

Primary Biliary Cholangitis Alters Functional Connections of the Brain's Deep Gray Matter

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OBJECTIVES: Fatigue, itch, depressed mood, and cognitive impairment significantly impact the quality of life of many patients with primary biliary cholangitis (PBC). Previous neuroimaging studies of non-hepatic diseases suggest that these symptoms are often associated with dysfunction of deep gray matter brain regions. We used resting-state functional magnetic resonance imaging (rsfMRI) to determine whether PBC patients exhibit altered functional connections of deep gray matter.

METHODS: Twenty female non-cirrhotic PBC patients and 21 age/gender-matched controls underwent rsfMRI. Resting-state functional connectivity (rsFC) of deep gray matter brain structures (putamen, thalamus, amygdala, hippocampus) was compared between groups. Fatigue, itch, mood, cognitive performance, and clinical response to ursodeoxycholic acid (UDCA) were assessed, and their association with rsFC was determined.

RESULTS: Relative to controls, PBC patients exhibited significantly increased rsFC between the putamen, thalamus, amygdala, and hippocampus, as well as with frontal and parietal regions. Reduced rsFC of the putamen and hippocampus with motor and sensory regions of the brain were also observed. Fatigue, itch, complete response to UDCA, and verbal working memory performance were also associated with altered rsFC of deep gray matter. These rsFC changes were independent of biochemical disease severity.

CONCLUSIONS: PBC patients have objective evidence of altered rsFC of the brain's deep gray matter that is in part linked to fatigue severity, itch, response to UDCA therapy, and cognitive performance. These results may guide future approaches to define how PBC leads to altered brain connectivity and provide insight into novel targets for treating PBC-associated brain dysfunction and behavioral symptoms.

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INTRODUCTION

Primary biliary cholangitis (PBC) is a cholestatic liver disease characterized by inflammatory destruction of the interlobular bile ducts that can progress to cirrhosis, liver failure, and transplantation/death.¹ PBC is also associated with itch and behavioral symptoms, including fatigue,^{2–4} cognitive impairment (e.g., decreased memory and concentration),⁵ and depression.^{6,7} Severity of symptoms does not typically correlate with markers of liver disease severity,^{3,8} but they negatively impact quality of life.⁹ There is also evidence that fatigued PBC patients have more aggressive disease and increased mortality.^{10,11}

The only widely approved therapy for PBC is ursodeoxycholic acid (UDCA), which can delay disease progression¹²; however, UDCA is not effective for all PBC patients. Even in UDCA responders, there is typically little impact on itch or behavioral symptoms.^{13–15} Brain mechanisms underlying behavioral

symptom development are unknown, which has led to symptoms predominantly being left untreated. Thus there is a need to better understand brain mechanisms so that symptom-targeted therapies can be developed to improve quality of life.

In non-hepatic diseases, fatigue, depressed mood, and cognitive impairment have all been linked to functional disturbances of the brain's deep gray matter. Fatigue can occur when the central nervous system fails to integrate limbic input and motor function within the basal ganglia, thus affecting the striatal–thalamic–frontal cortical pathway^{16,17} and thereby potentially leading to symptom development, including fatigue and cognitive impairment.¹⁸ Functional magnetic resonance imaging (fMRI) of patients with chronic fatigue syndrome demonstrated reduced activity of the putamen (a region of the basal ganglia), relative to control subjects, that correlated with fatigue assessment.¹⁹ The thalamus has also been implicated.²⁰ fMRI studies have linked depression to

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Table 1 Patient demographic and clinical characteristics, Fatigue Severity Scale (FSS) scores and itch domain scores of the PBC-40

Patient	Age (years)	Years since diagnosis (years)	Fibroscan value (kPa)	Alkaline phosphatase (U/l)	UDCA complete responder	FSS score	Itch
1	72	8	3.7	251	No	10	5
2	59	14	8.9	89	Yes	19	3
3	54	15	13.4	208	No	51	8
4	64	8	4.8	183	No	43	0
5	53	4	4.2	35	Yes	20	2
6	60	4	4.3	154	No	12	3
7	53	10	8.6	100	Yes	37	11
8	53	6	8.0	115	No	16	3
9	68	9	4.8	147	No	15	0
10	46	6	11.6	189	No	18	1
11	58	9	14.1	217	No	47	3
12	52	2	3.0	162	Yes	48	0
13	38	2	6.9	132	No	37	0
14	60	8	4.0	121	Yes	27	5
15	54	2	8.0	169	No	62	3
16	39	3	4.3	172	No	44	6
17	54	8	4.5	125	Yes	13	3
18	35	2	5.6	108	Yes	11	3
19	58	13	6.1	122	Yes	47	4
20	53	2	4.0	122	Yes	14	0
Median	54	7	5.2	139.5	—	23.5	3

UDCA, ursodeoxycholic acid.
FSS scores indicating fatigue (i.e., > 36) are italicized.

disruptions of the limbic system, including the amygdala and hippocampus,^{21–23} as well as the thalamus.²⁴ Patients with Parkinson’s disease, a motor disease attributed to basal ganglia degeneration, commonly exhibit apathy, fatigue, and cognitive deficits,²⁵ suggesting a dysfunctional interaction between deep gray matter regions. Although these diseases and disorders have diverse etiologies, they encompass a similar pattern of behavioral symptoms associated with functional disruption of deep gray matter regions. Hence, investigating the function of these regions in PBC patients may provide important insight into the etiology of disease-associated symptoms.

Itch has been shown to activate regions of the brain, including the somatosensory cortex, premotor cortex, insular cortex, and the supplementary motor area.^{26,27} Additionally, functional connectivity between the anterior insular cortex and globus pallidus is stronger when imagining itch as compared with pain.²⁸ The impact of itch (pruritus) on the brain in PBC patients is unknown but is important for understanding itch perception and central modulation in PBC.

Resting-state fMRI (rsfMRI) allows for the assessment of brain functional connections. Spontaneous fluctuations of rsfMRI signals are highly synchronous between brain regions that constitute a functional network, and the degree of synchrony indicates functional connection strength (termed resting-state functional connectivity (rsFC)).²⁹ To date, no rsFC studies have investigated PBC patients in the context of commonly reported symptoms. In the present study, we investigated rsFC of the putamen, thalamus, hippocampus, and amygdala of patients with PBC. We hypothesized that PBC patients would exhibit altered rsFC between these brain regions, as well as with frontal and parietal cortices responsible for cognitive processing. In exploratory analyses, we investigated the association between rsFC and fatigue severity, clinical response to UDCA treatment, itch, and cognitive performance.

METHODS

Participants. This study was approved by the Conjoint Health Research Ethics Board of the University of Calgary. All participants provided written informed consent. Twenty female patients with PBC, all of whom were taking UDCA for at least 6 months (mean dose 15.0 mg/kg/day; range = 13–19), were recruited from the University of Calgary Liver Unit. All patients met standard criteria for the diagnosis of PBC, including anti-mitochondrial antibody positivity and abnormal cholestatic liver biochemistry prior to the initiation of UDCA. Patients were intentionally selected to be non-cirrhotic based on biochemistry (i.e., normal International Normalized Ratio and normal serum bilirubin and albumin concentrations) and liver stiffness (<16.9 kPa³⁰) as measured by transient elastography (Fibroscan; Echosens, Paris, France). Patients were excluded if they had significant medical comorbidities (diabetes, neurological disease, mood disorder, cardiac or respiratory disease) or contraindications to MRI scanning. Complete UDCA response was defined as a sustained normalization of serum alkaline phosphatase levels.³¹ Patient characteristics are provided in Table 1. Prior to rsfMRI, all PBC patients completed the Fatigue Severity Scale³² (score of > 36 = fatigued³³) and the PBC-40 assessment scale.³⁴ Twenty-one female age-matched healthy volunteers were recruited through local advertisements (range = 33–63, median = 52, interquartile range = 7).

Resting-state fMRI. rsfMRI was performed using a 3 Tesla GE Discovery MR750 scanner equipped with a 12-channel receive-only phased array head coil (GE Healthcare, Waukesha, WI) and a gradient-recalled echo, echo planar imaging sequence (repetition/echo time = 2,500/30 ms, flip angle = 75°, 150 total volumes, 64 × 64 matrix, 3-mm isotropic voxels). Participants were asked to keep their eyes open

and focused on a fixation cross.³⁵ Anatomical images for registration were collected using a three-dimensional magnetization-prepared rapid gradient echo sequence (inversion/repetition/echo time = 550/8.2/3.2 ms, 0.8 × 0.8 × 1.3 mm³ voxels).

rsfMRI images underwent standardized preprocessing, including brain extraction, motion correction, intensity normalization, slice-timing correction, anatomical registration, and 6-mm Gaussian kernel spatial smoothing using the image analysis software (FSL; <http://www.fmrib.ox.ac.uk/fsl/>). To permit group comparisons, rsfMRI images were registered to anatomical images and subsequently to the Montreal Neurological Institute's (MNI) standard brain template using FSL's linear registration tool.^{36,37}

The putamen, thalamus, amygdala, and hippocampus, as defined by the MNI template, were chosen as regions of interest (ROIs). For each participant, the average rsfMRI time varying signal of each bilateral ROI was obtained using FSL's command-line tools and was subsequently used in separate time series analyses to determine rsFC, as implemented in FEAT v6.0 (part of FSL). The time series of voxels within cerebral spinal fluid and white matter were used as nuisance signals. Comparisons of rsFC between groups were performed using a mixed-effects analysis in FEAT, and results were presented as a brain map of the Z-score of the difference in rsFC between groups. Each map was corrected for multiple comparisons using a false discovery rate threshold of 0.05, corresponding to a cluster volume of >212 voxels, as determined by *AlphaSim* (part of the AFNI software package, <http://afni.nimh.nih.gov/afni>).

For patient rsfMRI data, additional mixed-effects analyses were conducted to determine the association between rsFC and: (i) fatigue (Y/N), (ii) complete biochemical response to UDCA therapy (Y/N), and (iii) total score on the itch domain of the PBC-40. Results were presented as a brain map of the Z-score of the association, corrected for multiple comparisons using a false discovery rate threshold of 0.05, corresponding to a cluster volume of >340 voxels, as determined by *AlphaSim*.

Cognitive testing and assessment. Partway through study recruitment, the Hamilton Depression Rating Scale (HAM-D) and a set of cognitive tests were added for exploratory analysis purposes. A subset of our cohort (15 PBC patients and 12 controls) completed the HAM-D, as well as the Trail Making Test (A and B), to assess visual attention and task switching,³⁸ the Digit Span Test (forward and back) to assess verbal/auditory working memory (part of the Wechsler adult intelligence scale), and the Corsi Block-Tapping Test (forward and back) to assess spatial working memory.³⁹ Total score on each test was compared between groups by one-way analysis of covariance, with age as a covariate. For scores significantly different ($P < 0.05$) between groups, a mixed-effects analysis of the PBC rsFC data was performed using the rank-order of the PBC group scores as the contrast of interest with age as a covariate in order to determine any association between score and rsFC. An analysis of covariance was also used to determine if scores differed between fatigued and non-fatigued patients, as well as between UDCA responders and non-responders. As only

Table 2 Cognitive testing and HAM-D scores

	Trails (A+B)	Digit Span (forward +backward)	Corsi Block (forward +backward)	HAM-D
<i>Patient</i>				
1	141	45	78	0
2	65	77	108	2
3	62	54	65	1
4	72	56	117	1
5	150	36	108	0
6	89	75	94	2
7	78	84	100	13
8	65	65	137	5
9	94	91	94	0
10	45	93	94	0
11	110	115	130	1
12	46	32	102	1
13	60	49	100	3
14	74	68	130	1
20	71	67	102	0
Avg (s.d.)	81.5 (31.1)	67.1* (22.8)	103.9 (19.2)	2.0 (3.3)
<i>Control</i>				
1	44	48	156	0
2	49	75	114	4
3	48	147	164	1
4	70	126	108	2
5	62	91	118	2
6	48	96	108	0
7	56	118	75	0
8	50	177	154	0
9	108	61	83	0
10	86	51	108	0
11	74	46	84	1
12	124	147	94	0
Avg (s.d.)	68.3 (25.8)	98.6 (44.3)	113.8 (29.8)	0.8 (1.3)

HAM-D, Hamilton Depression Rating Scale.
 Only Digit Span significantly differed between groups (* $P = 0.04$). Only PBC Patient 7 scored above normal on the HAM-D, in the mild depression range.

one PBC patient had a HAM-D score >7 (see Table 2), no association was investigated between rsFC and depressed mood.

RESULTS

Group differences. As shown in Figure 1 and summarized in Supplementary Table S1 online, relative to controls, rsFC of the putamen of PBC patients was increased with the amygdala bilaterally, left posterior insula and hippocampus, primary somatosensory cortex, and inferior parietal lobe bilaterally, as well as anterior and posterior cingulate cortices. Decreased rsFC was observed with right hemisphere structures, including the thalamus and superior frontal, precentral, lateral occipital, and inferior parietal gyri, as well as inferior frontal gyri (IFG) bilaterally.

Relative to controls, PBC patients exhibited increased rsFC of the thalamus with the left putamen, right hippocampus and amygdala, left primary motor cortex, and right primary somatosensory cortex (Figure 1 and Supplementary Table S2 online). Increased rsFC was also observed with the superior parietal lobe bilaterally and right superior temporal, inferior lateral occipital, and lingual gyri.

As shown in Figure 1 (summarized in Supplementary Table S3 online), relative to controls, patients exhibited increased rsFC of the amygdala with the putamen, thalamus, temporal

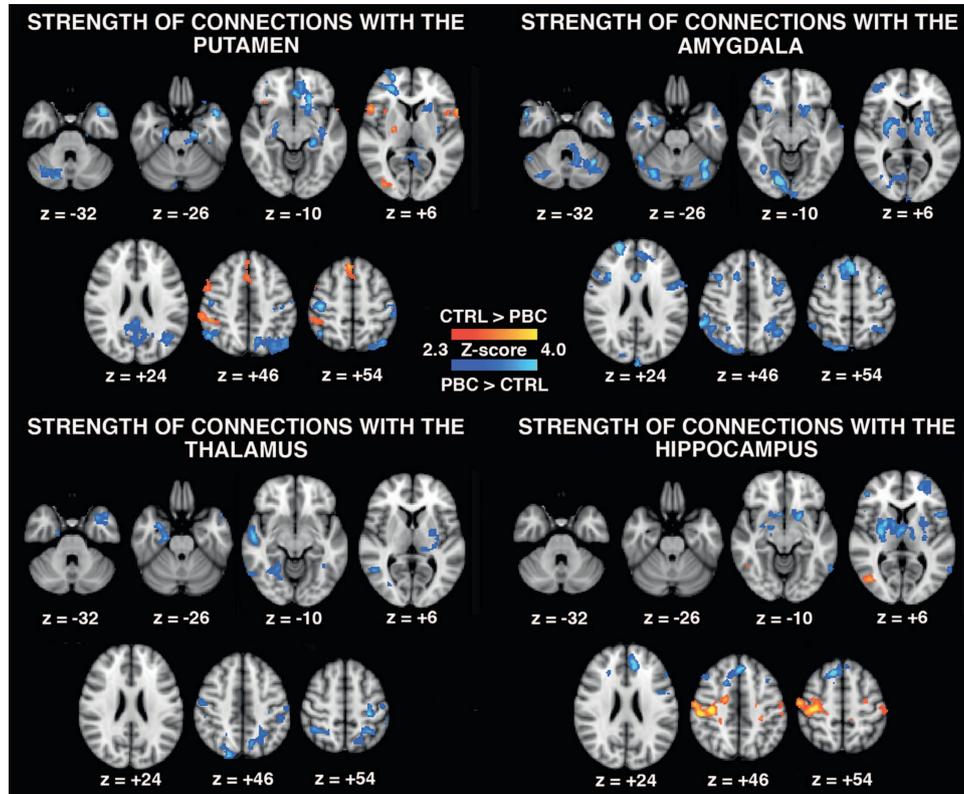


Figure 1 Top left. Brain regions where rsFC with the putamen (top left), amygdala (top right), thalamus (bottom left), and hippocampus (bottom right) significantly differed between primary biliary cholangitis (PBC) patients and control subjects. Red–yellow (blue–light blue) indicates regions where rsFC was less (greater) in patients than in controls, expressed as a Z-score. All regions are listed in Supplementary Tables S1–S4 online.

pole, and cerebellum bilaterally, and well as left premotor and superior parietal regions and right precuneus, dorsolateral prefrontal cortex, and superior and middle frontal cortices.

Relative to controls, patients exhibited increased rsFC of the hippocampus with the putamen, thalamus, and IFG bilaterally, as well as right dorsolateral prefrontal cortex and left middle frontal and temporal gyri (Figure 1, Supplementary Table S4 online). Decreased rsFC was observed with primary motor and somatosensory cortices bilaterally as well as the right inferior lateral occipital cortex.

Association with fatigue, Itch, UDCA response, and cognitive performance. Eight PBC patients were fatigued (Table 1). Fatigued patients exhibited stronger rsFC of the thalamus with right superior parietal and premotor regions, relative to non-fatigued patients (Figure 2 top). Reduced rsFC of the thalamus was observed with the ventral and dorsal anterior cingulate cortex and bilateral ventral lateral prefrontal cortex. Increased rsFC of the putamen was observed with the supplementary motor area and the left premotor cortex. No significant differences between fatigued and non-fatigued patients were observed with respect to rsFC of the amygdala and hippocampus.

Itch severity (Table 1) was associated with decreased rsFC of the amygdala and the hippocampus with the right sensorimotor and premotor cortices. Itch severity was also associated with decreased rsFC of the thalamus and the putamen with the anterior cingulate cortex (Figure 2 bottom).

Complete UDCA response was associated with increased rsFC of the hippocampus with the right amygdala and thalamus and left middle temporal gyrus (Figure 3 top). Complete response to UDCA was also associated with increased rsFC of the thalamus with the left amygdala and the putamen bilaterally, as well as superior temporal gyri bilaterally and left angular gyrus.

The subset of participants who underwent cognitive testing did not differ in age between groups ($t(25) = 1.33, P = 0.20$). PBC patients did not differ from controls on the Trail Making ($F(1,24) = 0.20, P = 0.66$) or Corsi Block Tapping ($F(1,24) = 0.60, P = 0.45$) tests (Table 2). Digit Span test scores for PBC patients were significantly lower than controls ($F(1,24) = 5.06, P = 0.03$). Digit Span scores did not differ between fatigued and non-fatigued patients ($F(1,12) = 0.12, P = 0.74$) nor between UDCA responders and non-responders ($F(1,12) = 0.37, P = 0.55$). Patients with lower Digit Span scores had increased rsFC of the amygdala with the right insula (Figure 3 bottom). Lower scores were also associated with decreased rsFC of the amygdala with the right inferior lateral occipital cortex, left IFG, and dorsomedial prefrontal cortex. Lower scores were not associated with rsFC of the putamen, thalamus, or hippocampus.

DISCUSSION

We have shown that deep gray matter regions of the brain (i.e., putamen, amygdala, hippocampus, and thalamus)

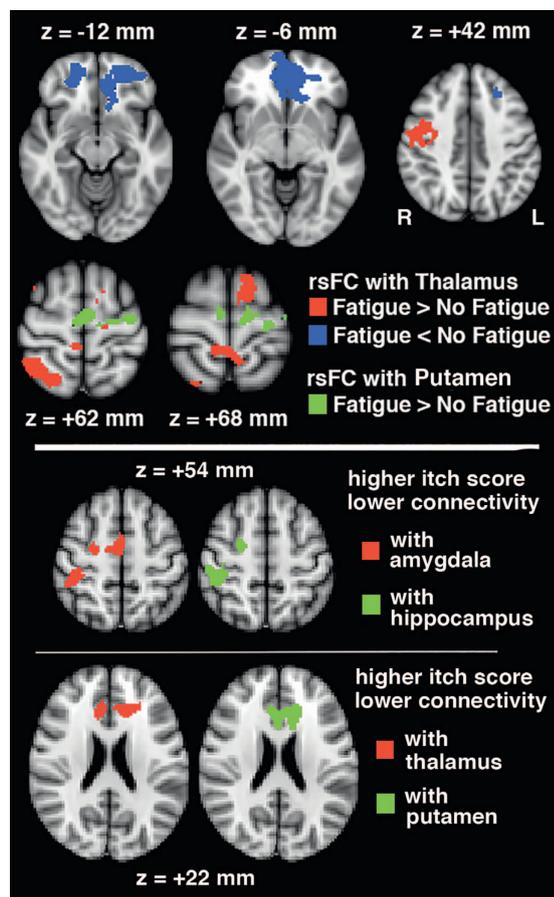


Figure 2 Brain regions where resting-state functional connectivity (rsFC) significantly differed ($P < 0.05$) between fatigued and non-fatigued primary biliary cholangitis patients (top) and in association with itch severity (bottom). L, left; R, right.

exhibit altered rsFC in patients with PBC. In the majority of cases, rsFC was increased in PBC patients relative to controls. This suggests that changes in brain functional connectivity may occur as part of a compensatory homeostatic response within the brain, which is in turn a response to chronic immune-mediated signaling from the liver to the brain in PBC patients.⁴⁰

The amygdala, hippocampus, and putamen are important for memory, learning, and emotional processing,^{41,42} and an overall disturbance in rsFC between these regions could therefore contribute to behavioral symptoms commonly reported by PBC patients.⁵ The hippocampus and amygdala also showed increased rsFC with the dorsolateral prefrontal cortex, a brain area involved in many executive functions, including memory and planning.^{43,44}

The thalamus is a key sensory relay station of the brain.^{45,46} With the exception of olfaction, every sensory system has a nucleus within the thalamus that is responsible for relaying sensory signals to appropriate cortical areas.⁴⁷ Therefore, it is plausible that altered sensory input to the brain in response to chronic immune-mediated liver injury in PBC contributes to the development of alterations in thalamic rsFC. We also observed decreased rsFC of the thalamus with regions of the anterior

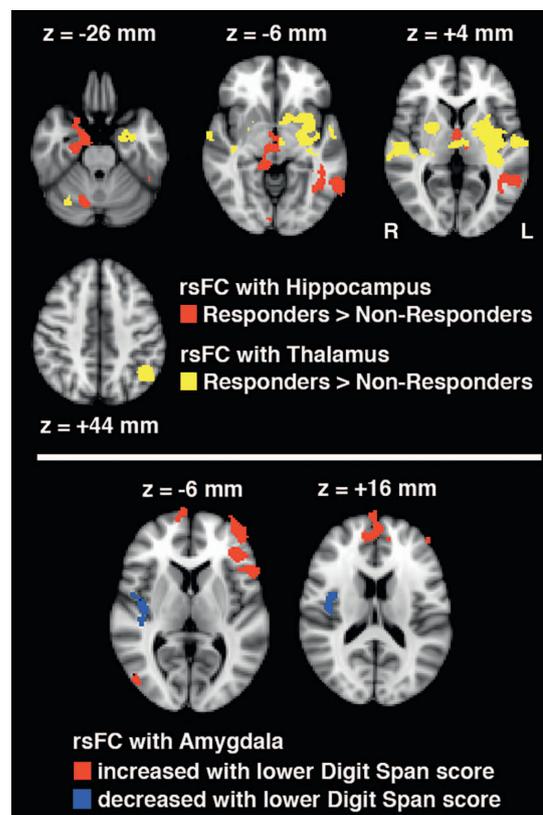


Figure 3 Brain regions where resting-state functional connectivity (rsFC) significantly differed ($P < 0.05$) between ursodeoxycholic acid responders and non-responders (middle) and in association with Digit Span score (bottom). L, left; R, right.

cingulate and increased rsFC with premotor brain regions in association with fatigue. Fatigued PBC patients have been reported as having a reduced threshold for exercise duration and lack postcontraction cortical depression typical of an exercise task.⁴⁸ If fatigue is associated with motor cortex dysfunction, increased rsFC may reflect a physiological response of the brain to attempt to compensate for this dysfunction in PBC patients.

Relative to control subjects, the putamen of PBC patients showed decreased rsFC with the precentral gyrus, an area of the brain containing the motor cortex. Connections between the putamen and motor cortex are important for the regulation of locomotion. Although our findings could be related to possible subtle unreported motor deficits in PBC patients, given the purported role of the putamen in the genesis of fatigue,¹⁹ these changes in rsFC are more likely linked to the development of fatigue as a symptom. This suggestion is also consistent with the previous demonstration of an association of motor cortex dysfunction with fatigue.⁴⁸ Our findings support this hypothesis, as we observed increased rsFC between the putamen and supplementary motor and premotor regions in fatigued patients. In our PBC patients, the putamen also exhibited decreased rsFC with regions involved in cognitive processing, including the IFG (language processing and attentional control⁴⁹), the superior frontal gyrus and inferior parietal gyrus (working memory^{50,51}), and the lateral occipital cortex (image interpretation⁵²).

rsFC of the amygdala demonstrated a direct association with working memory performance of our PBC patients. Increased rsFC was observed with the right insula, and decreased rsFC was observed with left IFG and dorsomedial prefrontal cortex. These findings suggest that an alteration in the functional connections between regions associated with emotional processing (amygdala, insula), sense of self (dorsomedial prefrontal cortex) and awareness (insula), and language processing (IFG) may contribute to deficits in working memory in the presence of chronic immune-mediated liver injury in PBC patients.

The hippocampus also exhibited decreased rsFC with motor and somatosensory brain regions in PBC patients, relative to controls. There is evidence to suggest that, in addition to its well-established role in memory, the hippocampus is also involved in locomotion.^{53,54} This suggestion is also supported by the widely reported positive effects of exercise on both mood and memory.^{55,56} A decrease in rsFC between the hippocampus and somatosensory cortex could therefore possibly be linked to cognitive deficits⁵ and reduced exercise duration tolerance⁴⁸ that have been previously reported in PBC patients.

In PBC patients, complete response to UDCA was associated with increased rsFC between the hippocampus, thalamus, and putamen, as well as with regions of the left parietal and temporal lobes. Even though UDCA response has not typically been associated with improvements in PBC-related symptoms, it is possible that complete normalization of liver biochemistry and associated improvement in ongoing liver injury may decrease hepatic immune-driven sensory input to the brain via the thalamus and/or hippocampus that in turn may strengthen rsFC of these regions.

The association between itch severity and functional connectivity of the sensory and premotor cortices is consistent with previous studies showing itch-related activation of these regions.^{26,27} Additionally, we found an association between itch severity and functional connectivity of the anterior cingulate cortex, a region known to regulate emotional reaction to pain.⁵⁷ Our findings suggest that itch (pruritus) severity in PBC patients correlates with a reduction in functional connectivity within brain networks known to regulate itch. These findings expand our current understanding of how itch impacts the brain in PBC.

Our study had several limitations. Each of our deep gray matter ROIs possess subregions that serve related, though distinct, functions. By selecting ROIs as a whole for our rsFC analyses, we have potentially decreased our ability to detect subdivision-specific alterations in rsFC or decreased the sensitivity of our analysis by combining subregions into a single ROI. Our study was also potentially limited by relatively small sample sizes, which makes addressing these ROI issues challenging and limits comparisons within the patient group. However, despite our relatively small sample sizes, we have robust evidence of differences in rsFC of deep gray matter between PBC patients and controls, as well as preliminary evidence that rsFC is associated with cognitive performance, fatigue, and complete response to UDCA.

Although comparing PBC patients to healthy controls determines the sensitivity of group differences, it does not establish their specificity. Fatigue, in particular, can be

classified as a non-specific symptom. Multiple sclerosis patients experiencing fatigue exhibit reduced activation in a variety of brain regions, including the left thalamus, bilateral striatum, left pallidus, and left substantia nigra, relative to controls.⁵⁸ Significant volume reduction of the putamen, caudate, pallidus, and thalamus in fatigued relapsed–remitting multiple sclerosis patients has also been observed.⁵⁹ In patients with chronic fatigue syndrome, the caudate nucleus exhibited lower levels of activation in response to visual imagery and motor imagery tasks.⁶⁰ These studies suggest that the altered functional connectivity observed in our PBC cohort may not be specific to PBC but may be linked to the symptom of fatigue in general. It would be of interest in future studies to compare findings in fatigued PBC patients in relation to those found in other liver in which fatigue is commonly reported.

An improved understanding of the association between liver disease and brain function has important clinical implications for the treatment of PBC-related symptoms. Altered functional brain connections may represent potential novel targets for pharmacological^{61,62} or non-pharmacological intervention in PBC patients with modalities, such as exercise or cognitive remediation. If functional connections and associated behavioral symptoms can be prevented, improved, or normalized, this may in turn improve the quality of life of PBC patients.

CONFLICT OF INTEREST

Guarantor of the article: Bradley G. Goodyear, PhD.

Specific author contributions: Victoria A.L. Mosher: conducted the study, collected and analyzed study data, interpreted findings, and drafted the manuscript. Mark G. Swain: enrolled patients, aided in planning of study, interpreted findings, and edited the manuscript. Jack X.Q. Pang: aided in conducting the study, administration of questionnaires, and cognitive testing of patients. Gilaad G. Kaplan: aided in planning of study and editing of the manuscript. Keith A. Sharkey and Glenda M. MacQueen: aided in planning of study, interpretation of findings, and editing of the manuscript. Bradley G. Goodyear: planning and conducting the study, analysis and interpreting data, and drafting and editing the manuscript. All authors approved the final draft submitted.

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Potential competing interests: None.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Fatigue, cognitive impairment, and altered mood significantly impact quality of life of many primary biliary cholangitis (PBC) patients.
- ✓ These symptoms have often been labeled as emotional reactions and have therefore been left untreated.
- ✓ In non-hepatic diseases, symptoms have been linked to dysfunction of deep gray matter brain regions.

WHAT IS NEW HERE

- ✓ PBC patients exhibit altered functional connections of the putamen, thalamus, amygdala, and hippocampus.
- ✓ Fatigue, itch, and working memory performance are associated with altered functional connections of deep gray matter.
- ✓ These findings help guide future study of the impact of hepatic diseases on brain function.

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