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Studying cognition impairment in patients with minimal hepatic encephalopathy through functional connectivity analysis: evidence from resting-state functional MRI

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AIM: This study aimed to investigate functional connectivity (FC) alterations between the insula and other brain regions in minimal hepatic encephalopathy (MHE) patients and their association with cognitive deficits.

MATERIALS AND METHODS: The study included 23 MHE patients and 25 healthy control (HC) individuals. All participants underwent resting-state functional magnetic resonanceimaging (fMRI), neuropsychological testing, and cognitive scale assessments. FC analysis was performed to investigate the connectivity between the insula and the whole brain in the context of MHE. *Pearson* correlation analysis was conducted to assess the association between changes in FC and cognitive scale scores.

RESULTS: The HC and MHE groups showed significant differences in cognitive performance measures, such as the Number Connection Test-A (NCT-A), Digit symbol Test (DST), and Montreal Cognitive Assessment (MoCA) score ($P < 0.01$). The MHE group demonstrated elevated FC between the right insula and the right superior frontal gyrus, right middle frontal gyrus, and right precentral gyrus compared with the HC group. Furthermore, the left insula showed increased FC with the left middle frontal gyrus and the right middle frontal gyrus ($P < 0.05$, corrected). Notably, these changes in FC among MHE patients were significantly correlated with the MoCA score ($P < 0.05$, corrected).

CONCLUSION: This study emphasises the FC alterations between the insula and the prefrontal cortex in MHE patients, which have a close association with cognitive functions. FC could potentially serve as a biomarker for diagnosing and assessing the severity of cognitive impairments in MHE.

Abbreviations: MHE, minimal hepatic encephalopathy; QSM, quantitative susceptibility mapping; MRI, magnetic resonance imaging; MWM, Morris water maze; EPM, elevated plus mazes; TAA, thioacetamide.

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Introduction

The term “minimal hepatic encephalopathy” was introduced by Collie *et al.*, in 2005.[1] It is typically associated with “normal” psychological symptoms.[2] Despite appearing to exhibit “normal” behaviours, these patients may experience subtle cognitive functional changes that impair their daily functioning and have a significant impact on their personal lives, including work and learning.[3] The global prevalence of minimal hepatic encephalopathy (MHE) varies significantly among different regions and populations, but it is generally elevated in patients with liver cirrhosis.[4,5] The emergence of MHE presents substantial challenges for clinicians and patients, especially considering the increasing number of individuals with liver cirrhosis. This raises important questions about the underlying pathogenesis and clinical significance of subclinical (minimal) hepatic encephalopathy-associated biological and behavioural changes, such as disruptions in sleep and cognition. Therefore, it is crucial for clinicians to accurately identify and manage this condition through early detection, monitoring of MHE progression, and a comprehensive understanding of its neurobiological mechanisms.

The insula is situated deep within the lateral fissure and is surrounded by the frontal, parietal, and temporal lobes.[6] The cortical region of the insula acts as a central hub in the brain due to its structural connectivity with other brain regions, enabling effective communication and functional integration throughout the brain.[7] Due to its distinctive anatomical location and intricate fibre connections, the insula has long been considered one of the most mysterious cortical regions.[6,8] Because of the intricate structure of the insula, its function is likewise highly complex. It plays a crucial role in cognition, emotion, visual processing, and sensorimotor functions, and is considered an information relay station in the brain.[9–11] Furthermore, it has extensive connections with multiple brain networks, including the default mode network and central executive network, playing an important role in the interactions of brain networks.[12,13] Meanwhile, researchers found that,[14] compared with patients with MHE, the amplitude of low frequency fluctuations (ALFF) in the default mode network area of patients with overt hepatic encephalopathy decreased, while the ALFF in the posterior insular cortex increased. These pieces of evidence indicate the important role of the insular cortex in MHE.

In recent years, researchers have used functional magnetic resonanceimaging (fMRI) technology to study the neurobiological mechanisms of MHE-related disorders. The findings indicate a link between MHE and extensive cortical involvement of the frontal, temporal, and parietal lobes.[15–17] Some studies have also explored the relationship between abnormal neuronal activity and volume changes in the insula with the clinical symptoms and behavioural alterations observed in MHE.[14,18–20] These research findings indicate that aberrant neuronal activity and volume alterations in the insula are associated with cognitive and behavioural abnormalities in MHE patients. However, the alterations in insular connectivity in the context of MHE remain unclear, despite evidence indicating the insula’s crucial role in MHE. This prompts us to further consider the potential link between functional connectivity (FC) changes in the insula and the occurrence of MHE, in other words, the role of insular connectivity in the clinical and behavioural manifestations resulting from MHE.

This study aims to fill this gap by utilising fMRI data to conduct a comprehensive FC analysis. Specifically, our objective is to clarify the importance of the insula in the wider brain network of MHE patients and investigate its association with cognitive dysfunction. Our hypothesis is that changes in insular FC, reflecting disrupted functional integration, are strongly associated with the cognitive impairments seen in MHE patients and may also serve as an indicator of disease severity. Through systematic investigation of changes in insular FC, we aim to enhance our understanding of the neurobiological basis of MHE and potentially discover new targets for diagnosis and treatment.

Materials and methods

Subjects

This prospective study obtained approval from the local ethics committee (KYLL-2021-2021-841), and a clinical trial was conducted (ChiCTR21000050880). All participants obtained consent and signed informed consent forms prior to their inclusion.

Between October 2020 and June 2022, 27 patients diagnosed with hepatitis B virus-related cirrhosis with MHE were included. Additionally, a healthy control (HC) group of 30 volunteers with matched gender, age, and educational level was recruited during the same period. The inclusion criteria for this study are as follows: (a) right-handedness; (b) education level exceeding 6 years; (c) absence of any brain parenchymal lesions (such as tumours, infectious diseases, or trauma) based on medical history and routine imaging examinations; (d) no contraindications for magnetic resonance imaging (MRI) examinations; (e) no recent history of acute infections, cardiovascular diseases, respiratory system diseases, or neurological diseases; (f) no presence of liver cancer or other types of hepatitis viruses; and (g) no history of substance abuse and no unhealthy habits such as smoking or alcohol consumption.

Cognitive function assessment

Two experienced radiologists conducted tests and diagnoses on all patients based on the guidelines in the "Hepatic encephalopathy-Definition, nomenclature, diagnosis, and quantification" from the working party at the 11th World Congresses of Gastroenterology in Vienna.[21] Patients who tested negative on both the Number Connection Test-A (NCT-A) and DST scales, which are neuropsychological assessment tools, were classified into the MHE group. The criteria for NCT-A and DST positivity were referenced from WANG *et al.*[22]

All participants underwent cognitive level assessment using the MoCA. The MoCA is widely employed to evaluate cognitive function, especially in individuals with mild cognitive impairment. It consists of multiple subtests that cover a wide range of cognitive domains, including attention, memory, processing speed, executive function, language abilities, and visuospatial skills. The maximum score on the scale is 30, with a score below 26 indicating cognitive impairment in the individual being evaluated.[23]

MR data acquisitions

We conducted data acquisition using the GE Architect 3.0 T MR scanner and a 48-channel head coil. Before the fMRI scan, all participants underwent a routine MRI scan to check for any brain pathology. During the MRI scan, participants were instructed to lie still, close their eyes, remain awake, and their head was secured using a foam pad to minimise head motion. Earplugs were also used to reduce the impact of noise on the participants. The routine MRI sequences included 3D-T1 volumetric scanning (3D-T1 BRAVO) (Repetition Time (TR) = 7.7ms, Echo Time (TE) = 3.1ms), T1FLAIR (TR = 2000ms, TE = 20ms) and T2WI (TR = 4000ms, TE = 107ms). Thereafter, resting-state BOLD-fMRI was performed using Ax BOLD rest 36sl sequence, TR = 2000, TE = 30, flip angle = 90°, Field of View (FOV) = 250 × 250mm, matrix = 64 × 64, number of layers = 35, slice thickness = 3.6mm, scan dynamics = 180 times, and scan time = 6min.

Image preprocessing

The original image processing after collection is based on Matlab 2012a and utilises the fMRI data processing package [DPABI_V 4.3, 5.0, advanced edition (http://rfmri.org/DPABI)] for preprocessing fMRI data. The processing procedure and steps are referenced by Yan *et al.*[24] Specifically, (1) remove data from the first 10 time points; (2) time correction, by interpolating data collected at different time points for each subject to adjust for differences in layer collection times; (3) head motion correction, excluding images with translations greater than 2.0 mm or rotations exceeding 2.0°; (4) spatial normalisation and resampling, we register each subject's functional images to the EPI template from the Montreal Neurological Institute and resample the voxel size to 3 × 3 × 3mm^3; (5) image smoothing, using a 6 mm full-width half-height Gaussian kernel for spatial smoothing; (6) we use detrending and band-pass filtering to obtain time series between 0.01 and 0.08 Hz.

Functional connectivity analysis

In order to assess alterations in FC within the bilateral insula and its interactions with other brain regions in individuals diagnosed with MHE, we selected the bilateral insula as the region of interest (ROI) for subsequent FC analysis (Supplementary Fig 1). Firstly, we obtained the average time series of all voxels within each ROI. Then, we calculated the *Pearson* correlation coefficient between the average time series of each ROI and the time series of each voxel within the rest of the brain. [25] Subsequently, we obtained the pattern of *Pearson* correlation coefficients between the bilateral insula and other brain regions and transformed them into z-values using Fisher's transformation to improve normality.

Statistical analysis

Data visualisation was performed using GraphPad Prism 9.3 (GraphPad Software, USA), while statistical analysis was conducted using SPSS 23.0 (International Business Machines Corporation, USA). Normally distributed continuous variables were analysed using Student's *t* test, with results reported as mean ± standard deviation ($\overline{x} \pm s$). Non-normally distributed continuous variables were analysed using the Mann–Whitney *U* test, with results reported as median (interquartile range) [M (Q1, Q3)]. Categorical variables were presented as percentages, and group comparisons were conducted using the Chi-square test. The significance level was set at $P < 0.05$.

fMRI data analysis was conducted using the DPABI toolbox.[26] A one-sample *t* test was performed to obtain the whole-brain FC patterns of the bilateral insular cortex in the HC and MHE groups. A two-sample *t* test was used to compare the FC differences between the HC and MHE groups. Gaussian random field (GRF) correction was applied with voxel $P < 0.001$ and cluster $P < 0.05$ for multiple comparisons (GRF correction method considers the spatial smoothness of functional magnetic resonance imaging data, and adjusts the critical threshold of importance according to the estimated smoothness of the data, thus reducing the possibility of identifying false positives).

To explore the potential clinical correlations between FC changes and cognitive impairment in MHE, *Pearson* correlation analysis was conducted. First, the brain regions showing FC differences with the bilateral insula compared with the rest of the brain in both HC and MHE groups were defined as ROI. Then, the average z-value of each subject's ROI was calculated. Finally, we will perform *a Pearson* correlation analysis between the average z-values of ROI and cognitive test scores. For each ROI, we used its FC strength with the insular cortex region as the index for receiver operating characteristic (ROC) curve analysis. We estimated the area under the ROC curve (AUC) to assess the discriminative performance between HC and MHE groups, and

Table 1
Demographic and physiologic data of studied cohort.

Parameter	HC group (n = 25)	MHE group (n = 23)	χ^2/Z/t value	*Peak P*-value
Sex (% of male patients)	56.0%	60.9%	0.117	0.732[§]
Age (years)	43.2 ± 9.4	48.1 ± 9.8	-1.758	0.085[&]
Years of education	8.0 (7.0, 9.0)	7.0 (6.0, 9.0)	-1.942	0.052[#]
NCT-A (seconds)	36.8 ± 9.0	71.6 ± 20.6	-7.669	<0.001[&]
DST (score)	46.8 ± 9.6	22.1 ± 6.9	10.203	<0.001[&]
MoCA (score)	27.08 ± 2.22	20.22 ± 3.69	7.881	<0.001[&]

Note: HC, healthy control; MHE, minimal hepatic encephalopathy; DST, digit symbol test; NCT-A, number connection test of type A; MoCA, Montreal Cognitive Assessment; [§]χ^2 test of two groups; [#] *Mann–Whitney U* test; [&] *Student's t test.*

carried out data analysis using MedCalc (Broekstraat, Mariakerke, Belgium) with a statistical threshold set at $P < 0.05$.

Results

Clinical data characteristics

All participants were divided into two groups: the HC group (n = 30) and the MHE group (n = 27). Five healthy volunteers and four MHE patients were excluded during the scanning and data preprocessing process due to excessive motion or poor image resolution. In the fMRI imaging process, the scanner uses magnetic fields and radiofrequency pulses to acquire image data. If the patient exhibits any movement (including small movements of the head or other parts of the body), it can lead to image blurring or distortion. These motion artifacts can severely affect the spatial localisation of brain activity, significantly reducing the accuracy of the data. Ultimately, 25 healthy volunteers and 23 MHE patients were included. The demographic and clinical characteristics of all participants are shown in Table 1. There were no significant differences between the HC and MHE groups in terms of gender ratio, age, and education level ($P > 0.05$). Except for the NCT-A and DST scale scores, which showed differences compared with the HC group, the MoCA score of the MHE group was also significantly lower than that of the HC group ($P < 0.05$) (Table 1).

Functional connectivity patterns of the bilateral insula in two groups

Right insula: Supplementary Fig 2a displays the FC patterns of the right insula with other brain regions in the HC and MHE groups. In the HC group, the brain regions include the following: right precentral gyrus, bilateral middle frontal gyrus, and bilateral inferior frontal gyrus. In the MHE group, the brain regions include the following: bilateral precentral gyrus, right postcentral gyrus, bilateral middle frontal gyrus, bilateral inferior frontal gyrus, and right middle occipital gyrus (GRF correction, voxel $P < 0.001$, cluster $P < 0.05$).

Left insula: Supplementary Fig 2b illustrates the FC patterns of the left insula with other brain regions in the HC and MHE groups. In the HC group, the brain regions primarily include: left precentral gyrus, bilateral postcentral gyrus, and bilateral middle frontal gyrus. In the MHE group, the brain regions primarily include the following: bilateral precentral gyrus, bilateral postcentral gyrus, bilateral middle frontal gyrus, bilateral inferior frontal gyrus, and bilateral superior temporal gyrus (GRF correction, voxel $P < 0.001$, cluster $P < 0.05$).

Differences in functional connectivity

When the right insula was used as the ROI, the study revealed three prominent clusters showing changes between the HC and MHE groups. Compared with the HC group, the MHE group exhibited significantly increased FC between the right insular lobe and the right superior frontal gyrus, right middle frontal gyrus, and right precentral gyrus (GRF correction, $P < 0.05$) (Table 2; Figs 1a; 2a).

When the left insula was used as the ROI, the results showed that compared with the HC group, the MHE group exhibited significantly increased FC between the left insula and the left middle frontal gyrus, as well as the right middle frontal gyrus (GRF correction, $P < 0.05$) (Table 2; Figs 1b; 2b). For the between-group FC map comparison, we added age, years of education, gender, and head motion as covariates in the processing for normalisation.

Result of correlation analysis

In this study, the correlation analysis was conducted to explore the relationship between changes in insula FC and cognitive and behavioural alterations in MHE patients. The study found that the FC changes between the right insula and the right superior frontal gyrus ($r = -0.451$, $P = 0.031$), as well as the right middle frontal gyrus ($r = -0.527$, $P = 0.010$), were negatively correlated with MoCA score. Additionally, the FC changes between the left insula and the left middle frontal gyrus showed a significant negative

Table 2
Brain regions with significantly altered functional connectivity with insula between two groups.

Brain regions	MNI (X Y Z)	Cluster size	*t* value
Seed: right insula			
Right superior frontal gyrus	23 -5 64	125	3.94
Right middle frontal gyrus	35 14 42	148	3.35
Right precentral gyrus	35 -11 62	59	5.33
Seed: Left insula			
Left middle frontal gyrus	-35 18 38	140	3.67
Right middle frontal gyrus	33 16 43	146	4.23

Note: MNI, Montreal Neurological Institute.

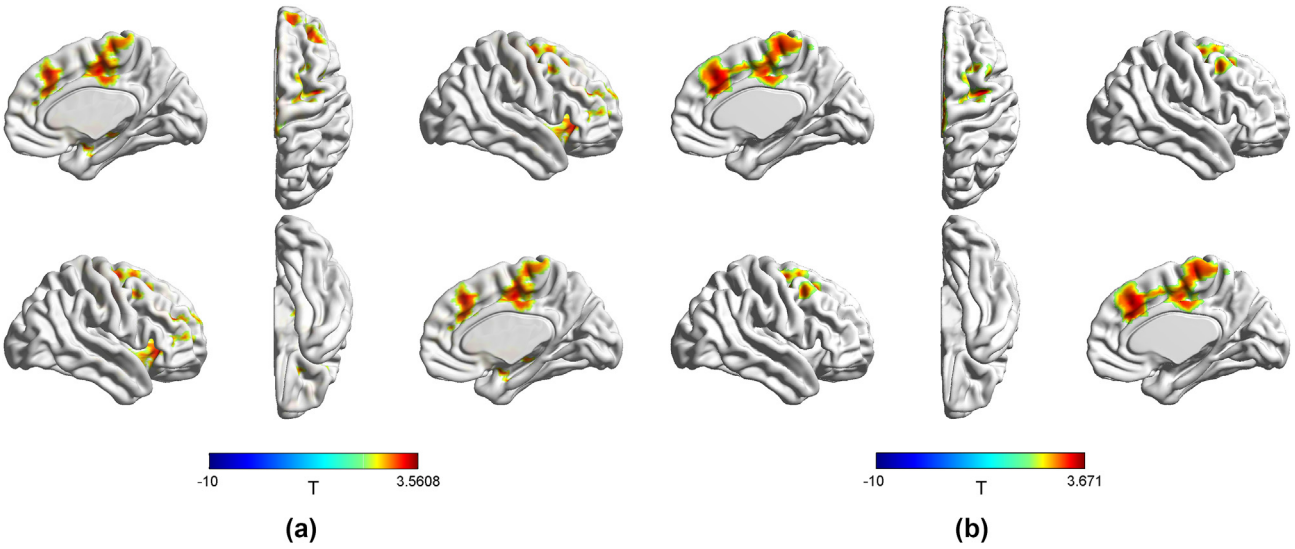

Figure 1 FC maps show differences between patients in HC and MHE groups. (a) The differences in FC of the right insula between the two groups; (b) The differences in FC of the left insula between the two groups. The red colour represents increased FC, while the blue colour represents decreased FC. FC, functional connectivity; HC, healthy control; MHE, minimal hepatic encephalopathy.

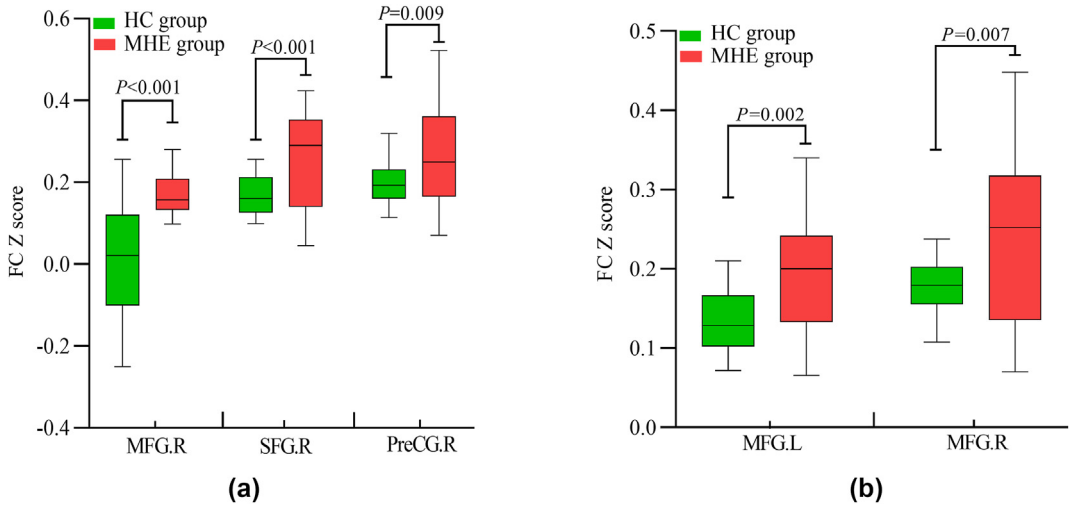

Figure 2 The comparisons of FC value in HC and MHE groups. (a) right insula; (b) left insula. FC, functional connectivity; HC, healthy control; INS.R., right insula; INS.L., left insula; MFG.R., right middle frontal gyrus; MFG.L., left middle frontal gyrus; MFG.R., right middle frontal gyrus; MHE, minimal hepatic encephalopathy; PreCG.R. right precentral gyrus; SFG.R. right superior frontal gyrus; .

correlation with MoCA score ($r = -0.530$, $P = 0.009$). None of the remaining FC changes exhibited a significant correlation with the MoCA score ($P > 0.05$) (GRF correction, Fig 3, Fig 4).

Diagnosis of MHE patients using ROC analysis

As shown in Fig 5, assessing the value of FC values in early diagnosis of MHE through analysing alterations in bilateral insular FC. The results revealed that the FC alterations in the right insula and right middle frontal gyrus exhibited the highest diagnostic value for MHE (AUC = 0.856). Furthermore, the FC changes between the right insula and right superior frontal gyrus, as well as the right precentral gyrus, also demonstrated robust predictive value (All $P < 0.05$). Conversely, the FC changes between the left insula and right middle frontal gyrus did not exhibit significant discriminatory predictive potential (AUC = 0.671, $P = 0.06$).

Discussion

This study represents the pioneering application of resting-state FC analysis to investigate alterations in connectivity patterns involving the insula and other brain regions in patients with MHE. Importantly, these outcomes suggest that bilateral insula FC changes may play a crucial role in the development of cognitive impairment observed in MHE patients. Moreover, our findings suggest that alterations in FC between the insula and frontal cortices

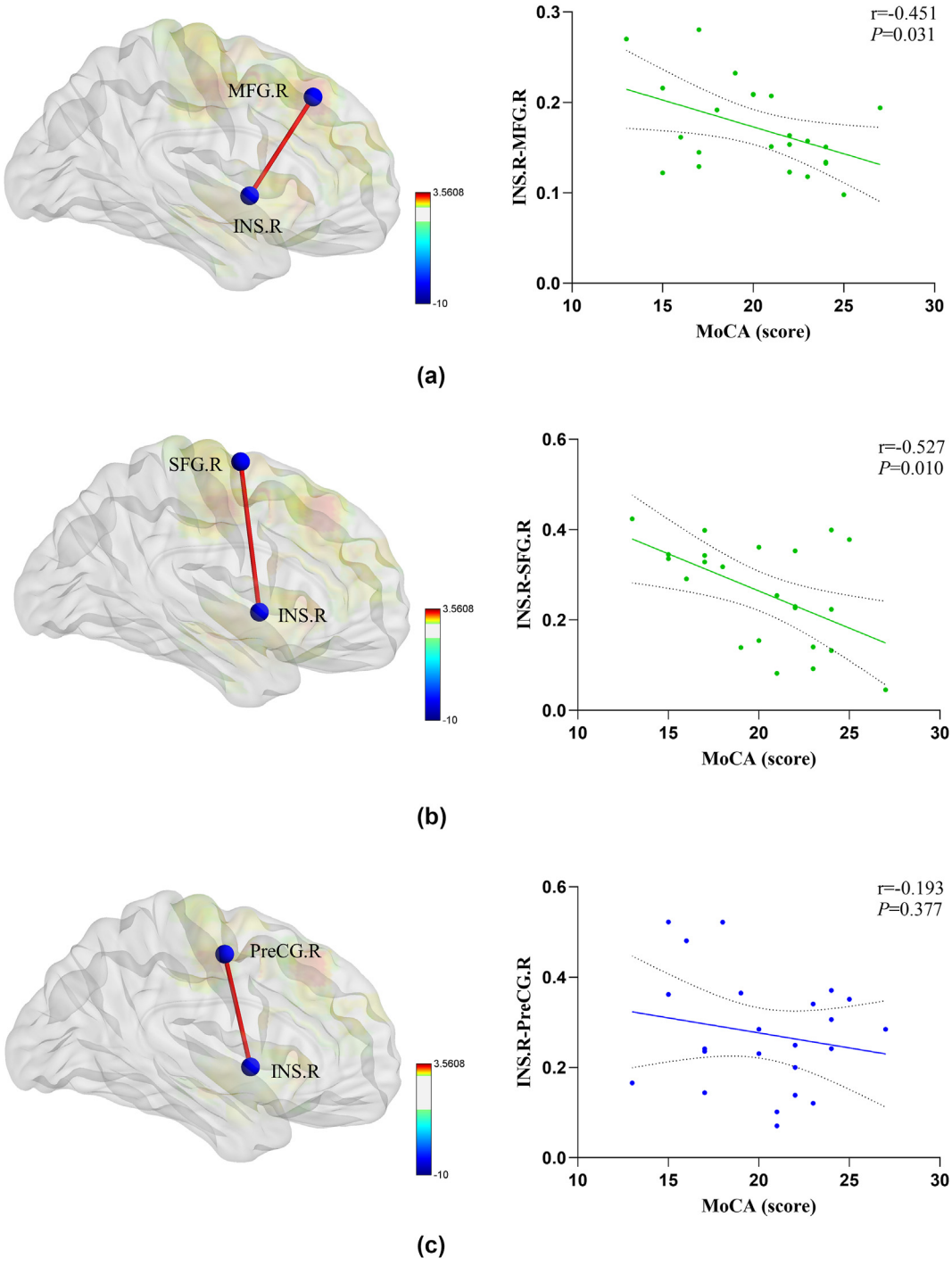

Figure 3 FC maps show correlation between MoCA score and FC value in MHE patients of right insula. (a) Significant negative correlation between FC value of the right insula to the right middle frontal gyrus and MoCA score ($P < 0.05$, corrected); (b) Significant negative correlation between FC value of the right insula to the right superior frontal gyrus and MoCA score ($P < 0.05$, corrected); (c) No correlation between FC value of the right insula to the right precentral gyrus and MoCA score ($P > 0.05$, corrected). The red colour represents a positive correlation, while the green colour represents a negative correlation. INS.R. right insula; L., left; MFG.R., right middle frontal gyrus; MHE, minimal hepatic encephalopathy; MoCA, Montreal Cognitive Assessment; PreCG.R., right precentral gyrus; R., right; SFG.R. right superior frontal gyrus.

exhibit a robust discriminatory capacity in distinguishing between HC and MHE patients. These results underscore the promising potential of FC changes as a valuable biological marker for the timely identification, intervention, and management of MHE, thereby contributing to improved patient outcomes.

An important finding of this study was that differential FC patterns between the bilateral insula and other brain

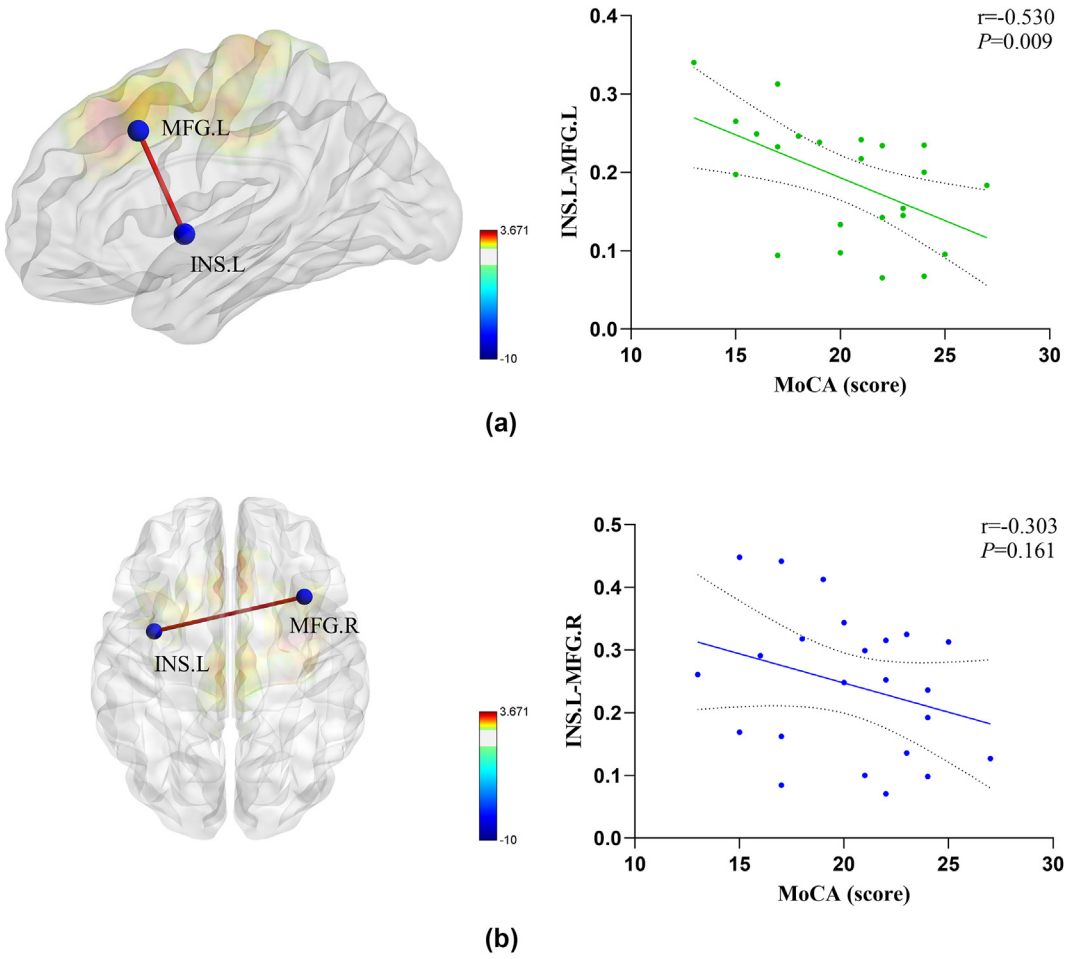

Figure 4 FC maps show a correlation between MoCA score and FC value in MHE patients of left insula. (a) Significant negative correlation between FC value of the left insula to the left middle frontal gyrus and MoCA score ($P < 0.05$, corrected); (b) No correlation between the FC value of the left insula to the right middle frontal gyrus and MoCA score ($P > 0.05$, corrected). The red colour represents a positive correlation, while the blue colour represents a negative correlation. FC, functional connectivity; INS.L., left insula; L., left; MFG.L., left middle frontal gyrus; MFG.R., right middle frontal gyrus; MHE, minimal hepatic encephalopathy; MoCA, Montreal Cognitive Assessment; R. right.

regions in two groups of patients (Fig 1). This finding is similar to the differential results observed by Qi *et al.*[27] in sleep-deprived individuals. On one hand, this difference may be attributed to developmental variations in the bilateral insula: the left insula has a larger surface area compared with the right insular cortex, and the right insula ceases growth earlier.[28] On the other hand, although the bilateral insula is located in similar positions, it may exhibit subtle structural differences, such as variations in fibre connections and fibre tract orientations. Furthermore, as previous studies have demonstrated, there is functional lateralisation between the left and right cerebral hemispheres in cognitive and language processing. The left insula is more prominently associated with language-related functions, including language processing and semantic comprehension, while the right insula may play a more prominent role in spatial perception and emotional processing.[29,30] This functional lateralisation may contribute to the distinct FC patterns between the right and left insula. Additionally, we observed that in each insula, the standardised FC values were higher in the MHE group compared with the HC group (Supplementary Fig 2). This finding may be indicative of the increased cognitive compensation needed by patients with MHE to alleviate the impact of cognitive impairment.

Another important finding is that abnormal FC in the left and right insula is distributed in different brain regions (superior frontal gyrus, middle frontal gyrus, and precentral gyrus) (Figs 1; 2), which are involved in social behaviour and emotion regulation, cognition (executive functions, attention, memory, and reaction time), decision-making, and sensory-motor functions.[31,32] According to the study by Shen *et al.*,[33] the superior frontal gyrus is considered a key node connecting the central executive network and the default network, and it is interconnected with the cognitive control network and the cognitive executive network. Serving as a bridge between these two networks, the superior frontal gyrus not only facilitates information transmission and coordination but also plays an important role in cognitive tasks and decision-making. The middle frontal gyrus has been shown to play a critical role in emotion processing and expression, self-awareness, decision-

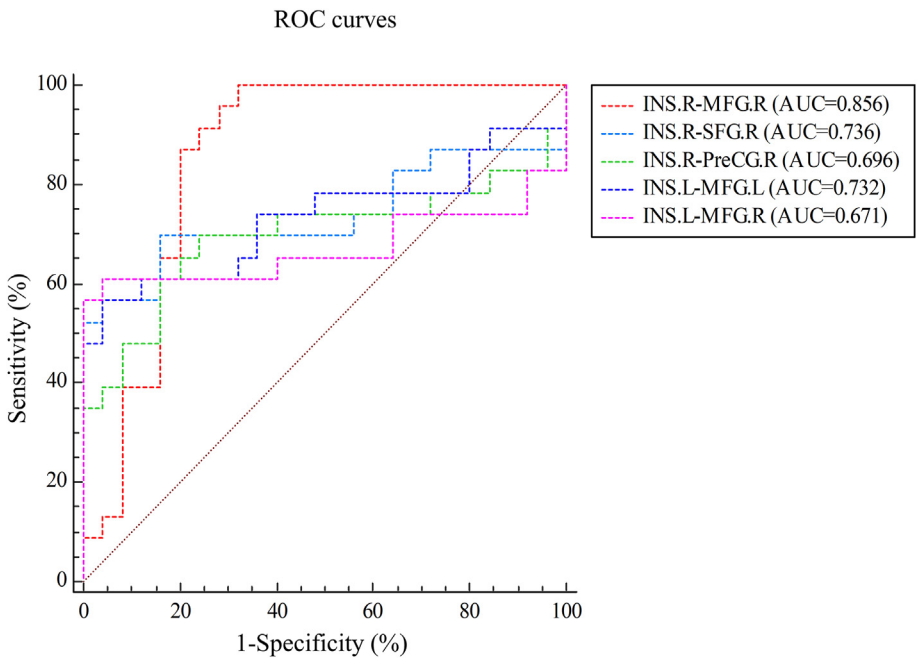

Figure 5 Results of ROC curve analysis. INS.R. right insula; INS.L. left insula; MFG.R. right middle frontal gyrus; MFG.L. left middle frontal gyrus; MFG.R. right middle frontal gyrus; PreCG.R. right precentral gyrus; ROC, receiver operating characteristic; SFG.R. right superior frontal gyrus.

making, autonomic regulation, and cognitive flexibility, exerting profound influences on an individual's cognitive, emotional, and behavioural processes.[34,35] As evidence, our research findings demonstrate significant changes in FC between the left insula and the left middle frontal gyrus, right middle frontal gyrus, as well as between the right insula and the right superior frontal gyrus, right middle frontal gyrus, and right precentral gyrus. Previous studies have also indicated that the prefrontal cortex is highly susceptible to MHE disease.[17] Given that the insula and prefrontal cortex are crucial brain regions involved in cognition and emotion, the enhanced FC observed between these two regions after the onset of MHE may be related to cognitive redistribution following the disease. Additionally, it is conceivable that the heightened FC between the insula and frontal cortex serves as a compensatory mechanism to mitigate the deleterious effects on cognitive and emotional processes resulting from insular and frontal cortical dysfunction, this compensatory mechanism aims to preserve the integrity and functionality of cognitive and emotional processes. Therefore, the connectivity alterations between the insula and prefrontal cortex revealed in our study may be associated with functional compensation following the occurrence of MHE.

Comparatively, we also observed a significant increase in FC between the insula and the precentral gyrus (Figs 1a, 2a). The precentral gyrus, as part of the prefrontal cortex, is located near the anterior central sulcus and is considered a core region of the mirror neuron system, playing a crucial role in action understanding and imitation,[36] as well as being involved in various cognitive functions. Previous studies have indicated a decrease in homogeneity within motor-related cortical areas following MHE.[37] Our findings, however, have unveiled an augmented FC between the insula and the motor control regions. This discovery potentially signifies an impaired integration of motor information in patients with MHE, necessitating the activation of compensatory mechanisms and the reinforcement of interconnections with cerebral regions intimately associated with motor function. Moreover, it implies that the neural circuitry connecting the insula and the precentral gyrus may represent a promising avenue for future research into MHE-related pathologies.

Encouragingly, when further analysing the correlation between abnormal FC of the insula and cognitive impairments in patients with MHE, we found that the FC abnormalities between the bilateral insula and the prefrontal cortex were significantly associated with cognitive deficits in MHE patients. Specifically, the FC changes between the right insula and the right superior frontal gyrus, as well as the right middle frontal gyrus, were negatively correlated with MoCA score (Fig 3). Similarly, the FC changes between the left insula and the left middle frontal gyrus showed a significant negative correlation with MoCA score (Fig 4). These findings suggest that the FC alterations between the insula and the prefrontal cortex, specifically involving the superior frontal gyrus and middle frontal gyrus, are implicated in the cognitive impairments of MHE patients. Moreover, they imply that the FC between the superior frontal gyrus and middle frontal gyrus may serve as potential imaging biomarkers for quantifying the severity of cognitive impairment in MHE patients. Consequently, we speculate that the FC changes within the insula-prefrontal cortex network may underlie cognitive dysfunction and hold promise as crucial factors for both research and treatment strategies related to MHE (Fig 5).

We acknowledge several limitations of this study. Firstly, the small sample size may introduce some bias in the results. Nevertheless, the current study is still actively recruiting eligible patients and volunteers, intending to conduct a large-scale study in the future. Secondly, this study employed a cross-sectional design, collecting data and conducting analysis at a single time point. However, a long-term longitudinal study would provide a better understanding of the neuro changes and disease progression in MHE patients. Lastly, although this study included healthy volunteers as a control group, it did not compare the FC changes in MHE patients with those in other liver disease patients or patients with different types of brain disorders. Therefore, the comparison with other diseases is lacking, limiting the comprehensive understanding of the unique neural mechanisms underlying MHE.

Conclusions

This study has yielded several noteworthy findings. Firstly, our investigation revealed disrupted FC patterns in the bilateral insula among patients with MHE, thus affirming the substantial impact of MHE on FC alterations between the insula and other brain areas. These aberrant FC patterns in the insula are implicated in the cognitive impairments observed in MHE patients, underscoring their pivotal role in the pathophysiology of this condition. Furthermore, they may serve as valuable indicators for assessing the severity of cognitive deficits exhibited by affected individuals. The present study thus offers novel insights that can inform future investigations into the underlying neuropathological mechanisms contributing to cognitive impairments in MHE.

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Author contribution

1. Guarantor of integrity of the entire study: X. Wang.
2. Study concepts and design: X. Wang, X. Ding, X. Yang.
3. Literature research: X. Yang, M. Wang.
4. Clinical studies: X. Yang, W. Liu, J. Zheng, W. Ma, X. Ding.
5. Experimental studies / data analysis; Manuscript preparation: X. Yang.
6. Statistical analysis: X. Yang, W. Liu.
7. Manuscript editing: X. Yang, X. Wang, X. Ding.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statements

The research work has not been completed yet, and some data are in the confidential stage. If researchers need data, applicants need to provide documents' approval from the researcher's local ethics committee.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crad.2025.106922.

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