CFS patients compared with HC

Brain region	QFS Mean (95% CI)	CFS Mean (95% CI)	HC Mean (95% CI)	QFS vs. HC Mean difference (95% CI), *P* value [a]	CFS vs. HC Mean difference (95% CI), *P* value [a]
Brainstem	1.49 (1.00-1.98)	1.42 (1.06–1.78)	1.79 (1.34–2.24)	-0.30 (-1.00-0.40), 0.84	-0.37 (-1.11-0.37), 0.63
Orbitofrontal cortex	1.04 (0.77–1.31)	0.89 (0.60–1.18)	1.20 (0.93–1.48)	-0.16 (-0.60-0.28), 1.00	-0.31 (-0.77-0.15), 0.28
Middle frontal gyrus	0.99 (0.66–1.32)	0.76 (0.48–1.04)	1.02 (0.69–1.35)	-0.04 (-0.53-0.46), 1.00	-0.27 (-0.79-0.26), 0.61
Straight frontal gyrus	0.89 (0.66–1.12)	0.81 (0.54–1.09)	1.07 (0.82–1.32)	-0.18 (-0.57-0.20), 0.70	-0.26 (-0.67-0.15), 0.35
Inferior frontal gyrus	1.05 (0.72–1.38)	0.81 (0.54–1.08)	1.09 (0.82–1.37)	-0.44 (-0.51-0.42), 1.00	-0.29 (-0.78-0.21), 0.44
Superior frontal gyrus	0.88 (0.66–1.10)	0.71 (0.42–0.99)	1.02 (0.70–1.33)	-0.14 (-0.56-0.28), 1.00	-0.31 (-0.75-0.14), 0.26
Primary motor cortex	0.92 (0.66–1.18)	0.70 (0.42–0.97)	0.99 (0.68–1.31)	-0.08 (-0.51-0.36), 1.00	-0.30 (-0.76-0.16), 0.33
Rostral anterior cingulate	0.84 (0.59–1.09)	0.81 (0.55–1.07)	1.14 (0.75–1.53)	-0.30 (-0.77-0.17), 0.33	-0.33 (-0.82-0.17), 0.30
Caudal anterior cingulate	1.04 (0.76–1.31)	0.91 (0.60–1.22)	1.24 (0.91–1.57)	-0.20 (-0.67-0.27), 0.83	-0.33 (-0.83-0.16), 0.29

Brain region	QFS Mean (95% CI)	CFS Mean (95% CI)	HC Mean (95% CI)	QFS vs. HC Mean difference (95% CI), *P* value [a]	CFS vs. HC Mean difference (95% CI), *P* value [a]
Posterior cingulate	1.14 (0.81–1.46)	1.00 (0.68–1.32)	1.32 (1.00-1.65)	-0.18 (-0.69-0.33), 1.00	-0.32 (-0.85-0.22), 0.43
Insula	1.02 (0.74–1.30)	0.96 (0.69–1.23)	1.26 (0.93–1.58)	-0.23 (-0.69-0.22), 0.60	-0.29 (-0.77-0.19), 0.39
Hippocampus	1.04 (0.71–1.37)	0.90 (0.62–1.19)	1.27 (0.95–1.60)	-0.23 (-0.73-0.27), 0.73	-0.37 (-0.90-0.16), 0.25
Amygdala	1.01 (0.68–1.35)	0.98 (0.67–1.29)	1.28 (0.96–1.60)	-0.26 (-0.77-0.25), 0.60	-0.30 (-0.83-0.24), 0.50
Temporal lobe	0.92 (0.67–1.16)	0.83 (0.58–1.09)	1.08 (0.76–1.39)	-0.16 (-0.58-0.26), 1.00	-0.24 (-0.69-0.20), 0.52
Primary somatosensory cortex	0.87 (0.59–1.15)	0.65 (0.41–0.90)	0.95 (0.60–1.29)	-0.08 (-0.53-0.38), 1.00	-0.30 (-0.77-0.18), 0.37
Parietal lobe	0.84 (0.62–1.10)	0.75 (0.51–0.99)	0.97 (0.63–1.32)	-0.13 (-0.54-0.28), 1.00	-0.23 (-0.66-0.21), 0.58
Occipital lobe	0.95 (0.73–1.18)	0.94 (0.70–1.18)	1.15 (0.79–1.52)	-0.20 (-0.63-0.23), 0.73	-0.21 (-0.66-0.24), 0.71
Caudate nucleus	0.47 (0.27–0.66)	0.42 (0.14–0.70)	0.61 (0.38–0.84)	-0.14 (-0.50-0.22), 0.98	-0.19 (-0.57-0.19), 0.61
Nucleus accumbens	1.39 (0.96–1.81)	1.23 (0.87–1.58)	1.53 (1.19–1.88)	-0.15 (-0.76-0.46), 1.00	-0.31 (-0.95-0.34), 0.69

Brain region	QFS Mean (95% CI)	CFS Mean (95% CI)	HC Mean (95% CI)	QFS vs. HC Mean difference (95% CI), *P* value [a]	CFS vs. HC Mean difference (95% CI), *P* value [a]
Lentiform nucleus	1.17 (0.84–1.50)	1.09 (0.77–1.40)	1.44 (1.04–1.83)	-0.26 (-0.80-0.27), 0.64	-0.35 (-0.91-0.22), 0.38
Thalamus	1.43 (1.01–1.84)	1.31 (1.02–1.61)	1.63 (1.24–2.02)	-0.20 (-0.80-0.39), 1.00	-0.32 (-0.94-0.31), 0.61
Cerebellum	1.08 (0.78–1.38)	0.98 (0.68–1.28)	1.26 (0.92–1.59)	-0.18 (-0.66-0.31), 1.00	-0.28 (-0.79-0.23), 0.52
Midbrain	1.45 (1.03–1.86)	1.38 (1.02–1.73)	1.78 (1.37–2.18)	-0.33 (-0.95-0.29), 0.54	-0.40 (-1.06-0.25), 0.38
Pons	1.58 (1.04–2.11)	1.44 (1.05–1.83)	1.88 (1.41–2.34)	-0.30 (-1.05-0.45), 0.95	-0.44 (-1.23-0.36), 0.51

Correlation between symptom severity scores and BP_{ND} of [^{11}C]-PK11195

Significant correlations between symptom severity scores and BP_{ND} of [^{11}C]-PK11195 in various brain regions of both CFS patients and QFS patients are shown in Table 3. For CFS patients; the CDC questionnaire, subscale on complaints, and the CIS questionnaire, subscale fatigue severity, negatively correlated with BP_{ND} in the caudate nucleus (-0.73, $P < 0.05$ and − 0.78, $P < 0.05$, respectively) (Table 3) (Figs. 2 and 3). For QFS patients; the CDC questionnaire, subscale on complaints, positively correlated with BP_{ND} in the brainstem (0.66, $P < 0.05$), caudal anterior cingulate (0.64, $P < 0.05$), insula (0.65, $P < 0.05$), amygdala (0.71, $P < 0.05$), and pons (0.69, $P < 0.05$), and the CIS questionnaire, subscale on fatigue severity, positively correlated with BP_{ND} in the orbitofrontal cortex (0.70, $P < 0.05$), middle frontal gyrus (0.83, $P < 0.01$), inferior frontal gyrus (0.78, $P < 0.01$), superior frontal gyrus (0.64, $P < 0.05$), primary motor cortex (0.74, $P < 0.05$), temporal lobe (0.78, $P < 0.01$), primary somatosensory cortex (0.83, $P < 0.01$), and parietal lobe (0.77, $P < 0.01$) (Table 3) (Figs. 2 and 3). In healthy controls, no significant correlations were found between symptom severity scores and BP_{ND} of [^{11}C]-PK11195 (Table 3).

Table 3
Correlation between BPND of [11C]-PK11195 in various brain regions with symptom severity scores in QFS patients, CFS patients, and HC

QFS				
Brain Region	**BDI-PC**	**CDC Complaints**	**CIS Fatigue**	**SIP-8**
Brainstem	0.17	0.66*	0.17	0.43
Orbitofrontal cortex	0.03	0.46	0.70*	0.49
Middle frontal gyrus	0.34	0.39	0.83**	0.39
Straight frontal gyrus	0.00	0.59	0.45	0.50
Inferior frontal gyrus	0.10	0.21	0.78**	0.39
Superior frontal gyrus	0.08	0.47	0.64*	0.54
Primary motor cortex	0.16	0.44	0.74*	0.43
Rostral anterior cingulate	0.01	0.61	0.56	0.48
Caudal anterior cingulate	0.13	0.64*	0.46	0.52
Posterior cingulate	0.07	0.57	0.27	0.45
Insula	0.92	0.65*	0.47	0.48
Hippocampus	0.03	0.60	0.27	0.38
Amygdala	0.04	0.71*	0.21	0.51
Temporal lobe	0.02	0.35	0.78**	0.40
Primary somatosensory cortex	0.30	0.38	0.83**	0.37
Parietal lobe	0.21	0.42	0.77**	0.36
Occipital lobe	-0.02	0.47	0.53	0.37
Caudate nucleus	-0.37	0.17	0.08	-0.12
Nucleus accumbens	0.08	0.56	0.34	0.44
Lentiform nucleus	0.05	0.62	0.39	0.42
Thalamus	0.06	0.54	0.21	0.36
Cerebellum	0.09	0.54	0.37	0.40
Midbrain	0.08	0.57	0.11	0.34
Pons	0.20	0.69*	0.17	0.39
CFS				

QFS				
Brain Region	BDI-PC	CDC Complaints	CIS Fatigue	SIP-8
Brainstem	0.16	-0.38	-0.24	0.01
Orbitofrontal cortex	0.05	-0.30	-0.38	-0.24
Middle frontal gyrus	0.09	-0.38	-0.45	-0.20
Straight frontal gyrus	0.06	-0.27	-0.30	-0.18
Inferior frontal gyrus	0.09	-0.31	-0.42	-0.25
Superior frontal gyrus	0.07	-0.35	-0.49	-0.20
Primary motor cortex	0.03	-0.38	-0.51	-0.24
Rostral anterior cingulate	0.21	-0.52	-0.40	-0.21
Caudal anterior cingulate	0.22	-0.40	-0.41	-0.30
Posterior cingulate	0.14	-0.43	-0.47	-0.30
Insula	0.18	-0.42	-0.36	-0.19
Hippocampus	0.15	-0.36	-0.25	-0.18
Amygdala	0.18	-0.34	-0.23	-0.13
Temporal lobe	0.04	-0.38	-0.40	-0.13
Primary somatosensory cortex	0.01	-0.41	-0.51	-0.24
Parietal lobe	-0.00	-0.47	-0.54	-0.16
Occipital lobe	0.01	-0.49	-0.48	-0.12
Caudate nucleus	0.07	-0.73*	-0.78*	-0.27
Nucleus accumbens	0.12	-0.45	-0.39	-0.14
Lentiform nucleus	0.11	-0.41	-0.35	-0.07
Thalamus	0.20	-0.59	-0.46	-0.05
Cerebellum	-0.03	-0.33	-0.42	-0.07
Midbrain	0.05	-0.48	-0.41	0.05
Pons	-0.08	-0.18	-0.19	0.22
HC				
Brain Region	BDI-PC	CDC Complaints	CIS Fatigue	SIP-8
Brainstem	-	-0.14	0.43	-

QFS				
Orbitofrontal cortex	-	-0.12	0.37	-
Middle frontal gyrus	-	-0.03	0.51	-
Straight frontal gyrus	-	-0.19	0.32	-
Inferior frontal gyrus	-	-0.02	0.53	-
Superior frontal gyrus	-	-0.02	0.53	-
Primary motor cortex	-	-0.03	0.55	-
Rostral anterior cingulate	-	-0.14	0.39	-
Caudal anterior cingulate	-	-0.08	0.39	-
Posterior cingulate	-	-0.10	0.41	-
Insula	-	-0.42	0.48	-
Hippocampus	-	-0.14	0.43	-
Amygdala	-	-0.10	0.46	-
Temporal lobe	-	-0.08	0.46	-
Primary somatosensory cortex	-	0.01	0.52	-
Parietal lobe	-	0.00	0.54	-
Occipital lobe	-	-0.01	0.51	-
Caudate nucleus	-	0.11	0.67	-
Nucleus accumbens	-	-0.21	0.26	-
Lentiform nucleus	-	-0.08	0.43	-
Thalamus	-	-0.06	0.42	-
Cerebellum	-	-0.12	0.46	-
Midbrain	-	-0.08	0.45	-
Pons	-	-0.10	0.46	-

Discussion

In this study, we aimed to investigate neuroinflammation in CFS and QFS patients by using the TSPO ligand [^{11}C]-PK11195 for PET neuroimaging. No signs of neuroinflammation were seen in either CFS or QFS patients. Our findings contradict previous findings in CFS patients by Nakatomi *et al*. who found significantly increased BP_{ND} values in the cingulate, hippocampus, thalamus, midbrain, and pons (5).

Even though no signs of neuroinflammation were found, similar correlations between BP_{ND} of [^{11}C]-PK11195 and scores on questionnaires were found in the amygdala of QFS patients, but not CFS patients.

Although the set-up of this study was similar to that of Nakatomi *et al*., using the same TSPO ligand ([^{11}C]-PK11195) (5), a number of important differences can be discerned. First of all, for reasons of homogeneity, our study only included women. Around 75% of CFS patients are female and, although the percentage of women in QFS is lower (52%) (11, 31), we felt that we should avoid a gender effect in a study with such a small sample size. Nakatomi *et al*. included 30–40% males without presenting separate data for men and women (31). This is important as inflammatory responses are generally higher in males (32). Also, in experimental mouse studies of traumatic brain injury, male mice are more likely to exhibit neuroinflammation compared to female mice (33). One could argue that neuroinflammation is more likely to occur, and perhaps even persist, in males compared to females. However, if neuroinflammation is indeed present, the high percentage of female CFS patients contradicts with this hypothesis. A second difference between our study and that of Nakatomi *et al*. is that we distinguished CFS patients, with often heterogenic aetiologies (31), from post-infectious fatigue syndrome patients, i.e., QFS patients. Thirdly, we used a neighbourhood control group with healthy women that were matched with CFS and QFS patients in terms of age and geographical area in order to accomplish optimal matching and avoid bias due to confounding. Also, patients, especially those with CFS, that were included in our study had a longer duration of illness than those included in the study by Nakatomi *et al* (reported mean of 62.4 months). When using small numbers of included patients, as is the case in both studies, subtle differences like these might contribute to the different outcomes that are seen. This brings us to a fifth and final difference, i.e., the method used for determining the binding of [^{11}C]-PK11195. We used pharmacokinetic binding with an arterial input function whereas Nakatomi *et al*. used the cerebellum as a reference region in reference tissue modelling. We feel that the latter is methodologically less sound as no brain region is devoid of TSPO, meaning that the cerebellum is not an objective reference region, and the cerebellum may actually be involved in the disease process. Whether binding of the [^{11}C]-PK11195 ligand is considered enhanced, normal or even lowered, may be explained by this difference in methodology.

Regarding the effect of disease duration, Hornig *et al*. previously reported that the inflammatory response, determined by cytokine measurements, is lower in CFS patients with a long duration, i.e., > 39 months, of illness than in those with a short duration, i.e., < 39 months, of illness (34). In a previous study however, we were unable to confirm these findings (13). We found that CFS patients, who had fatigue for a median of 240 months, show less signs of neuroinflammation than QFS patients, who were fatigued for a median of 84 months. Given previous findings by Hornig *et al*. and our observation that healthy controls generally showed a stronger signal of TSPO binding than patients, one could speculate that neuroinflammation wanes off over time and is followed by a refractory period with decreased expression of TSPO. Furthermore, studies on peripheral inflammatory cell metabolism in CFS and QFS patients have repeatedly shown that mitochondria of these cells are likely to be affected (35–37). As TSPO is

expressed in the outer mitochondrial membrane, it could be conceived that its expression is similarly affected in chronically fatigued patients. It would be interesting to longitudinally investigate TSPO expression in the mitochondrial membrane of chronically fatigued patients and relate findings to symptom severity scores.

An inherent problem in CFS research is the presumed heterogeneity of the disorder. We addressed this problem in several ways. As mentioned above, we only enrolled adult women. Secondly, we used a validated test panel of instruments to assess fatigue and disability. Thirdly, we included a group of patients with post-infectious fatigue related to antecedent Q fever (QFS). As in the latter group an infectious, and therefore inflammatory, aetiology was the precipitating factor, we would have expected this group in particular to exhibit signs of neuroinflammation. However, even though BP_{ND} of [^{11}C]-PK11195 was generally higher than in CFS patients, even in this well-defined group we could not detect neuroinflammation. For future perspective, we should avoid previous mistakes in CFS research and continue investigating neuroinflammation in chronic fatigue by using strict and uniform in- and exclusion criteria, together with well-defined control groups (4, 38).

Our study has some limitations. We chose to use the [^{11}C]-PK11195 ligand as this was the ligand used by the only neuroinflammation PET imaging study in CFS by Nakatomi *et al*. Nowadays, a new generation of more sensitive ligands such as [^{11}C]-PBR28 and [^{18}F]-DPA-714 are available, and perhaps even preferable, when taking allelic dependence of affinity into account (16). Using [^{11}C]-PBR28 for example, signs of neuroinflammation have been found in functional somatic syndromes such as fibromyalgia and Gulf War Illness (26, 39). The former study included mostly women while the latter included mostly men, but with complaints for up to 30 years. Another limitation is the large amount of correlations that were conducted which increases the risk of a type 1 error (while post-hoc analyses increase the risk of a type 2 error). A final limitation is the small number of subjects included in both our study and the study by Nakatomi *et al*. One could argue that a more sensitive new generation ligand would be better suited when using such small numbers (40). Other than imploring a larger study or using more sensitive TSPO ligands, we should keep an eye on current investigations on other targets than TSPO for PET neuroimaging (41).

Conclusion

In contrast to what was previously reported, our well-controlled study shows no significant difference in BP_{ND} of [^{11}C]-PK11195 when comparing both CFS and QFS patients to healthy controls. A larger study, including both men and women together with well-matched controls, is needed to confirm whether or not chronically fatigued patients exhibit neuroinflammation. For this study, we propose using a new generation ligand with kinetic modelling via an arterial input function.

List of abbreviations

CFS - Chronic fatigue syndrome

QFS - Q fever fatigue syndrome

TSPO – 18kD translocator protein

PET - Positron emission tomograpgy

MINI - Mini-international neuropsychiatric interview

BP_{ND} - Non-displaceable binding potential

BBB - Blood brain barrier

MRS - Magnetic resonance spectroscopy

CSF - Cerebral spinal fluid

[^{11}C]-PK11195 – *11*C-(*R*)-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline-carbox-amide

ECCF - Expert Center for Chronic Fatigue

CIS - Checklist Individual Strength (questionnaire)

SIP-8 - Sickness Impact Profile-8 (questionnaire)

MRI - Magnetic resonance imaging

BDI-PC - Beck depression inventory for primary Care

CDC - Centers for disease control

VOI - Volume of interest

HC - Healthy controls

CI - Confidence interval

SD - Standard deviation

Declarations

Declarations

Declarations

Ethics approval and consent to participate

All participants provided written informed consent and the study was approved by the Medical Ethical Review Committee of the University Medical Center Groningen (UMCG NL51194.042.15).

Consent for publication

Not applicable.

Declarations

Competing interest

The authors declare that they have no competing interests.

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Authors' contributions

RR contributed to the design of the study, collection of data, analysis, interpretation of data, and writing of the manuscript. MR contributed to the design of the study, collection of data, analysis, interpretation of data, and writing of the manuscript. SK contributed to the interpretation of data, and writing of the manuscript. LJ contributed to the interpretation of data, and writing of the manuscript. MN contributed to the interpretation of data, and writing of the manuscript. JvdM contributed to the design of the study, collection of data, analysis, interpretation of data, and writing of the manuscript. HK contributed to the interpretation of data and writing of the manuscript. H(C)K contributed to the design of the study. CB-R contributed to the design of the study, collection of data, interpretation of data, and writing of the manuscript. JD contributed to the design of the study, collection of data, analysis, interpretation of data, and writing of the manuscript.

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Figures

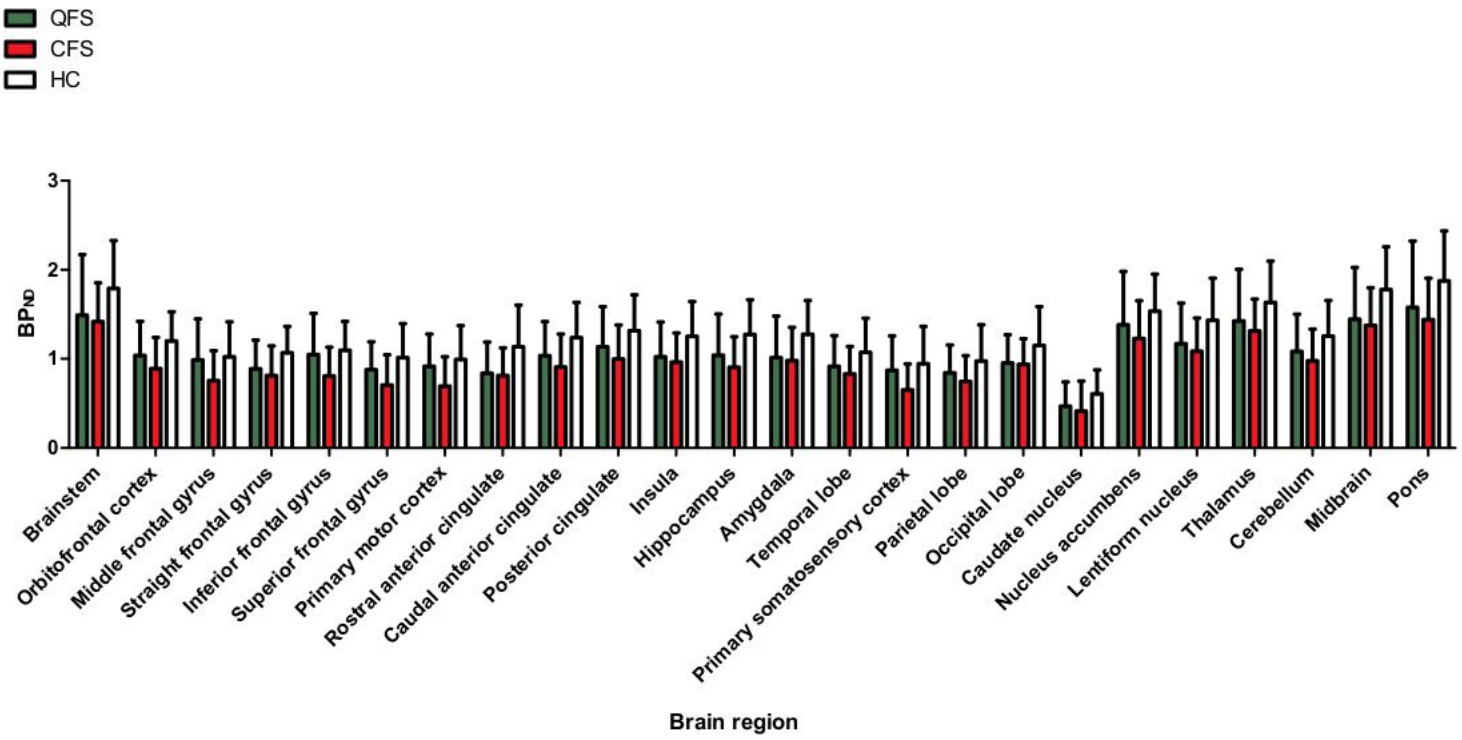

Figure 1

BPND of [11C]-PK11195 for various brain regions in QFS patients, CFS patients, and HC Graph showing BPND of [11C]-PK11195 per brain region for QFS patients, CFS patients, and healthy controls (HC). Data are depicted as mean ± SD. Abbreviations: BPND = non-displaceable binding potential; QFS = Q fever fatigue syndrome; CFS = chronic fatigue syndrome; HC = healthy controls; SD = standard deviation.

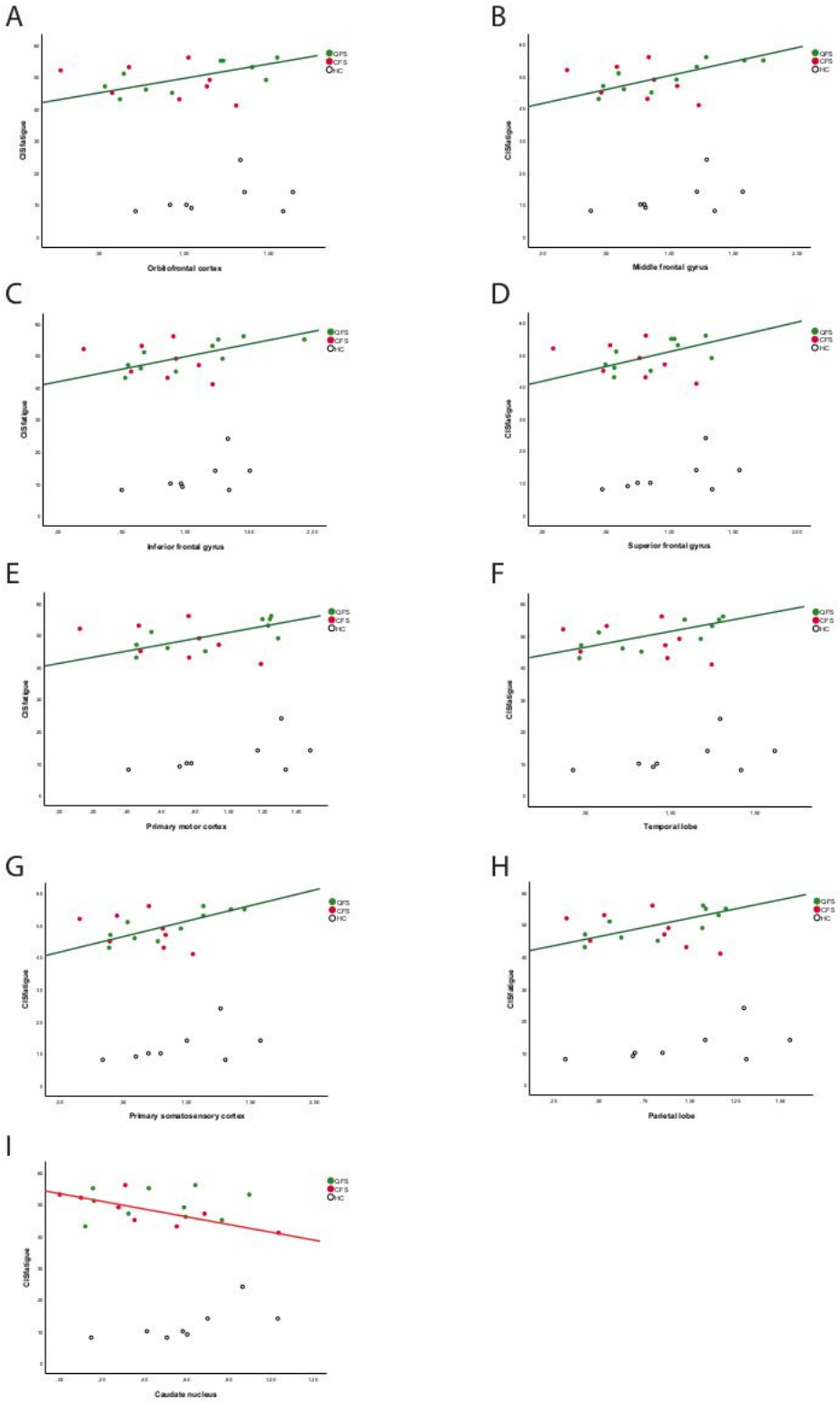

Figure 2

Correlation between BPND of [11C]-PK11195 in various brain regions with CIS questionnaire, subscale on fatigue severity, scores in QFS patients, CFS patients, and HC Scatter plots showing significant correlations between BPND of [11C]-PK11195 and fatigue severity, measured by CIS questionnaire, subscale on fatigue severity, for QFS patients; in the orbitofrontal cortex (A, R = 0.70*), middle frontal gyrus (B, R = 0.83**), inferior frontal gyrus (C, R = 0.78**), superior frontal gyrus (D, R = 0.64*), primary

motor cortex (E, R = 0.74*), temporal lobe (F, R = 0.78**), primary somatosensory cortex (G, R = 0.83**), and parietal lobe (H, R = 0.77**), and CFS patients; in the caudate nucleus (I, R = -0.78*). Abbreviations: BPND = non-displaceable binding potential; QFS = Q fever fatigue syndrome; CFS = chronic fatigue syndrome; HC = healthy controls. Statistical significance was attained if P < 0.05. * P < 0.05. ** P < 0.01.

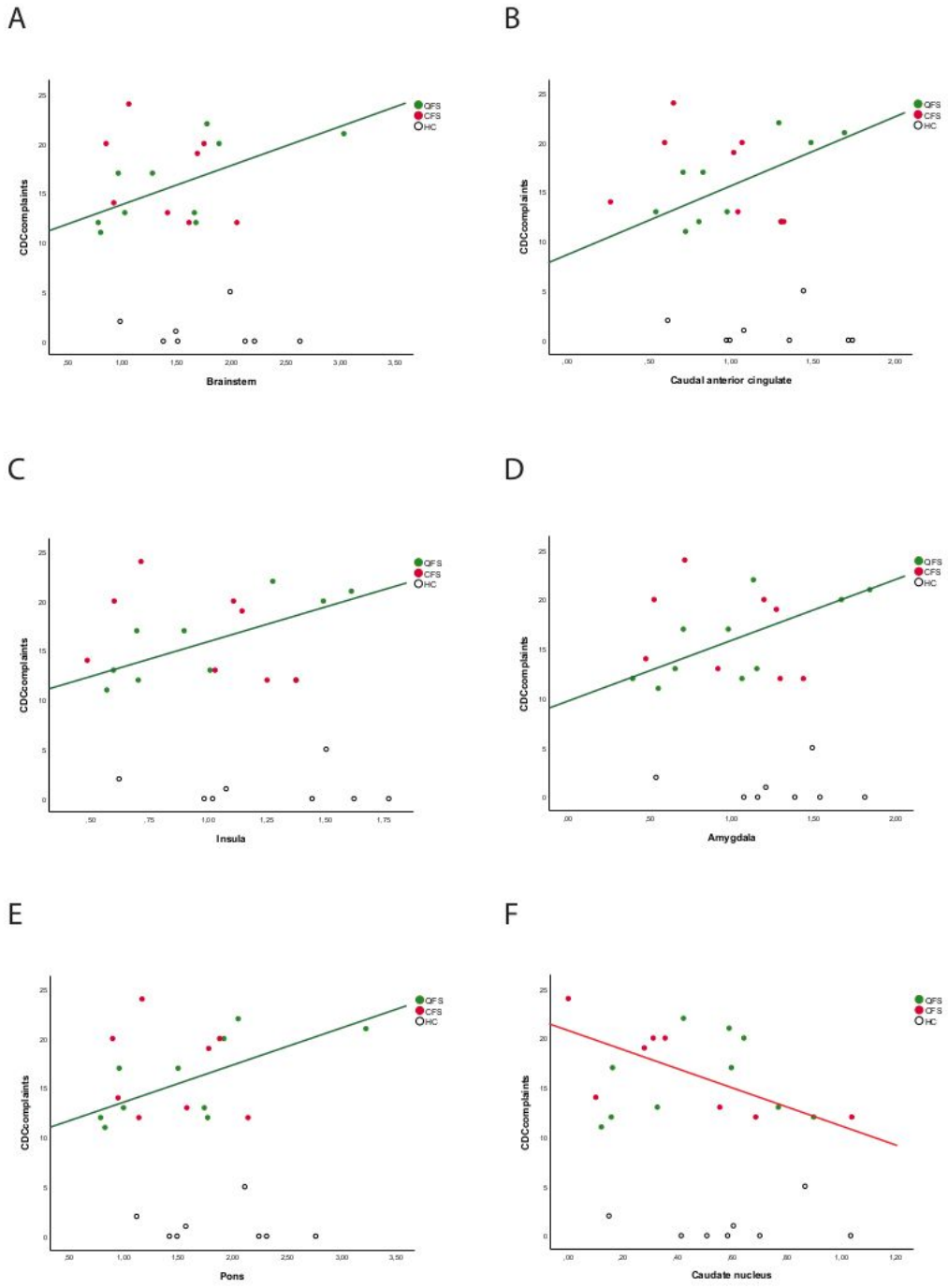

Figure 3

Correlation between BPND of [11C]-PK11195 in various brain regions with CDC questionnaire, subscale on complaints, scores in QFS patients, CFS patients, and HC Scatter plots showing significant correlations between BPND of [11C]-PK11195 and fatigue severity, measured by CDC questionnaire, subscale on complaints, for QFS patients; the brainstem (A, R = 0.66*), caudal anterior cingulate (B, R = 0.64*), insula (C, R = 0.65*), amygdala (D, R = 0.71*), and pons (E, R = 0.69*), and CFS patients; in the caudate nucleus (F, R = -0.73*). Abbreviations: BPND = non-displaceable binding potential; QFS = Q fever fatigue syndrome; CFS = chronic fatigue syndrome; HC = healthy controls. Statistical significance was attained if $P < 0.05$. * $P < 0.05$.

Supplementary Files

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