

# Peripheral and Central Nervous System Biomarkers of Inflammation in Functional Seizures: Assessment with Magnetic Resonance Spectroscopy

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**Purpose:** Inflammation may link trauma to clinical symptoms in functional seizures (FS). We compared brain temperature and metabolites in FS, psychiatric (PCs) and healthy controls (HCs) and quantified their associations with serum biomarkers of inflammation and clinical symptoms.

**Patients and Methods:** Brain temperature and metabolites were measured with whole-brain magnetic resonance spectroscopic imaging (MRSI) and compared between groups in regions of interest and the whole brain. Relationships with inflammatory biomarkers and symptoms were assessed with Pearson correlations.

**Results:** Brain temperature was higher in FS than HCs in the orbitofrontal cortex (OFC) and anterior cingulate gyrus (ACG) and lower in the occipital cortex and frontal lobe. PCs showed lower temperatures than HCs in the frontal lobe including precentral gyrus and in the cerebellum. Myo-inositol (MINO) was higher in FS than HCs in the precentral gyrus, posterior temporal gyrus, ACG and OFC, and choline (CHO) was higher in the occipital lobe. CHO was higher in PCs than HCs in the ACG and OFC, and N-acetylaspartate (NAA) was higher in the ACG. There were no significant correlations with the serum inflammatory biomarkers. In FS, brain temperature correlated with depression, quality of life, psychological symptoms, and disability, CHO correlated with disability, and MINO correlated with hostility, disability, and quality of life.

**Conclusion:** Some of the previously identified neuroimaging abnormalities in FS may be related to comorbid psychiatric symptoms, while others, such as abnormalities in sensorimotor cortex, occipital regions, and the temporo-parietal junction may be specific to FS. Overlapping MINO and temperature increases in the ACG and OFC in FS suggest neuroinflammation.

**Keywords:** brain temperature, magnetic resonance spectroscopy, functional seizures, inflammatory biomarkers, metabolites

## Introduction

Functional seizures (FS) are a subtype of functional neurological symptom disorder (FNSD). Their hallmark feature are recurrent episodes of altered awareness and abnormal motor behavior (ie, seizures) that clinically resemble epileptic seizures but are not attributable to epileptiform discharges. The etiology of FS is still being investigated and the field has seen an increased focus on identifying organic underpinnings of the disorder.<sup>1,2</sup> For example, neuroimaging studies have uncovered abnormal brain structure and function in networks supporting salience, emotion regulation, self-agency, attention, and sensorimotor functions.<sup>1</sup>

Lifetime exposure to trauma is common among individuals with FS, yet how traumatic events are linked to brain structure and function in FS remains unclear.<sup>3-5</sup> We recently proposed a two-hit model of FS pathophysiology which underscores the role of early life stressors (eg, physical, emotional) in later development of FNSD/FS in the setting of secondary trauma.<sup>2</sup> We hypothesized that neuroinflammation (NI) is the pathophysiological process linking traumatic

experiences with neural network alterations in FS.<sup>6</sup> In this model, physical or psychological trauma activates the hypothalamic-pituitary-adrenal axis to release catecholamines,<sup>7</sup> which causes the immune system to mount an inflammatory response. Interactions between the peripheral and central immune system lead to inflammatory responses in the brain, which leave the brain vulnerable to developing FS either spontaneously or in response to a second insult or trauma.<sup>8,9</sup> Importantly, this model can account for the proportion of FS patients who do not report a history of trauma because inflammatory processes can be initiated through mechanisms other than trauma. To date, only two studies have directly tested inflammatory biomarkers in FS.<sup>10,11</sup> Gledhill et al found elevations in TNF-related apoptosis-inducing ligand (TRAIL) and intercellular adhesion molecule (ICAM)-1 within 24 hours following FS compared to patients with epileptic seizures. In contrast, tumor necrosis factor (TNF) receptor 1 (TNF-R1) and monocyte chemoattractant protein (MCP)-1 were elevated following epileptic seizures compared to FS, supporting the idea that epileptic seizures and FS may have distinct inflammatory signatures.<sup>12</sup> We recently described relationships between inflammatory biomarkers and white matter microstructure in the current study sample, showing associations between TNF-R1, interleukin (IL)-6, and ICAM-1, and neurite morphology in the uncinate fasciculus of FS patients, among other findings.<sup>11</sup>

Although NI may be difficult to quantify directly, advanced neuroimaging techniques allow us to detect metabolic abnormalities that point to the presence of NI.<sup>13–15</sup> Magnetic Resonance Spectroscopic Imaging (MRSI) quantifies a number of brain metabolites that may be associated with NI, including choline (CHO), myo-inositol (MINO), and N-Acetyl-aspartate (NAA).<sup>13,16</sup> MRSI can also be used to measure brain temperature, which may be a proxy for NI because heightened metabolic demands during NI increase temperature in the microenvironment.<sup>17,18</sup>

To provide support for the above two-hit hypothesis, we applied MRSI to assess whether FS are associated with abnormally elevated brain temperature and metabolites. We compared brain temperature, CHO, MINO, and NAA between 24 patients with FS, 27 psychiatric controls (PCs), and 18 healthy controls (HCs). We also assessed their relationships to serum levels of TNF-R1, TRAIL, IL-6, TNF- $\alpha$ , ICAM-1, and MCP-1, and to questionnaires assessing psychiatric symptoms, stressful life events, disability, and quality of life (QoL).

We hypothesized that brain temperature, CHO, and MINO would be higher and NAA lower in FS compared to PCs and HCs. We also hypothesized that brain temperature, CHO, and MINO would be higher and NAA lower in PCs compared to HCs. We hypothesized that higher brain temperature, CHO, and MINO, and lower NAA would be associated with higher levels of inflammatory biomarkers, more severe symptoms, and lower QoL in FS and PCs.

## Materials and Methods

### Study Design

The current study was a prospective, cross-sectional observational study comparing brain temperature and metabolites (CHO, MINO, NAA) and serum inflammatory biomarkers between FS, PCs, and HCs. We recruited two control groups to determine whether any identified brain abnormalities are specific to FS or could be due to the presence of psychiatric comorbidities, such as depression, anxiety, trauma, and dissociation, many of which have previously been linked to NI.<sup>19,20</sup> TRAIL and ICAM-1 were measured due to their previously identified roles in FS and epileptic seizures.<sup>10</sup> TNF- $\alpha$ , a pro-inflammatory cytokine, and its receptor, TNF-R1, have been shown previously to be elevated in depression and post-traumatic stress disorder (PTSD),<sup>21–23</sup> and IL-6, a pro-inflammatory cytokine, has been linked to depression.<sup>24</sup> Finally, MCP-1 is a chemokine involved in regulating the migration of monocytes and macrophages into the brain that has been linked to depression.<sup>25</sup>

The HC data have been previously reported as part of a repeatability analysis in Sharma et al.<sup>26</sup> The serum biomarker and questionnaire data in the FS and PC groups have been previously reported in Mueller et al<sup>11</sup> as part of an analysis investigating relationships between inflammatory biomarkers and white matter microstructure in FS.

### Participants

Participants aged 15–60 years were recruited from the epilepsy monitoring unit and outpatient psychology clinics at the University of Alabama at Birmingham (UAB). FS patients had a documented diagnosis of FS.<sup>27</sup> PCs were age-matched

to FS participants and had a diagnosis of depression, anxiety, or PTSD. Both groups were undergoing standard-of-care pharmacological and non-pharmacological treatments. HCs reported no medical or mental health conditions. PCs and HCs were recruited through online advertisements. A phone screen was conducted to confirm inclusion and exclusion criteria, and information was checked against medical records when available. The following criteria were exclusionary: 1) autoimmune, inflammatory, or neurological condition (eg, epilepsy, stroke, multiple sclerosis), 2) pregnancy or plans to become pregnant during the study, 3) psychotic disorder (eg, schizophrenia), 4) personality disorder, 5) substance use disorder, 6) contraindications to MRI (eg, metallic implants or claustrophobia), 7) blood clotting disorder, and 8) conditions that would prevent participation. HCs did not provide blood samples or questionnaires.

## Study Procedures

The study was conducted in accordance with the Declaration of Helsinki. The UAB Institutional Review Board approved the study procedures and written informed consent was obtained from all participants. Participants under 18 provided assent, and in these cases parents or legal guardians provided consent. Women of reproductive age underwent pregnancy testing. FS and PCs completed the Brief Trauma Questionnaire (BTQ),<sup>28</sup> Dissociative Experiences Scale, second edition (DES-II),<sup>29</sup> Somatoform Dissociation Questionnaire (SDQ),<sup>30</sup> Symptom Checklist 90 (SCL-90),<sup>31</sup> Holmes-Rahe Life Stress Inventory (LSI),<sup>32</sup> Hospital Anxiety and Depression Scale (HADS),<sup>33</sup> Quality of Life in Epilepsy short form (QOLIE-31),<sup>34</sup> the World Health Organization Disability Assessment Schedule (WHODAS2.0),<sup>35</sup> and a demographics form. FS patients reported the number of seizures experienced over the previous seven days.

Ten milliliters of blood were drawn into SST Vacutainers (BD, Franklin Lakes, NJ). Serum was separated by centrifugation and stored in  $-80^{\circ}\text{C}$  freezers. The following inflammatory biomarkers were quantified via multiplex electrochemiluminescence using a Meso Scale Discovery QuickPlex SQ 120 imager (MSD, Rockville, MD): IL-6, TNF- $\alpha$ , ICAM-1, and MCP-1. TNF-R1 and TRAIL were quantified via R&D ELISA (Minneapolis, MN).

## Neuroimaging

Neuroimaging was performed on a 3T Siemens Prisma Magnetom MRI with 20-channel head and neck coil (Siemens Healthineers, Erlangen, Germany). A T1-weighted Magnetization Prepared Gradient Echo (MPRAGE) sequence was acquired for anatomical reference with the following parameters: TR 2400ms, TE 2.22ms, flip angle:  $8^{\circ}$ , slice thickness 0.8mm, number of slices: 208, matrix:  $256 \times 256$ , voxel resolution:  $0.8 \times 0.8 \times 0.8\text{mm}$ . A 3-dimensional echoplanar spectroscopic imaging (EPSI) sequence was acquired with TR<sub>1</sub> 1550ms, TR<sub>2</sub> 511ms, TE 17.6ms, inversion time for lipid signal suppression 198ms, GRAPPA acceleration factor 1.3,  $71^{\circ}$  flip angle,  $280 \times 280 \times 180\text{mm}$  field-of-view,  $50 \times 50 \times 18$  matrix, and  $5.6 \times 5.6 \times 10\text{mm}$  voxel resolution. A saturation band covered the eyes and sinuses and iterative shimming and off-resonance frequency adjustment were performed prior to acquisition.

## Image Processing

MRSI data were processed with the Metabolite Imaging and Data Analysis System (MIDAS).<sup>16</sup> The fully automated processing pipeline performs reconstruction of the spectroscopic data, magnetic field inhomogeneity correction, smoothing, and spectral fitting. Metabolite spectra were integrated with the water reference image. Voxel-wise temperature was calculated with the following formula:  $T_{\text{CRE}} = -102.61 \times \Delta_{\text{H2O-CRE}} + 206.1^{\circ}\text{C}$ , where  $\Delta_{\text{H2O-CRE}}$  is the difference between locations of the water and creatine (CRE) peaks on the ppm scale. Temperature was corrected for gray and white matter content based on segmentation of the T1-weighted images. Metabolites (NAA, CHO, MINO) were expressed as CRE ratios using the *SINorm* module and interpolated to  $4.375 \times 4.375 \times 5.625\text{mm}$  resolution. Temperature and metabolite maps were non-linearly warped into MNI 2mm space. The spatially normalized maps were exported from MIDAS and smoothed with AFNI's *3dMedianFilter* (2-voxel radius) followed by smoothing with an 8mm Gaussian kernel in SPM12 (<https://www.fil.ion.ucl.ac.uk/>) for MATLAB (version R2021a, MathWorks, Natick, MA). Only voxels with linewidths of 2.0 to 12.00 and cerebrospinal fluid content of  $<30\%$  were used. Exported temperature maps are scaled so that a value of 0 corresponds to a temperature of  $32^{\circ}\text{C}$ . Voxels with temperatures outside the  $35.0^{\circ}\text{C}$  to  $44.0^{\circ}\text{C}$  range (raw values from 3 to 12) are physiologically impossible and were excluded from the analysis.

## Statistical Analyses

Statistical analyses of region-of-interest (ROI)-based imaging outcomes, serum biomarkers, and questionnaire scores were conducted in IBM SPSS Statistics for Macintosh, v28.0 (IBM Corp, Armonk, NY). Group differences in continuous and categorical variables were assessed with independent-samples *t*-tests and Chi-square tests, respectively. Statistical significance was assumed at  $p < 0.05$  (two-tailed).

Mean temperature, CHO/CRE, MINO/CRE, and NAA/CRE were extracted with AFNI's *3dROIstats* function from the bilateral anterior cingulate gyrus (ACG), orbitofrontal cortex (OFC; Brodmann areas 10, 11, and 47), inferior frontal gyrus, precentral gyrus, temporo-parietal junction (TPJ; including the posterior superior temporal, supramarginal, and angular gyri), superior parietal lobe, and insula. The ROIs were selected based on prior literature showing abnormalities in FS in these regions.<sup>2</sup> One-way analyses of variance (ANOVAs) were used to compare temperature (°C) and metabolites in the 14 ROIs (seven per hemisphere) among the three groups. ANOVAs were considered significant at  $p < 0.0154$  (corrected for 14 tests).<sup>36</sup>

Voxelwise group differences were assessed with AFNI's *3dttest++*. Three tests were run for each outcome testing the FS-PC, FS-HC, and PC-HC difference, respectively. Cluster thresholds were determined with the *3dFWHMx* and *3dClustSim* functions in AFNI. A voxel-level threshold of  $p < 0.01$  was applied, followed by the cluster extent threshold with nearest-neighbor 1 clustering, bi-sided thresholding, and  $\alpha = 0.05$ . The mean temperature and metabolite concentration were extracted from significant clusters for visualization. To determine relationships between brain outcomes, inflammatory biomarkers, and questionnaire outcomes, Pearson correlation coefficients were obtained in FS and PCs.

## Results

### Participant Characteristics

We screened 84 potential participants with FS, 42 of whom met inclusion criteria and were scheduled for the in-person visit. Thirteen FS participants withdrew before their visit, and five did not tolerate the MRI. The remaining 24 FS patients provided usable data. We screened 47 PCs, 31 of whom were scheduled. Four PCs withdrew before the visit, and 27 provided usable data. Twenty-two FS and 25 PCs provided blood and were included in correlations.

Table 1 shows demographic information and questionnaire means for FS and PCs. The mean age of HCs was 34.94 (SD=12.41), and did not differ from FS ( $t(40)=0.735$ ,  $p=0.467$ ) or PCs ( $t(43)=0.968$ ,  $p=0.338$ ). There were no differences in age, sex, or racial makeup between FS and PCs. The FS group reported higher HADS depression and anxiety, worse QoL related to seizure worry, emotional functioning, cognitive functioning, medication side effects, social functioning, and overall QoL than PCs. FS patients also reported higher somatization, obsessive-compulsive symptoms, depression, anxiety, phobic anxiety, psychoticism, and global psychological symptoms than PCs. Everyday dissociative experiences (DES-II) were more prevalent in FS compared to PCs, as was somatoform dissociation (SDQ). On the WHODAS2.0, FS patients reported greater disability than PCs in all six subdomains, and greater total disability. Significantly more FS (23/23 completed questionnaires) than PCs (21/27) reported lifetime trauma ( $p=0.016$ ).

At the time of study participation, 20/24 (83.3%) of FS patients and 20/27 (74.1%) PCs were taking psychotropic medications, and this proportion was not significantly different between groups ( $X^2=0.644$ ,  $p=0.422$ ). The most commonly taken psychotropic medications were duloxetine (7 FS; 2 PCs), escitalopram (2 FS; 5 PCs), trazodone (6 FS; 2 PCs), sertraline (4 FS; 2 PCs), fluoxetine (3 FS; 2 PCs), and lamotrigine (5 FS; 0 PCs).

### ROI-Based Analyses

The ANOVAs did not reveal brain areas with significant differences in temperature, CHO, MINO, or NAA among the three groups (all  $p > 0.0148$ , corrected).

### Whole-Brain Group Differences

Results from the whole-brain analyses are shown in Table 2. There were no clusters with significant differences in temperature, MINO, CHO, or NAA between FS and PCs. When assessing differences between FS and HCs, there were no significant differences in temperature or NAA surviving thresholds. MINO was higher in FS compared to HCs in the

**Table I** Demographic Information and Questionnaire Data for the Final Sample

	FS (N=24)		PCs (N=27)		t/X <sup>2</sup>	p
	Mean	SD	Mean	SD		
Age	37.67	11.48	38.67	12.78	0.292	0.771
Sex					0.514	0.473
Female (N)	22	–	23	–		
Male (N)	2	–	4	–		
Race					1.856	0.603
Asian (N)	1	–	0	–		
Black/African-American (N)	5	–	8	–		
White (N)	17	–	17	–		
Other (N)	1	–	2	–		
<b>Questionnaires</b>						
<b>HADS</b>						
Depression	7.96	4.78	4.74	3.48	–2.770	0.008**
Anxiety	11.54	4.70	10.11	3.92	–1.185	0.242
<b>LSI</b>						
Total score	210.83	139.87	194.37	140.03	–0.419	0.677
<b>QOLIE-31<sup>††</sup></b>						
Seizure Worry	33.26	30.43	97.74	10.84	9.572 <sup>†</sup>	<0.001***
Overall QoL	60.11	17.16	68.89	12.83	2.067	0.044*
Emotional	47.65	27.26	63.11	15.35	2.413 <sup>†</sup>	0.021*
Energy	35.22	26.05	44.81	18.27	1.525	0.134
Cognitive	40.47	29.66	69.37	21.92	3.861 <sup>†</sup>	<0.001*
Medication	66.18	36.92	94.15	18.28	3.190 <sup>†</sup>	0.003**
Social	52.46	23.29	89.81	17.76	6.428	<0.001***
<b>SCL-90</b>						
Somatization	1.63	1.10	0.46	0.31	–5.016 <sup>†</sup>	<0.001***
Obsessive-compulsive	1.77	1.19	0.99	0.77	–2.730 <sup>†</sup>	0.009**
Interpersonal sensitivity	1.21	1.17	0.70	0.53	–1.978 <sup>†</sup>	0.057
Depression	1.74	1.31	0.93	0.74	–2.664 <sup>†</sup>	0.012*
Anxiety	1.58	1.20	0.61	0.44	–3.724 <sup>†</sup>	<0.001***
Anger-hostility	0.90	1.16	0.43	0.48	–1.876 <sup>†</sup>	0.070
Phobic-anxiety	1.24	1.27	0.37	0.70	–2.980 <sup>†</sup>	0.005**
Paranoid ideation	0.99	1.15	0.69	0.93	–1.055	0.297

(Continued)

Table 1 (Continued).

	FS (N=24)		PCs (N=27)		t/X <sup>2</sup>	p
	Mean	SD	Mean	SD		
Psychoticism	0.99	1.06	0.33	0.42	-2.835 <sup>†</sup>	0.008**
Global Score	1.42	1.05	0.66	0.43	-3.309 <sup>†</sup>	0.002**
<b>DES-II</b>						
Total score	30.02	21.82	14.15	11.73	-3.124 <sup>†</sup>	0.004**
<b>SDQ</b>						
Total score	34.25	10.31	22.37	2.92	-5.451 <sup>†</sup>	<0.001***
<b>WHODAS 2.0</b>						
Cognition	35.22	22.74	17.41	13.33	-3.304 <sup>†</sup>	0.002**
Mobility	36.68	26.13	8.10	13.52	-4.734 <sup>†</sup>	<0.001***
Self-care	17.39	20.50	3.33	10.00	-2.999 <sup>†</sup>	0.005**
Getting along	34.42	31.91	16.98	20.35	-2.260 <sup>†</sup>	0.030*
Life activities	46.52	32.56	24.44	21.90	-2.849	0.006**
Participation	49.46	26.51	18.36	22.92	-4.449	<0.001***
Total score	38.37	22.08	15.22	13.54	-4.377 <sup>†</sup>	<0.001***
<b>BTQ</b>						
Trauma exposure (N)	23	–	21	–	5.808	0.016*
No trauma exposure (N)	0	–	6	–		
Not reported (N)	1	–	0	–		
<b>Serum biomarkers</b>						
IL-6	1.37	0.93	1.11	0.74	1.047	0.301
TNF-α	1.61	0.39	1.56	0.52	0.406	0.687
ICAM-1	590.23	184.47	520.73	102.99	1.566 <sup>†</sup>	0.127
MCP-1	250.55	148.14	234.88	55.73	0.468 <sup>†</sup>	0.644
TNF-R1	1359.95	244.21	1202.94	253.47	2.155	0.037*
TRAIL	96.75	20.43	95.91	27.06	0.119	0.906

Notes: <sup>†</sup>df adjusted for unequal variances. <sup>††</sup>Higher scores reflect better quality of life. \*p<0.05 \*\*p<0.01 \*\*\*p<0.001.

**Abbreviations:** BTQ, Brief Trauma Questionnaire; DES-II, Dissociative Experiences Scale, 2<sup>nd</sup> edition; HADS, Hospital Anxiety and Depression Scale; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin-6; LSI, Life Stress Inventory; MCP-1, monocyte chemoattractant protein 1; SCL-90, Symptom Checklist 90; SDQ, Somatoform Dissociation Questionnaire; QoL, Quality of Life; QOLIE-31, Quality of Life in Epilepsy, 31-item version; TNF-α, tumor necrosis factor α; TNF-R1, tumor necrosis factor receptor 1; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; WHODAS 2.0, World Health Organization Disability Assessment Schedule, 2nd edition.

precentral gyrus, TPJ and adjacent areas, and the ACG and OFC (Figure 1). CHO was also higher in FS versus HCs in the occipital lobe and adjacent regions (Figure 1).

There were no significant differences in temperature or MINO between PCs and HCs surviving cluster thresholds. CHO was higher in PCs than HCs in the ACG, OFC, and adjacent regions, and NAA was higher in PCs than HCs in the ACG and adjacent regions (Figure 2).

**Table 2** Clusters with Significant Differences in Brain Metabolites Between Functional Seizures (FS) and Healthy Controls (HC) and Between Psychiatric Controls (PC) and HCs

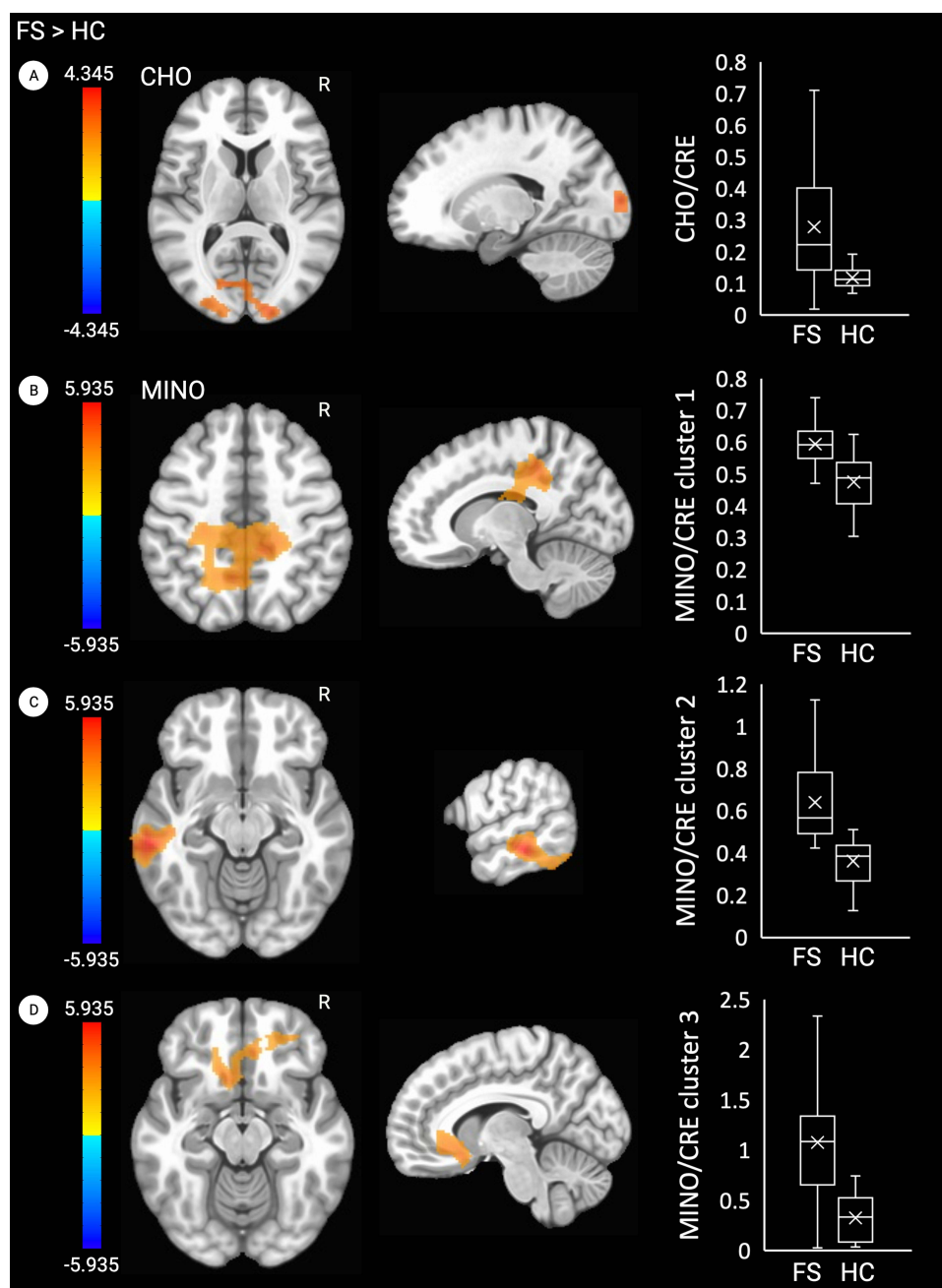
	Region	Hem	MNI Coordinates (Peak Signal)			Cluster Size (mm <sup>3</sup> )
			x	y	z	
FS vs HC						
Temperature						
Cluster 1*	Superior lateral occipital cortex, inferior lateral occipital cortex, occipital pole	L	+52	+78	+0	1680
Cluster 2*	Frontal pole, superior frontal gyrus, middle frontal gyrus	Bilateral	+32	−36	+44	819
Cluster 3*	<b>ACG, OFC</b> , frontal pole, frontal medial cortex, subcallosal cortex, paracingulate gyrus, genu of corpus callosum, anterior corona radiata, forceps minor	Bilateral	−10	−38	−8	547
CHO						
Cluster 1	Occipital pole, inferior lateral occipital cortex, intracalcarine cortex, lingual gyrus, occipital fusiform gyrus, forceps major	Bilateral	−18.0	+96.0	+10.0	1812
MINO						
Cluster 1	<b>Precentral gyrus</b> , posterior cingulate gyrus, precuneus, corpus callosum	Bilateral	−12.0	+36.0	+46.0	5171
Cluster 2	<b>Posterior superior temporal gyrus**</b> , posterior middle temporal gyrus, posterior inferior temporal gyrus, temporo-occipital inf. temporal gyrus, posterior temporal fusiform	Left	+60.0	+30.0	−12.0	1881
Cluster 3	<b>ACG, OFC</b> , frontal pole, medial frontal cortex, subcallosal cortex, paracingulate gyrus, corpus callosum, forceps minor	Bilateral	+8.0	−18.0	−14.0	1501
PC vs HC						
Temperature						
Cluster 1*	<b>Precentral gyrus</b> , superior frontal gyrus, middle frontal gyrus	L	+36	+12	+66	891
Cluster 2*	Cerebellum	R	−52	+66	−36	583
CHO						
Cluster 1	<b>ACG, OFC</b> , frontal pole, medial frontal cortex, subcallosal cortex, paracingulate gyrus, corpus callosum, forceps minor	Bilateral	−36.0	−46.0	−14.0	2279
NAA						
Cluster 1	<b>ACG</b> , frontal pole, medial frontal cortex, subcallosal cortex, paracingulate gyrus, corpus callosum, forceps minor	Bilateral	−10.0	−40.0	−6.0	2723

**Notes:** \*Did not survive cluster thresholding; \*\*Part of the temporo-parietal junction (TPJ). Only atlas regions with more than 3% overlap with the cluster are reported. Areas with previously identified abnormalities in FS are highlighted in bold.

**Abbreviations:** ACG, anterior cingulate; CHO, choline; Hem, hemisphere; MINO, myo-inositol; NAA, N-acetylaspartate.

Because many brain regions are smaller than the cluster thresholds that were determined for the temperature analyses (>2100 voxels), we performed additional analyses with a cluster threshold of 500 voxels (Table 2, Figure 3). This identified two clusters in the occipital and frontal lobes where temperatures were lower in FS than HCs, and one cluster in the frontal lobe including the OFC and ACG, where temperature was higher in FS than HCs. Additionally, PCs





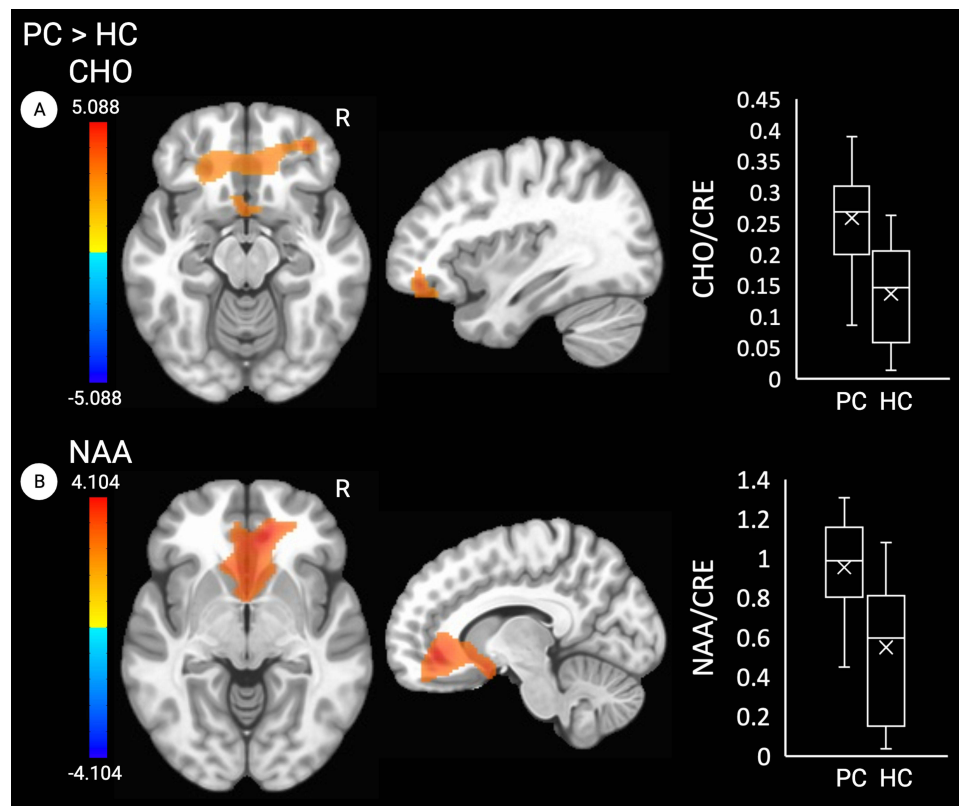
**Figure 1** Differences in choline (CHO) and myo-inositol (MINO) between functional seizures (FS) and healthy controls (HC). All contrasts are FS>HC. Images are shown in neurological convention (left = left). Color bars indicate t-values; CRE = creatine. **(A)** CHO increases (FS>HC) in the occipital pole, inferior lateral occipital cortex, intracalcarine cortex, lingual gyrus, occipital fusiform gyrus, and forceps major. **(B)** MINO increases (FS>HC) in the precentral gyrus, posterior cingulate gyrus, precuneus, and corpus callosum. **(C)** MINO increases (FS>HC) in the posterior superior temporal gyrus, posterior middle temporal gyrus, posterior inferior temporal gyrus, temporo-occipital inferior temporal gyrus, and posterior temporal fusiform. **(D)** MINO increases (FS>HC) in the anterior cingulate gyrus, orbitofrontal cortex, frontal pole, medial frontal cortex, subcallosal cortex, paracingulate gyrus, corpus callosum, and forceps minor.

exhibited lower temperatures than HCs in two clusters in the frontal lobe including the precentral gyrus and the cerebellum, respectively (Table 2, Figure 3).

## Associations with Serum Inflammatory Biomarkers

Mean serum biomarker levels in FS and PCs are shown in Table 1. The FS group had higher levels of TNF-R1 than PCs ( $t(45) = 2.155$ ,  $p = 0.036$ ). The remaining serum biomarkers did not differ between groups (all  $p > 0.05$ ). None of the





**Figure 2** Differences in choline (CHO) and N-acetylaspartate (NAA) between psychiatric controls (PC) and healthy controls (HC). All contrasts are PC>HC. Images are shown in neurological convention (left = left). Color bars indicate t-values; CRE=creatine. **(A)** CHO increases (PC>HC) in the anterior cingulate gyrus, orbitofrontal cortex, frontal pole, medial frontal cortex, subcallosal cortex, paracingulate gyrus, corpus callosum, and forceps minor. **(B)** NAA increases (PC>HC) in the anterior cingulate gyrus, frontal pole, medial frontal cortex, subcallosal cortex, paracingulate gyrus, corpus callosum, and forceps minor.

clusters showing temperature or metabolite differences in FS versus HCs and in PCs versus HCs were correlated with serum biomarkers.

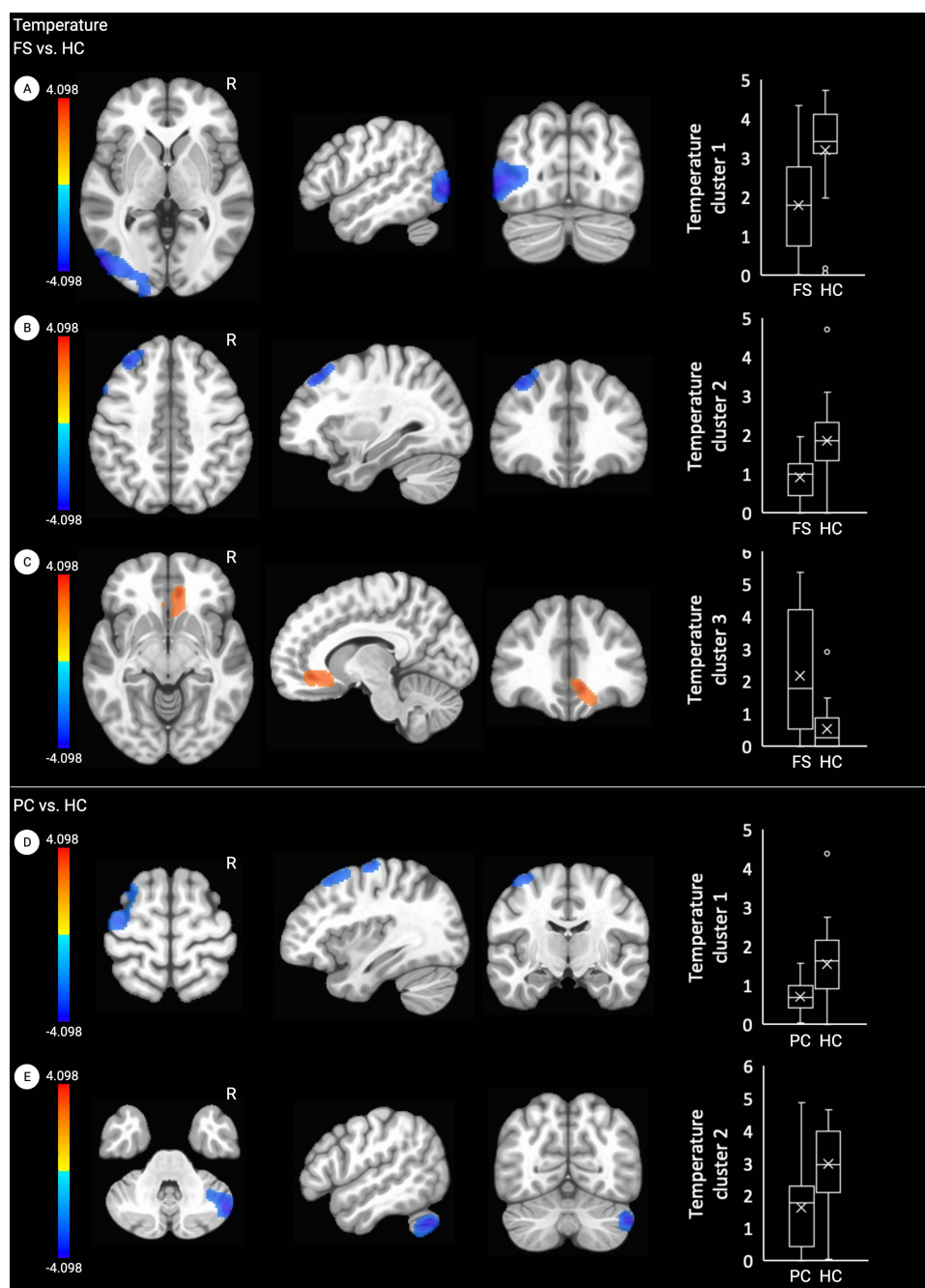
## Associations with Clinical Symptoms

Several correlations between temperature and metabolites and clinical symptoms were identified in the FS group (Figure 4). Lower temperatures in the occipital lobe (cluster 1) were correlated with higher depression (HADS-D;  $r=-0.448$ ,  $p=0.037$ ), lower emotional QoL ( $r=0.429$ ,  $p=0.047$ ), higher interpersonal sensitivity ( $r=-0.523$ ,  $p=0.013$ ), and psychoticism ( $r=-0.487$ ,  $p=0.021$ ) on the SCL-90, and higher disability related to getting along with others ( $r=-0.555$ ,  $p=0.007$ ) and total disability ( $r=-0.426$ ,  $p=0.048$ ). Higher temperatures in the frontal lobes including the ACG and OFC (cluster 3) were associated with decreased overall QoL ( $r=-0.460$ ,  $r=0.031$ ).

Increased CHO in the occipital lobe was correlated with higher disability related to getting along with others ( $r=0.437$ ,  $p=0.042$ ). Increased MINO in the precentral gyrus (cluster 1) was associated with increased hostility ( $r=0.426$ ,  $p=0.048$ ), and increased MINO in the posterior superior temporal gyri (cluster 2) was associated with higher hostility ( $r=0.527$ ,  $p=0.012$ ) and higher disability related to getting along with others ( $r=0.527$ ,  $p=0.012$ ). Increased MINO in the ACG and OFC (cluster 3) was related to lower overall QoL ( $r=-0.461$ ,  $p=0.031$ ) and lower emotional QoL ( $r=-0.425$ ,  $p=0.049$ ). CHO and MINO increases did not correlate with 7-day FS count. There were no significant correlations with clinical symptoms in PCs.

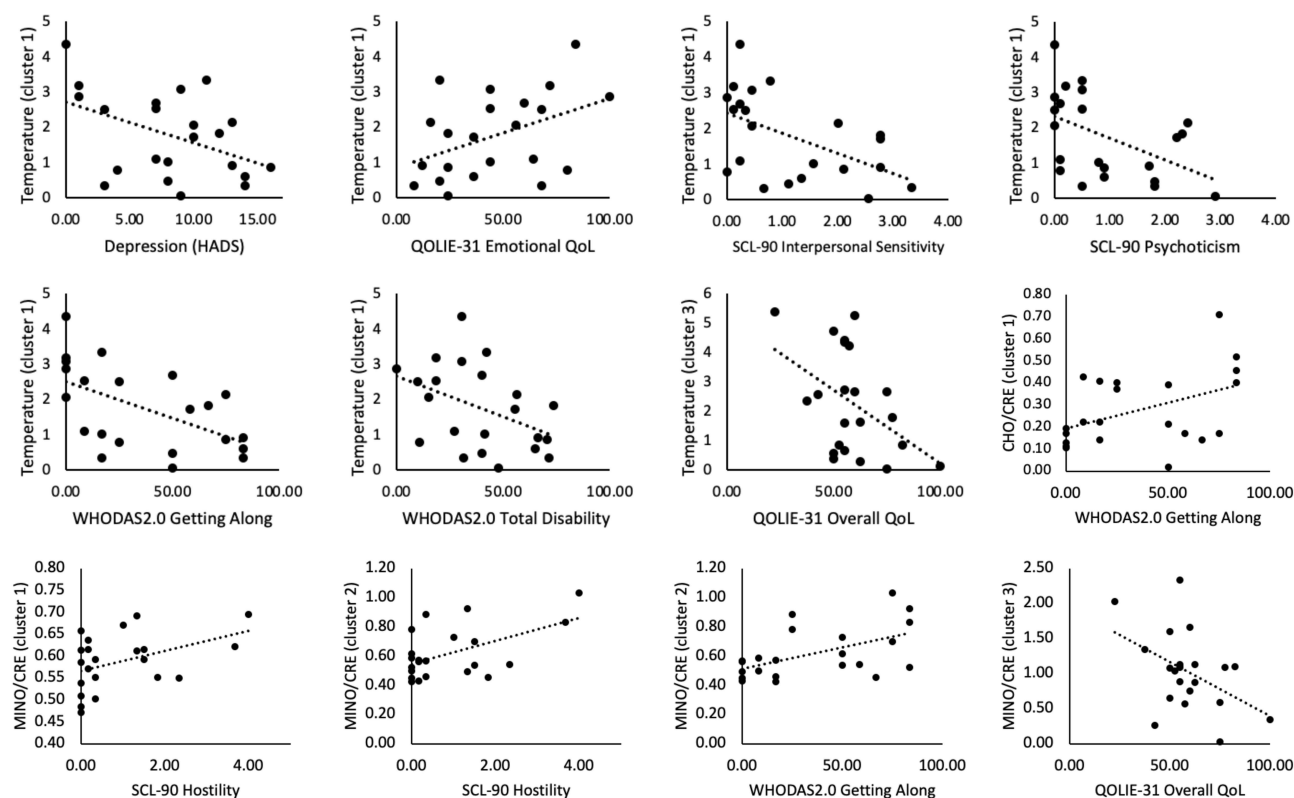
## Discussion

The aim of this study was to assess brain temperature and metabolic abnormalities in FS compared to PCs and healthy individuals. We compared brain temperature, CHO, MINO, and NAA in FS, PCs, and HCs in eight brain regions that



**Figure 3** Differences in brain temperature between functional seizures (FS) and healthy controls (HC) and psychiatric controls (PC) and HCs. Images are shown in neurological convention (left = left). Color bars indicate t-values. **(A)** Temperature decreases (FS<HC) in the superior lateral occipital cortex, inferior lateral occipital cortex, and occipital pole. **(B)** Temperature decreases (FS<HC) in the frontal pole, superior frontal gyrus, and middle frontal gyrus. **(C)** Temperature increases (FS>HC) in the anterior cingulate gyrus, orbitofrontal cortex, frontal pole, frontal medial cortex, subcallosal cortex, paracingulate gyrus, genu of corpus callosum, anterior Corona radiata, and forceps minor. **(D)** Temperature decreases (PC<HC) in the precentral gyrus, superior frontal gyrus, and middle frontal gyrus. **(E)** Temperature decreases (PC<HC) in the cerebellum.

have previously been implicated in FS. We did not identify the hypothesized differences between FS and PCs in the ROI-based or whole-brain exploratory analyses. When comparing the FS and PCs to HCs, we found differences in several brain regions. In FS, we found increased MINO in the precentral gyrus, the TPJ, ACG, and OFC, which corroborates previous study findings in FS.<sup>37</sup> In PCs, we observed increased CHO in the ACG and OFC and increased NAA in the ACG (PC>HCs). We also found lower temperature in FS in the occipital and frontal lobe (FS<HCs), and higher



**Figure 4** Significant correlations between brain temperature, choline (CHO), myo-inositol (MINO) and clinical symptoms in functional seizures.

**Abbreviations:** CRE, creatine; HADS, Hospital Anxiety and Depression Scale; QoL, quality of life; QOLIE-31, Quality of Life in Epilepsy; SCL-90, Symptom Checklist 90; WHODAS 2.0, World Health Organization Disability Assessment Schedule, 2nd edition.

temperature in the ACG and OFC (FS>HCs) that overlapped with MINO increases. In PCs, lower temperature was found in the precentral gyrus and cerebellum (PC<HCs), which did not overlap with metabolite abnormalities.

The FS group showed increased temperature and increased MINO in the ACG (FS>HC), with the MINO increases being associated with decreased emotional QoL. This may be an indication of NI-associated glial proliferation and could contribute to clinical outcomes in FS.<sup>38,39</sup> In PCs, increased CHO and NAA were found in the ACG, possibly reflecting different pathological substrates in the two groups. The lack of clinical associations in PCs could be the result of low clinical scores or narrow symptom range in our sample. The ACG is involved in emotion regulation, reward anticipation, and cognitive control, and has been implicated in FS pathophysiology in previous studies.<sup>40,41</sup> In our prior study in a different cohort of FS patients, we found decreased neurite density in the cingulum bundle,<sup>42</sup> the white matter tract that connects the cingulate gyrus to other cortical and subcortical regions. Additionally, hypometabolism, abnormal functional connectivity, and increased sulcal depth have been detected in the ACG of FS patients.<sup>43</sup> ACG abnormalities may underlie emotion regulation difficulties in FS, and may be similar to abnormalities seen in other psychiatric conditions.<sup>44</sup> Patients with other FNSDs also exhibit structural changes in the anterior cingulate that correlate with dissociative symptoms.<sup>45</sup> Because we found cingulate abnormalities in both FS and PCs when compared to HCs, but no differences between the two patient groups, cingulate abnormalities in FS could be due to psychiatric comorbidities rather than being specific to FS. This notion is supported by studies demonstrating changes in ACG activation after psychological treatment of functional tremor and depression.<sup>46</sup>

Another region that was differentially affected in FS and PCs was the OFC. We chose this as an ROI due to previous studies reporting structural abnormalities in FS in this region, including abnormal cortical thickness and sulcal depth.<sup>47</sup> We found increased OFC temperature and MINO in FS and increased CHO in PCs. In FS, the abnormalities were associated with lower emotional and overall QoL, whereas in PCs, no association with clinical symptoms was found. The

OFC plays a crucial role in action suppression and inhibition, thus supporting intentional behaviors. OFC abnormalities in FS may contribute to involuntary motor symptoms and altered awareness that are often observed during FS events.<sup>48</sup>

We also found increased MINO in the precentral gyrus in FS, but not in PCs. Abnormalities in cortical thickness,<sup>47</sup> functional connectivity,<sup>43</sup> and decreased neuronal activation<sup>49</sup> have been previously reported in the precentral gyrus of FS patients.<sup>50</sup> Since the precentral gyrus controls voluntary movement, abnormalities in this region may be contributing to the motor disturbances in FS. The only clinical correlation was found with hostility on the SCL-90, although we note that this cluster also included the posterior cingulate, precuneus, and corpus callosum, which could be driving the associations with hostility. Although we did not find an association between precentral gyrus abnormalities and FS frequency,<sup>50</sup> it is worth noting that our participants were receiving treatment and reported relatively few FS prior to study participation.

Further, we found increased MINO in the TPJ in FS compared to HCs, but not in PCs. The TPJ includes the supramarginal gyrus, angular gyrus, and the posterior portions of the superior temporal gyrus.<sup>51</sup> The TPJ has been implicated in regulating self-agency and theory of mind – processes that are disrupted in FS.<sup>51</sup> Self-agency is the subjective sense that one is causing one's own actions, and theory of mind is the ability to infer others' mental states (eg, emotions, intentions, thoughts). Previous research has consistently demonstrated abnormalities in the TPJ of FS patients, including decreased cortical folding<sup>37</sup> and decreased neuronal activation and functional connectivity.<sup>52</sup> Interestingly, the elevated MINO in the TPJ was related to increased hostility on the SCL-90, and to increased disability related to getting along with others. The TPJ supports the ability to interpret other people's intentions, which could explain the associations with hostility and problems getting along with others.

Increased CHO and lower brain temperature were found in the occipital lobe of FS patients compared to HCs. Although we did not define the occipital lobe as an ROI, previous studies have identified abnormalities in this region, including decreased functional connectivity<sup>43</sup> and cortical thickness<sup>47</sup> (FS < HCs). The occipital cortex supports visual processing and perception – processes that are disrupted in FS patients who report symptoms such as blurred or double vision, light sensitivity, and temporary loss of vision during seizures. The CHO abnormalities in the occipital lobe were correlated with increased disability in social interactions, which is not directly related to visual functioning. The temperature decreases were associated with higher depression, QoL, higher psychological symptoms, and disability. We note that the presence of decreased temperature in the occipital lobe does not support the idea that the symptoms are driven by NI, but given the overlapping CHO increases, this difference may be driven by abnormalities in cellular metabolism. Abnormalities in the occipital lobe have now been replicated in several FS studies<sup>43,47</sup> and warrant further investigation to delineate their relevance to FS symptoms.

We note that none of the brain temperature or metabolite differences in FS and PCs were associated with inflammatory biomarkers, suggesting that blood tests may not be a suitable substitute for measuring brain metabolite abnormalities in these groups. The null findings regarding differences between FS and PCs also suggest that NI may be a prominent feature of both conditions such that brain temperature alone may not be used to distinguish between the two. Because the brain temperature differences between FS and HCs did not survive the cluster thresholds calculated based on spatial noise in the maps, we caution against over-interpreting these, and we also note that the observed brain metabolite abnormalities could be driven by factors other than NI, such as neurodegeneration or mitochondrial dysfunction.

## Limitations

The current study had several limitations that should be considered. First, HCs did not provide blood samples, preventing a comparison of serum biomarkers between FS and age-matched HCs. Secondly, most FS patients were either undergoing or had recently completed psychological treatment, which is reflected in the low frequency of FS events reported by these patients at enrollment. The effects of psychotherapy on NI in this population are not known and need to be investigated, for example by comparing treated and untreated FS patients, or by tracking NI longitudinally before and after treatment. Regarding the current results, it is possible that the observed brain metabolite abnormalities are not generalizable to patients with untreated or more severe FS. If the two-hit model<sup>2</sup> is correct, we would speculate that NI would be more severe in untreated compared to treated cases. A related issue is regarding the effects of pharmacological treatment on NI. There are currently no medications approved for the treatment of FS, but many patients with FS take psychotropic medications to treat comorbid psychiatric conditions, and this could have impacted our results. The effects of psychotropic medications are somewhat mitigated in the current study by the fact that our two patient groups were similar with regard to psychotropic

medication use. However, when comparing patients to HCs, who were not on medication, the observed group differences could be related to the condition, the effects of medication, or a combination of both.

A third limitation is that this was a cross-sectional study and we did not time neuroimaging and biomarker collection in relation to seizures. One study has previously shown elevated serum inflammatory markers immediately following FS,<sup>10</sup> so our results may have been biased depending on the timing of FS patients' most recent seizure in relation to study participation. It is also likely that brain metabolites may be affected by a recent seizure, and this could be tested in longitudinal observational studies. Unlike other methods for imaging NI (eg, positron emission tomography), MRSI is completely non-invasive and does not require down-time to allow for radioactive decay, so it would be well suited for such longitudinal investigations.

## Conclusion

Brain temperature mapping is emerging as a valuable tool for non-invasively detecting NI in patients with various neurological and psychiatric conditions, but no prior study has attempted to distinguish between brain temperature elevations related to FS in particular and those related to comorbid psychiatric symptoms. To that end, this study revealed several differences in brain temperature and metabolites (FS vs HCs, PCs vs HCs) that may be used to distinguish patients with FS from those with other psychiatric conditions. Importantly, we also found that brain temperature was not abnormally elevated in patients with FS when compared to patients with other psychiatric conditions, but was altered when compared to HCs. Some abnormalities previously associated with FS, such as those found in the ACG and frontal lobe, may be related to comorbid psychiatric symptoms. Abnormalities observed in sensorimotor regions, the TPJ, and the occipital lobe appear more specific to FS.

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