

## RESEARCH ARTICLE

# Medial prefrontal cortex deficits correlate with unrefreshing sleep in patients with chronic fatigue syndrome

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Unrefreshing sleep is a hallmark of chronic fatigue syndrome/myalgic encephalomyelitis (CFS). This study examined brain structure variations associated with sleep quality in patients with CFS. 38 patients with CFS (34.8 ± 10.1 years old) and 14 normal controls (NCs) (34.7 ± 8.4 years old) were recruited. All subjects completed the Hospital Anxiety and Depression Scale, Pittsburgh Sleep Quality Index (PSQI), and Chalder Fatigue Scale (CFQ) questionnaires. Brain MRI measures included global and regional grey and white matter volumes, magnetization transfer  $T_1$  weighted (MT-T1w) intensities, and  $T_1$  weighted (T1w) and  $T_2$  weighted spin echo signal intensities. We performed voxel based group comparisons of these regional brain MRI measures and regressions of these measures with the PSQI and CFQ scales adjusted for age, anxiety and depression, and the appropriate global measure. In CFS patients, negative correlations were observed in the medial prefrontal cortex (mPFC) between PSQI and MT-T1w intensities (family-wise error corrected cluster,  $P_{FWE} < 0.05$ ) and between PSQI and T1w intensities ( $P_{FWE} < 0.05$ ). In the same mPFC location, both MT and T1w intensities were lower in CFS patients compared with NCs (uncorrected voxel  $P < 0.001$ ). This study is the first to report that brain structural differences are associated with unrefreshing sleep in CFS. This result refutes the suggestion that unrefreshing sleep is a misperception in CFS patients and further investigation of this symptom is warranted.

**KEYWORDS**

inferior fronto-occipital fasciculus, medial prefrontal cortex, MRI, sleep quality, voxel based morphometry

## 1 | INTRODUCTION

Chronic fatigue syndrome (CFS)/myalgic encephalomyelitis (ME) is a medically unexplained debilitating illness characterized by prolonged, post-exertional fatigue together with a range of other core symptoms.<sup>1,2</sup> There is ongoing debate as to whether CFS is the expression of somatic complaints accompanying a psychiatric syndrome, or a consequence of a viral infection, hormonal dysregulation, or auto-immune disease and their interactions with the central nervous system and the autonomic nervous system.<sup>3</sup>

Sleep plays an important role in human health and well-being, with its recuperative, restorative, and learning consolidation properties.<sup>4,5</sup> Unrefreshing sleep is a hallmark of ME/CFS that causes patients to wake up feeling tired even after extended periods of sleep, to experience excessive daytime sleepiness, and to have difficulty falling asleep and staying asleep. Therefore, unrefreshing sleep results at least in worsening fatigue symptoms and adds to the 'illness burden' in CFS patients.<sup>6</sup> Roughly 91% of CFS patients report unrefreshing sleep regardless of adequate sleep duration.<sup>7</sup> The effect of unrefreshing sleep is of importance in CFS aetiology because sleep disturbance can cause fatigue, myalgia, and poor attention.<sup>8</sup> A recent path analysis study, exploring the interrelationship of different symptoms in CFS, found that poor sleep quality directly impacted on

**Abbreviations used:** A&D, anxiety and depression; CFQ, Chalder Fatigue Scale; CFS, chronic fatigue syndrome; DARTEL, diffeomorphic anatomical registration through exponentiated Lie algebra; FWE, family-wise error; GM, grey matter; HADS, Hospital Anxiety and Depression Scale; IFOF, inferior fronto-occipital fasciculus; ME, myalgic encephalomyelitis; MNI, Montreal Neurological Institute; mPFC, medial prefrontal cortex; MT-T1w, magnetization transfer  $T_1$  weighted; NC, normal control; PSG, polysomnography; PSQI, Pittsburgh Sleep Quality Index; REM, rapid eye movement; SPM, statistical parametric mapping; T1w,  $T_1$  weighted; T2w,  $T_2$  weighted; VBIS, voxel based iterative sensitivity; WM, white matter

both mental and physical quality of life.<sup>9</sup> Similarly, another study found that subjective- but not actigraphy-defined sleep quality predicts next-day fatigue in patients with CFS.<sup>10</sup>

Despite consistent complaints of unrefreshing sleep by CFS patients, studies of sleep quality in CFS patients are limited. A review of 24 papers on sleep in CFS noted a discrepancy between objectively measured sleep architecture using polysomnography (PSG) and the multiple sleep latency test, and the subjective sleep quality reported by CFS patients.<sup>11</sup> Differences in PSG measures of total sleep time between CFS patients and healthy controls were limited. A twin study of 22 pairs of monozygotic twins where one member of the pair had CFS and the other did not found that the twins did not differ in objective PSG measures of sleep latency, total sleep time, sleep efficiency, arousal number, arousal index, hypnogram awakenings, rapid eye movement (REM)-sleep latency, or percentages of Stages 1, 2, and 3–4 (delta) sleep.<sup>12</sup> However, the percentage of REM sleep was increased in the CFS twins compared with the healthy twins. Other studies suggested that sleep homeostasis, reflected by the slow wave electroencephalogram, was impaired in CFS.<sup>13,14</sup>

Associations between sleep disturbance and brain structure changes have been observed in other diseases and aging. Mander et al. found that decreased sleep slow wave activity with age was mediated by a reduction in medial prefrontal cortex (mPFC) grey matter (GM).<sup>15</sup> Similarly, sleep disturbance is associated with distinct alterations in default mode network functional connectivity in brain regions underpinning salient memory and sleep systems.<sup>16</sup> More specifically, sleep deprivation caused widespread changes in white matter (WM) microstructure.<sup>17</sup> Therefore, previous studies have shown that sleep disturbance was associated with brain abnormalities.<sup>15–17</sup> Given that unrefreshing sleep is a hallmark of CFS, we hypothesized that brain changes would be associated with unrefreshing sleep in patients with CFS. Interrogation of brain structure associations with sleep disturbance can provide direct *in vivo* evidence on whether there is brain involvement in unrefreshing sleep in CFS.

To the best of our knowledge, there is no study of brain structure correlations with sleep quality in patients with CFS. Therefore, this study investigates if there are brain structural changes associated with unrefreshing sleep in CFS using MRI. In addition to global and regional GM and WM volumes, we performed a voxel based quantitative analysis of magnetization transfer  $T_1$  weighted (MT-T1w),  $T_1$  weighted (T1w), and  $T_2$  weighted (T2w) spin echo signal intensities. T1w hypointensities are associated histopathologically with severe tissue destruction, including axonal loss.<sup>18</sup> Similarly it has been shown that MT-T1w intensity is sensitive in depicting tissue lesions.<sup>19</sup> MT-T1w and T1w spin echo imaging are regarded as qualitative modalities because their intensities may be affected by factors such as shim, coil loading, and receiver gain settings, and can vary markedly across imaging sessions and subjects. Therefore, we adopted the voxel based iterative sensitivity (VBIS) method,<sup>20</sup> an objective statistical method for evaluation of signal intensity in our routinely acquired MT-T1w, T1w and T2w spin echo images. The VBIS method has been validated against relaxation time imaging to effectively perform inter-group comparisons of MR spin echo intensities.<sup>20</sup> After signal normalization, T1w and T2w images are ideal for cross-sectional studies because of their low noise, high resolution, and minimal distortion from patient induced and instrumentation induced magnetic field inhomogeneities.<sup>21</sup>

## 2 | MATERIALS AND METHODS

The study protocol was approved by the Human Research Ethics Committee of The Queen Elizabeth Hospital in compliance with the National Statement on Ethical Conduct in Human Research, and all subjects gave informed written consent for examinations.

### 2.1 | Subjects

Thirty-eight CFS subjects from community based specialist and general practice, meeting Canadian Consensus Criteria<sup>22</sup> for CFS, were assessed. Fourteen healthy, normal controls (NCs), unrelated to the CFS subjects, were recruited by public advertisement. The CFS group included 27 females and 11 males aged  $34.8 \pm 10.1$  (mean  $\pm$  SD) years. The NC group included 10 females and 4 males aged  $34.7 \pm 8.4$  years old. There was no significant difference in age ( $P = 0.26$ ) between the CFS and NC groups. No CFS or NC subject had developed a significant medical condition, including hypertension, or psychiatric illness during the research period. No subject was taking centrally acting medication. All subjects were right handed, not smokers, and not substance abusers, ascertained during clinician (RK) interview.

### 2.2 | Symptom scores

CFS severity was measured by the Chalder Fatigue Scale (CFQ), which discriminated reliably between the clinical and non-clinical conditions in 361 patients with CFS and 1615 NCs.<sup>23</sup> Higher CFQ scores indicate more severe disease. To determine their levels of depression and anxiety, all subjects completed the reliable and simple Hospital Anxiety and Depression Scale (HADS) questionnaire.<sup>24</sup> Sleep quality was evaluated using the Pittsburgh Sleep Quality Index (PSQI), which has a sensitivity of 89.6% and specificity of 86.5% in distinguishing good and poor sleepers.<sup>25</sup> The PSQI is inversely proportional to the sleep quality; i.e., higher scores indicate poorer sleep quality.

### 2.3 | MRI acquisition

MR images were acquired on a Philips 1.5 T Intera MR scanner (Philips, Eindhoven, The Netherlands) with a body transmit coil and birdcage receive coil. Transverse anatomic images were acquired using a three-dimensional spoiled gradient-echo sequence with  $T_R = 5.76$  ms,  $T_E = 1.9$  ms, flip

angle = 9°, resolution =  $0.938 \times 0.938 \times 1 \text{ mm}^3$ . Transverse T1w MR images with 3 mm thick contiguous slices of  $0.82 \times 0.82 \text{ mm}^2$  pixels were acquired with  $T_R = 600 \text{ ms}$ ,  $T_E = 15 \text{ ms}$ , flip angle = 90°. MT-T1w MR images were acquired with *off-resonance* excitation followed by  $T_1$  fast spin echo read-out for 3 mm thick contiguous slices of  $1.19 \times 1.19 \text{ mm}^2$  pixels and  $T_R = 600 \text{ ms}$ ,  $T_E = 15 \text{ ms}$ , flip angle = 90°. Transverse T2w MR image with 3 mm thick contiguous slices of  $0.82 \times 0.82 \text{ mm}^2$  pixels were acquired with  $T_R = 4000 \text{ ms}$ ,  $T_E = 80 \text{ ms}$ , flip angle = 90°.

## 2.4 | MRI processing

The three-dimensional anatomic images were segmented into GM, WM, and cerebrospinal fluid images using SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK), in which a unified probabilistic framework that combined image segmentation, tissue classification, and bias correction within the same generative model was implemented.<sup>26</sup> The empirical parameter for Markov random field removal of isolated voxel assignments was set to 4, in accordance with our previous validation results.<sup>27</sup> Global GM and WM volumes were computed in SPM12 by summing all voxel values in the GM and WM images.

Voxel based morphometry (VBM) in SPM12 was used to evaluate regional volume changes in the brain. VBM is a well-established tool to examine patterns of regional anatomical brain changes in neurological diseases and neuroanatomical correlates of CNS disease symptoms. VBM spatially normalizes GM and WM images for each subject from their native space to Montreal Neurological Institute (MNI) space, and the local GM and WM volume changes associated with their non-linear deformations are incorporated into their GM and WM image intensities.<sup>28</sup> More specifically, a study specific template was constructed from the GM images of all subjects normalized using DARTEL (diffeomorphic anatomical registration through exponentiated Lie algebra) implemented in SPM12.<sup>29</sup> Deformations to the new template were refined over six iterations. The study specific template was then normalized to a standard anatomical space defined by the MNI atlas. The DARTEL flow field images representing individual image deformations to the final study specific template, and the transformation of the template to MNI space, were combined. Then the regional GM and WM volumetric changes associated with the local composite deformation were encoded onto their intensities to form 'modulated' images, followed by a Gaussian smoothing with an  $8 \text{ mm} \times 8 \text{ mm} \times 8 \text{ mm}$  kernel.

The T1w, MT-T1w, and T2w images were first co-registered to their corresponding anatomic images. Second, co-registered T1w, MT, and T2w images were normalized to MNI space using the combined deformations computed above and smoothed with an  $8 \text{ mm} \times 8 \text{ mm} \times 8 \text{ mm}$  Gaussian kernel. Global signal levels for each individual and each modality were computed for each subject as the mean voxel value in a mask (brain region) generated using the VBIS method.<sup>20</sup> The MT-T1w, T1w, and T2w signal intensities were normalized using their corresponding global signal levels.

## 2.5 | Statistical analysis of symptom scores and global volumes

The following statistical tests of symptom scores and global GM and WM volumes were performed using SPSS22 (IBM, New York, USA). An independent two sample two tailed *t* test was used to compare inter-group differences in clinical measures (CFQ, anxiety and depression (A&D) from HADS, and PSQI), global GM volumes, and global WM volumes. The Bonferroni correction was used to counteract multiple comparisons of six characteristics ( $P < 0.0083$ ). Pearson correlations between each clinical measure and global GM and WM volumes from all subjects and separately from patients with CFS were calculated. Bivariate tests of significant difference in correlation coefficient from zero with two tails were followed with Bonferroni correction for multiple comparisons of 15 pairs to test for uncorrected ( $P < 0.05$ ) and corrected ( $P < 0.003$ ) statistical significance.

## 2.6 | Voxel based statistical analysis of MRI

Voxel based statistical analysis of the processed MRI was performed with SPM12. Cluster statistical inference was used for the primary detection of group differences and correlations with clinical variables.

A two sample *t* test was used to compare the CFS and NC groups for regional GM and WM volumetric differences (modulated GM images and WM images) and regional differences in signal intensity for T1w, MT-T1w, and T2w. All comparisons incorporated age as a nuisance covariate. The significance of inter-group differences was tested using family-wise error (FWE) corrected cluster *P* value ( $P_{\text{FWE\_corr\_cluster}} < 0.05$ ) with a cluster forming voxel threshold of uncorrected  $P < 0.001$  ( $P_{\text{uncorr\_voxel}} < 0.001$ ).

Voxel-wise statistical parametric mapping (SPM) regressions were performed between images from the CFS group against the symptom scores of CFQ and PSQI. All regressions incorporated the covariates of age, the appropriate global value, and HADS A&D scores. The significance of correlation was tested using  $P_{\text{FWE\_corr\_cluster}} < 0.05$  with cluster forming threshold of  $P_{\text{uncorr\_voxel}} < 0.001$ .

When a significant cluster was detected in either a group level difference or a correlation in CFS group analysis, a *secondary* statistical assessment was conducted at its location looking at *voxel* statistics in order to test whether the correlation also existed in all subjects (CFS + NC) and in the NC group. Thus, detection of a significant cluster in a *group* test was followed by examination of voxel *P* from the *correlation* tests at the same location in that image type, and vice versa. Because of the a priori nature of locations assessed in this second step, uncorrected voxel *P* values were used and the SPM voxel significance threshold was  $P_{\text{uncorr\_voxel}} < 0.001$ .

For the regions with significant correlations with symptom scores in the CFS group, voxel-wise SPM regressions were performed for all subjects (CFS + NC) versus the same symptom scores. Similar regressions were also performed using images from NCs only. Further, voxel based regressions with symptom scores in images from CFS were performed without adjustment for A&D in HADS. For these regions, the significances of

correlation were tested using  $P_{\text{uncorr\_voxel}} < 0.001$ . The inter-group comparisons were also revisited with a relaxed significant threshold of  $P_{\text{uncorr\_voxel}} < 0.001$  in these regions.

### 3 | RESULTS

#### 3.1 | Symptom score and global GM and WM volumes

The mean values and the significances of differences between CFS and NC groups for symptom scores and global GM and WM volumes are summarized in Table 1. There was no significant difference in total GM and WM volumes between CFS and NC. All symptom scores were significantly different between CFS and NC. Bivariate correlations between any pair of symptom scores or total GM and WM volumes in CFS patients only and in all subjects (CFS + NC) are summarized in Table 2. In the CFS group, significant correlations were detected between total GM and WM volumes and between depression and anxiety. In addition, CFQ and PSQI, CFQ and anxiety, CFQ and depression, and PSQI and anxiety were correlated, with uncorrected  $P < 0.05$ . Except for the correlation between total GM and WM volumes, these correlations were stronger when the CFS and NC groups were pooled.

#### 3.2 | Voxel-wise SPM of inter-group differences

Regional WM volumes in the left inferior fronto-occipital fasciculus (IFOF) were significantly lower ( $P_{\text{FWE\_corr\_cluster}} = 0.005$ ) in patients with CFS than those in NCs (Figure 1). The SPM statistics for this regional difference were as follows: cluster size 291 voxels, peak  $T$  score 5.73, peak

**TABLE 1** Means of symptom scores and global GM and WM volumes of CFS and NC groups<sup>§</sup>

Characteristics	$M_{\text{CFS}} \pm SD_{\text{CFS}}$	$M_{\text{NC}} \pm SD_{\text{NC}}$	P
GM	666.3 ± 71.9 ml	697.2 ± 94.2 ml	0.28
WM	504.4 ± 66.4 ml	514.0 ± 45.4 ml	0.55
CFQ	22.5 ± 7.2	3.5 ± 3.3	<0.008
PSQI	9.7 ± 4.0	4.0 ± 2.4	<0.008
Anxiety	7.1 ± 4.2	4.4 ± 2.3	<0.008
Depression	6.1 ± 3.5	1.8 ± 2.1	<0.008

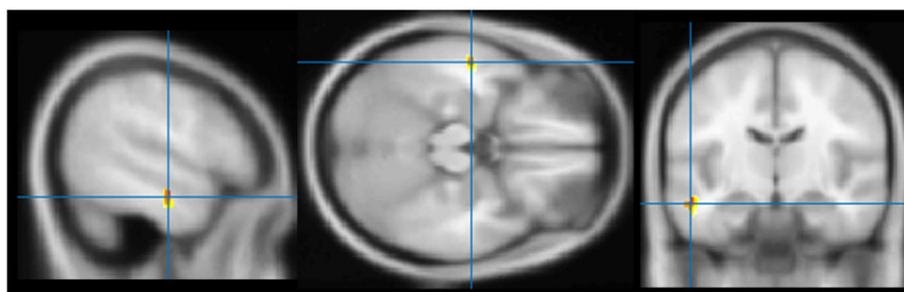
<sup>§</sup>M, mean value; SD, standard deviation; P, significance level of differences between CFS and NC groups.

**TABLE 2** Bivariate correlations among symptom scores and total GM and WM volumes<sup>§</sup>

Characteristics	GM	WM	CFQ	PSQI	Anxiety	Depression
GM		<b>0.64<sup>a</sup></b>	-0.15	0.07	-0.06	0.03
WM	<b>0.67<sup>a</sup></b>		-0.10	0.12	-0.02	0.14
CFQ	-0.13	-0.14		<b>0.60<sup>a</sup></b>	<b>0.49<sup>a</sup></b>	<b>0.63<sup>a</sup></b>
PSQI	0.23	0.31	<b>0.31<sup>b</sup></b>		<b>0.45<sup>a</sup></b>	<b>0.48<sup>a</sup></b>
Anxiety	-0.05	0.01	<b>0.44<sup>b</sup></b>	<b>0.33<sup>b</sup></b>		<b>0.63<sup>a</sup></b>
Depression	-0.06	0.16	<b>0.41<sup>b</sup></b>	0.26	<b>0.61<sup>a</sup></b>	

<sup>§</sup>The bivariate correlation coefficients in the CFS group only are listed to the left and below the diagonal. Coefficients for all subjects (CFS + NC) are listed in the shaded cells to the right and above the diagonal.

<sup>a</sup>Correlation significance level  $P < 0.002$ ; <sup>b</sup>correlation significance level  $P < 0.05$ .



**FIGURE 1** Results from voxel based comparison of regional WM volumes between CFS and NC groups. The cluster (CFS < NC) is superimposed on sections through its peak voxel of the 3D T1w MNI brain atlas. Colour coding from yellow to red represents  $T$  statistic values from 3.0 to 7.0

MNI coordinates  $[-52-12-16]$ . The secondary statistical assessment showed an adjacent negative correlation between regional WM volumes and PSQI ( $P_{\text{uncorr\_voxel}} < 0.001$ ): cluster size 24 voxels, peak  $T$  score 3.73, peak MNI coordinates  $[-56-15-22]$  in the CFS group. There were no significant differences ( $P_{\text{FWE\_corr\_cluster}} > 0.05$ ) in regional GM volume, MT-T1w, T1w, or T2w between the CFS and NC groups.

### 3.3 | Voxel-wise PSQI regressions

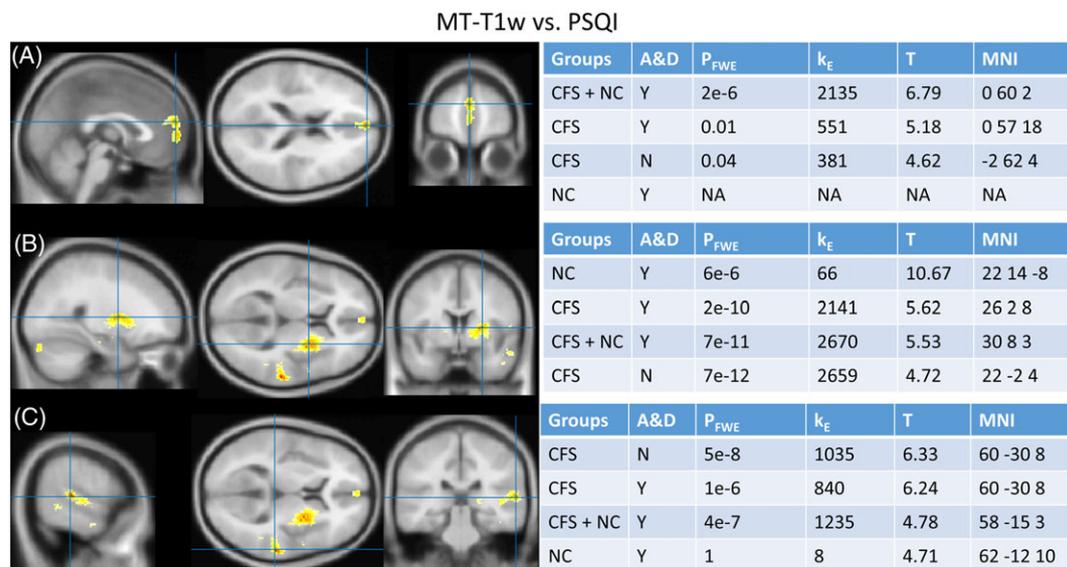
The MT-T1w and T1w intensities were significantly and negatively correlated with PSQI ( $P_{\text{FWE\_corr\_cluster}} < 0.05$ ) in multiple regions, including the mPFC, internal capsule, and right IFOF (Figures 2 and 3). The secondary statistical assessment of inter-group differences showed lower MT-T1w and T1w intensities ( $P_{\text{uncorr\_voxel}} < 0.001$ ) in the mPFC in CFS. The relative locations of all mPFC results are shown in Figure 4. There was no inter-group difference ( $P_{\text{uncorr\_voxel}} > 0.001$ ) of MT-T1w or T1w intensity in the right internal capsule or right IFOF. We did not observe significant PSQI correlations ( $P_{\text{FWE\_corr\_cluster}} > 0.05$ ) for regional GM volumes, WM volumes, or T2w intensities.

### 3.4 | Voxel-wise CFQ regressions

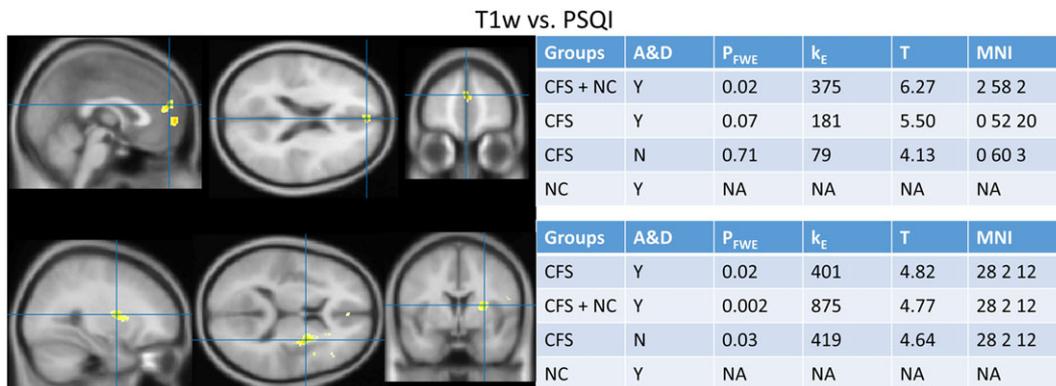
T1w intensities in the right insula were significantly and negatively correlated ( $P_{\text{FWE\_corr\_cluster}} = 0.002$ ) with CFQ in CFS: cluster size 534 voxels, peak  $T$  score 6.38, peak MNI coordinates  $[46-8-14]$  (Figure 5). When all subjects were included, the peak  $T$  score became lower but with increased cluster size:  $P_{\text{FWE\_corr\_cluster}} = 1.4 \times 10^{-5}$ , cluster size 1263 voxels, peak  $T$  score 5.49, peak MNI coordinate  $[45-8-9]$ . There was no significant correlation ( $P_{\text{uncorr\_voxel}} > 0.001$ ) between T1w intensities and CFQ in the right insula in images from NCs only. No significant inter-group difference of T1w intensities between CFS and NC groups ( $P_{\text{uncorr\_voxel}} > 0.001$ ) in the right insula was observed and we did not observe any significant CFQ correlations with regional GM volumes, WM volumes, MT-T1w intensities, or T2w intensities in the CFS group.

## 4 | DISCUSSION

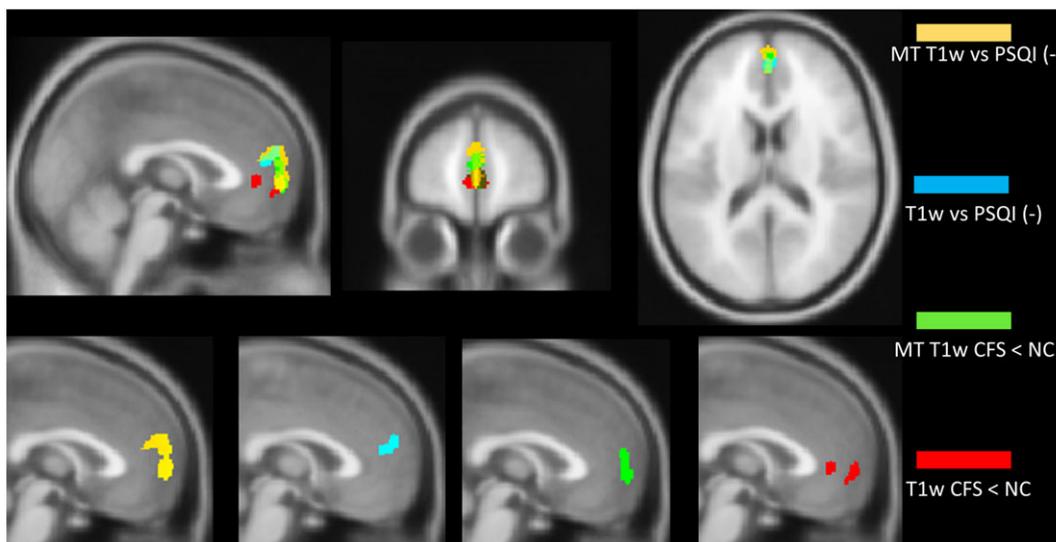
This is the first brain MRI study in CFS to test for brain structure associations with the frequent complaint of unrefreshing sleep as measured by the PSQI. There were three novel findings. (i) In the mPFC, MT-T1w and T1w intensities are significantly correlated with the sleep quality measure PSQI. Furthermore, the mPFC MT-T1w and T1w intensities were lower in patients with CFS than in NCs. (ii) The regional WM volumes in the left IFOF are significantly lower in patients with CFS than in NCs. In CFS subjects WM volumes in the left IFOF correlated with PSQI. (iii) The MT-T1w and T1w intensities in the right internal capsule were significantly correlated with PSQI both in the CFS group alone and in the combined CFS + NC groups, but not in the NC group alone. The T1w intensities in the right insula were significantly correlated with the CFS severity measure CFQ both in the CFS group and in the combined group. MT-T1w intensities in the right IFOF were also significantly correlated with PSQI in both the CFS and combined groups. However, no inter-group difference in MT-T1w or T1w level measures was observed in the corresponding regions.



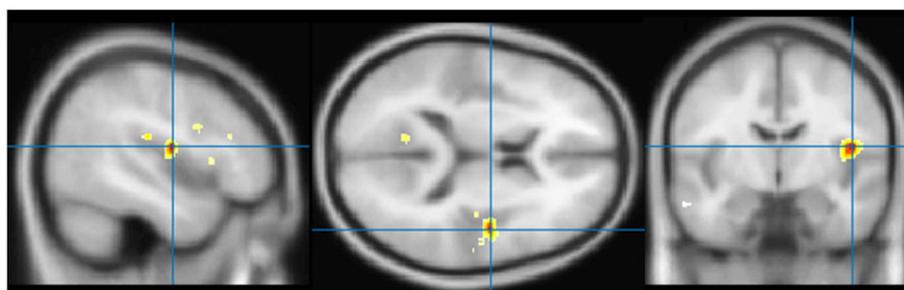
**FIGURE 2** Results from voxel based regressions of the PSQI with MT-T1w spin echo intensities. The images show three significant clusters from the negative correlation in the CFS group adjusted for A&D. Clusters were located in the mPFC (a), the internal capsule (B) and the right IFOF (C). Each cluster is superimposed on sections through its peak voxel of the 3D T1w MNI brain atlas. Colour coding from yellow to red represents  $T$  statistic values from 3.0 to 7.0. The tables on the right summarize MT-T1w versus PSQI cluster properties for regression analysis of pooled subjects (CFS + NC), CFS only (with and without A&D adjustment), and NC only.  $P_{\text{FWE}}$  is the FWE corrected cluster  $P$  value (cluster forming voxel  $P < 0.001$ , NA means no cluster formed);  $k_{\epsilon}$  is the cluster size in voxels;  $T$  is the  $T$  statistic of the peak voxel; MNI is the peak voxel coordinates in MNI space;  $a-b$  represents  $a \times 10^{-b}$



**FIGURE 3** Results from voxel based regressions of the PSQI with T1w intensities. The images on the left show two clusters where regional T1w intensities negatively correlate with PSQI in the CFS group after controlling for A&D. Clusters were located in the mPFC (a) and the internal capsule (B). Each cluster is superimposed on sections through its peak voxel of the 3D T1w MNI brain atlas. Colour coding from yellow to red represents  $T$  statistic values from 3.0 to 7.0. Tables on the right summarize cluster properties from T1w versus PSQI for NCs only, pooled subjects (NC + CFS), and CFS only (with and without A&D adjustment).  $P_{FWE}$  is the FWE corrected cluster  $P$  value (cluster forming voxel  $P < 0.001$ , NA means no cluster formed);  $k_E$  is the cluster size in voxels;  $T$  is the  $T$  statistic of the peak voxel; MNI is the peak voxel coordinates in MNI space



**FIGURE 4** Four clusters in the mPFC from MT-T1w and conventional T1w intensity analyses. For both MT-T1w and T1w, clusters are shown for the group comparison (CFS versus NC) and the CFS regression with the PSQI. Significant correlations (FWE corrected cluster  $P$  value,  $P_{FWE} < 0.05$ ) between MT-T1w intensities and the PSQI indices and between conventional T1w intensities and the PSQI indices (yellow and blue) are superimposed on the average  $T_1$  brain atlas in MNI space. Two sample  $t$  tests comparing MT-T1w and T1w intensities between CFS and NC groups (green and red) also showed significant differences (uncorrected voxel level  $P < 0.001$ ) in the same region



**FIGURE 5** Results from voxel based regressions of the CFQ with T1w intensities. The cluster shows the location in the right insula where regional T1w intensities correlate with CFQ in the CFS group adjusted for a&D. The cluster is superimposed on sections through its peak voxel of the 3D T1w MNI brain atlas. Colour coding from yellow to red represents  $T$  statistic values from 3.0 to 7.0

This study found that MT-T1w and T1w intensities were negatively correlated with PSQI in the mPFC, i.e., impaired sleep quality is associated with lower MT-T1w and T1w intensities. MT-T1w hypointensities indicate a reduced number of macromolecules in brain tissue or a reduced capacity for them to exchange magnetization with the surrounding water molecules. In WM this would most likely indicate changes in myelin. However, the mPFC locations involved here were in GM, where MT-T1w hypointensity has been interpreted to indicate tissue matrix damage and axon loss.<sup>30</sup> A post-mortem study in multiple sclerosis confirmed that T1w hypointensities were histologically correlated with tissue matrix damage and axon loss.<sup>18</sup> Therefore, our findings suggest that unrefreshing sleep is associated with mPFC matrix damage and axon loss. Our mPFC finding is consistent with previous findings that mPFC atrophy diminishes slow wave activity during non-REM sleep.<sup>15</sup> Experimental support for the role of mPFC in mediating sleep has been reviewed.<sup>31</sup> The correlations between mPFC MT-T1w intensities and PSQI were stronger with adjustment for A&D (Figure 2). Similarly, the correlations between T1w intensities and PSQI became more significant with adjustment for A&D (Figure 3). Thus, while A&D contribute variance to these MRI measures in the mPFC, this variance is not correlated with the variance associated with PSQI. Removal of A&D variance therefore strengthens the statistics of the MRI versus PSQI signal. Although PSQI correlates with depression and anxiety at the subject level, their correlations with local MRI measures are different.

Unrefreshing sleep is a defining characteristic of CFS. However, lack of objective evidence has led to questioning of subjective patient reports.<sup>12,32</sup> This study not only established negative correlations of MT-T1w with PSQI and T1w with PSQI in the mPFC, but also found lower mPFC MT-T1w and T1w intensities in CFS patients than in NCs. These two findings together confirmed that changes in MRI characteristics in the mPFC were associated with unrefreshing sleep in CFS patients, although causality between them could not be determined. It is not necessary, therefore, to resort to subject misperceptions to explain unrefreshing sleep in CFS. Our findings suggest that traditional sleep measures may not be able to detect sleep abnormality in CFS patients. The reasons for the changed MRI characteristics in the mPFC in CFS could not be determined in this study, although it is noteworthy that mPFC reduction of serotonin transporters and mPFC hypoperfusion were observed in previous studies of CFS.<sup>33,34</sup>

Regional IFOF WM volumes were significantly decreased here in patients with CFS compared with NCs. This finding supports our previous observation of *progressive* WM atrophy in the left IFOF in CFS,<sup>27</sup> although 15 of the 40 CFS patients and 10 of the 14 NCs in this study were also used in that longitudinal study. WM atrophy in the left IFOF also correlated with PSQI. To the best of our knowledge, the left IFOF does not play a role in mediating sleep, although a recent study found that frontoparietal connection strength predicts individual resistance to sleep deprivation.<sup>35</sup> Therefore, left IFOF WM atrophy could exacerbate poor sleep quality in patients with CFS.

We observed significant and negative correlations of MT-T1w and PSQI and of T1w and PSQI in right internal capsule WM in CFS patients. The statistical assessments suggested that this relationship does not derive from A&D and also exists in NCs. The internal capsule mainly contains the corticospinal tract, which carries sensorimotor information between the motor cortex neurons and motor neurons in the spinal cord that control limb muscles. This observation may explain the clinical presentation of difficulties in spatial sensing and coordination of motor function in CFS, although we are not able to make decisive conclusions given that no significant group difference was observed. The internal capsule also connects the thalamus with the insular cortex, comprising the interoceptive pathway for body state awareness and pain experience.<sup>36</sup> Therefore, these relationships consolidate the negative correlation of T1w and CFQ in the right insula cortex. These findings are also consistent with a recent report that an aberration of the salience network might play a role in CFS pathophysiology.<sup>37</sup> However, we did not observe inter-group differences in these regions. This could be due to the small sample size of NCs or indicate that these relationships exist in all individuals and are not specifically related to CFS. Similarly, we were not able to make a CFS-related conclusion on correlations between MT-T1w and PSQI in the right IFOF because inter group differences were not observed.

This study was limited in several aspects due to its retrospective nature. The MT-T1w, T1w, and T2w spin echo imaging are qualitative modalities because their intensities may be affected by factors such as shim, coil loading, and receiver gain settings, and can vary markedly across imaging sessions and subjects. Therefore, we adopted the VBIS analysis method, which had been validated against relaxation time imaging as effective in cross group comparison of MR spin echo intensities.<sup>20</sup> Although VBIS cannot correct for regional intensity differences caused by heterogeneity in receiving coil sensitivity, we argue that these differences would introduce additional variance and decrease statistical sensitivity, but would not introduce bias between groups. The sample size of the NC group is small in this study due to the retrospective nature of this analysis. Similarly, VBM can be sensitive to artifacts including normalization errors and misclassification of tissue types. All of these factors may confound the statistical analysis and decrease the sensitivity, but are less likely to increase the chance of false positives given that MRI data of CFS and NCs were collected and processed in an indistinguishable way. Furthermore, the overlapping of clusters in both MT-T1w and T1w regression and group difference analysis suggested a low probability of false positive findings. Therefore, although we are able to confirm mPFC as one brain structural underpinning of disrupted sleep in CFS, we cannot rule out that other structural changes may also be involved, especially in regions having correlations with PSQI that here show no inter-group difference.

## 5 | CONCLUSION

This is the first study to investigate brain structure associations with unrefreshing sleep in CFS. We found that mPFC MRI measures correlate with sleep quality and concluded that mPFC integrity may be affected in patients with CFS. The discrepancy between subjective sleep quality reports in

CFS and the objective instrumental measures most likely arises because traditional sleep monitoring methods do not respond to the mechanism that causes unrefreshing sleep in CFS. This study warrants the exploration in more depth of unrefreshing sleep in CFS patients.

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