Abnormal spontaneous brain activity in minimal hepatic encephalopathy: resting-state fMRI study

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PurposE
We aimed to assess the abnormality of baseline spontaneous brain activity in minimal hepatic encephalopathy (MHE) by amplitude of low frequency fluctuation (ALFF) and fraction ALFF (fALFF).

methoDs
A total of 14 MHE patients and 14 healthy controls were included in our study. Both ALFF and fALFF of functional magnetic resonance imaging were calculated for statistical analysis.

resuLtS
Compared with healthy controls, patients with MHE had significantly decreased ALFF in the bilateral medial prefrontal cortex (MPFC), left superior frontal gyrus, right precentral gyrus, left opercular part of inferior frontal gyrus, left gyrus rectus, bilateral precuneus, and the posterior lobe of right cerebellum; and they had significantly decreased fALFF in the bilateral MPFC, right middle frontal gyrus, right superior temporal gyrus, and the posterior lobe of left cerebellum.

conclusion
ALFF and fALFF changes in many brain regions demonstrate abnormality of the spontaneous neuronal activity in MHE. Especially the impairment of right precuneus and left MPFC may play a critical role in manifestation of MHE. Changes of ALFF and fALFF in the precuneus and the MPFC can be used as a potential marker for MHE.

M inimal hepatic encephalopathy (MHE), as an early stage of hepatic encephalopathy (HE), shows only mild attention deficit, visual motor dysfunction, cognitive control impairment and working memory network disorder, without any obvious neurologic manifestations (e.g., confusion, hepatic coma). As a transitional stage, 20%–80% of MHE cases develop from liver cirrhosis (1). Patients usually have weaker capabilities of handling an emergency situation and higher risk of accident (2). MHE is potentially reversible, because of the minor damage to the neurons (3).

Previously, diffusion tensor imaging and magnetic resonance spectrum were used to get an insight into HE. These modalities found neuron swelling or interstitial edema and abnormal metabolism in related brain regions (4–8).

Currently, blood oxygenation level dependent-functional magnetic resonance imaging (BOLD-fMRI), which has the ability to detect brain dysfunction, is a focus in the field of HE research. Some task-related fMRI studies found impairments in multiple cognitive and spatial memory functional regions of the brain (9, 10). Different from task-related fMRI, resting-state fMRI demonstrates spontaneous neuronal activity. Using resting-state fMRI, studies found decreased regional homogeneity in brain regions that are related to cognition and memory function, and the changes of regional homogeneity in the precuneus were closely related to the development of MHE (11, 12). Functional connectivity was also used in HE studies, which revealed disturbed brain networks (13–18).

A different approach from regional homogeneity and functional connectivity, amplitude of low frequency fluctuation (ALFF) analysis, which detects the alternation of spontaneous brain activity by measuring the energy of low frequency oscillation, was first used by Zang et al. (19) in a study of patients with attention deficit hyperactivity disorder, becoming a new method to study the brain function. Recently, Chen et al. (20) and Qi et al. (21) used it in MHE and revealed aberrant brain activity and continuous impairment. Based on the ALFF, the fraction ALFF (fALFF) proposed by Zou et al. (22) not only gets rid of physiological noise.
effectively, but also has a higher sensitivity and specificity compared with the ALFF. But until now, few studies employed the ALFF to investigate MHE and even fewer employed the fALFF. Moreover, study of MHE by means of ALFF and fALFF continues to be regarded as controversial. In this study, we used the ALFF and fALFF to investigate alternations of spontaneous brain activity in MHE.

Methods

Subjects
This study was approved by the Institutional Ethics Committee and written informed consent was obtained from all participants. In total, 25 patients with nonalcoholic liver cirrhosis and 19 healthy control subjects were recruited in our study. All participants were right-handed and had no alcohol or drug abuse history, related neurologic or/and psychiatric illness, or MRI contraindications. Healthy control subjects had no history of liver disease. Nonalcoholic liver cirrhosis was diagnosed through clinical manifestations, signs, and examinations (biochemical laboratory examinations and radiologic examinations such as computed tomography, MRI, and ultrasonography). The neuropsychologic tests including the number connection test A and digit-symbol test and fMRI were performed in all participants. MHE was diagnosed by two senior gastroenterologists based on the final report of the working party of 11th World Congress of Gastroenterology in Vienna in 1998 (23); nonalcoholic liver cirrhosis patients with abnormal neuropsychologic test scores (more than one standard deviation below for number connection test A or under the mean value for digit-symbol test) were regarded as MHE. The normal values of neuropsychiatric tests were determined in a sample of 120 healthy volunteers in a prior study (24). We excluded four cases of MHE and two healthy control subjects with intolerable motion (translation of more than 1.0 mm or rotation of more than 1.0°), one case of MHE with bad registration, two cases of MHE with artifacts and four cases of MHE and three healthy control subjects with intracranial complications in the data preprocessing. Finally, 14 MHE and 14 healthy control subjects were included in our study.

MRI data acquisition and preprocessing
MRI data was acquired on a 3.0 T scanner (Achieva Intera, Phillips Medical System) using an eight-channel head coil. The functional image was acquired by using an echo-planar imaging sequence (repetition time, 2000 ms; echo time, 30 ms; flip angle, 90°; matrix, 64×64; field of view, 240 mm×240 mm; section thickness, 4.0 mm; number of sections, 34; dynamics, 180). All participants were instructed to keep their head still and eyes closed. We provided the participants with earplugs and fixed their head with sponges of different thickness before MRI scanning.

Preprocessing was performed by the data processing assistant for resting-state fMRI (DPARSF) (http://www.restfmri.net). The procedures included removing the first 10 time points (for adaption to the scanning noise and the steady state of machine), slice-timing correction, realign correction, spatial normalization, smoothing (full width at half maximum, 4 mm), linear detrend and band-pass filtering (0.01–0.08 Hz). Then the time courses were converted to the frequency domain by using a fast Fourier transform and the averaged square root of the spectrum across 0.01–0.08 Hz at each voxel was taken as the ALFF. The fALFF was calculated as ALFF divided by the value of the entire detectable frequency band. For standardizing variability across the participants, mean ALFF was obtained as ALFF divided by the global mean ALFF; mean fALFF was also calculated in a similar manner. Finally, mean ALFF and mean fALFF maps of the whole brain of MHE and healthy control subjects, which reflected the degree of the raw value relative to the global mean value, were used for statistical analysis.

ALFF and fALFF analysis
The two-sample t-test was performed by Resting-State fMRI Data Analysis Toolkit v1.8 (REST1.8) (http://www.restfmri.net) to determine the mean ALFF and mean fALFF differences within the whole-brain between the two groups. We used the significant regions of ALFF or fALFF respectively to make a new union mask. Pearson’s correlation analysis was performed by REST1.8 software to find the correlation between the ALFF or fALFF and poor neurocognitive performances within the union mask. The gray matter volume and age were added as covariants for excluding the related effects. The threshold was set at P < 0.05 (corrected by using the AlphaSim program) and the minimum cluster size was 54 voxels.

Results
There were no significant differences in terms of age (P = 0.334) and education (P = 0.618) between MHE patients and healthy controls. The number connection test A and digit-symbol test showed significant differences within the two groups (P < 0.001) (Table 1).

Main points

- Minimal hepatic encephalopathy (MHE) is difficult to diagnose because of its mild brain dysfunction.
- It is important to be aware of MHE, given its higher risk of accidents and morbidity and potentially reversible nature.
- Amplitude of low frequency fluctuation (ALFF) and fraction ALFF (fALFF) changes in many brain regions demonstrate abnormality of the spontaneous neuronal activity in MHE, especially in the right precuneus and the left medial prefrontal cortex (MPFC).
- Changes of ALFF and fALFF in the precuneus and MPFC play a critical role in the manifestation of MHE.

Table 1. Summary of demographic information and neuropsychologic tests

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients with MHE (n=14)</th>
<th>Healthy controls (n=14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F ratio</td>
<td>7/7</td>
<td>7/7</td>
<td>0.334</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.57±10.57</td>
<td>50.86±9.38</td>
<td></td>
</tr>
<tr>
<td>Education (years)</td>
<td>10.4±3.50</td>
<td>11.00±2.39</td>
<td>0.618</td>
</tr>
<tr>
<td>NCT-A (seconds)</td>
<td>57.63±30.96</td>
<td>21.33±3.80</td>
<td>0.001</td>
</tr>
<tr>
<td>DST (raw score)</td>
<td>23.29±10.99</td>
<td>48.86±10.29</td>
<td>0.001</td>
</tr>
</tbody>
</table>

MHE, minimal hepatic encephalopathy; M/F, male/female; NCT-A, number connection test A; DST, digit-symbol test.
ALFF were located in the bilateral frontal lobes including the bilateral medial prefrontal cortex (MPFC), left superior frontal gyrus, right precentral gyrus, left opercular part of inferior frontal gyrus, left gyrus rectus, bilateral precuneus, and the posterior lobe of right cerebellum. These findings suggest the presence of abnormal brain spontaneous activity in MHE at the baseline.

Our study revealed that the ALFF and fALFF changes of the MHE group happened mainly in the frontal lobes. As we know, the frontal lobe is the most advanced part of the brain and it is closely related to working memory and cognition (25, 26). The prefrontal cortex is responsible for spatial memory (10, 25, 26). As the integration and processing center of spatial working memory, it has functions of noticing, inhibiting, coding, integrating, storing, extracting and processing the information from the outside (25). Decreased ALFF and increased fALFF in the frontal lobe reflect decreased brain spontaneous activity, which may lead to brain dysfunction and result in cognition deficits such as distracted attention, decreased memory, and slow reaction of MHE patients. A MHE study by Liao et al. (10) also suggested the impairment of prefrontal lobes as the mechanism of dysfunction of spatial working memory.

As we know, default mode network includes the precuneus and cuneus, post cingulum cortex, MPFC, bilateral inferior parietal lobule, and the lateral temporal lobes. It attends the basic functional activities in the resting state, such as processing the episodic memory, monitoring the cognitive control, and switching the state of external stimuli and internal brain activity (27–29). The precuneus mainly participates in the process of visual spatial information integration (30). Our study showed that patients with MHE had lower ALFF in the bilateral precuneus and MPFC, while they had lower fALFF in the bilateral MPFC and higher fALFF in the precuneus. This finding indicates decreased neuronal activity in those regions led by reduced cerebral oxygen consumption and cerebral blood flow (31). Previous MHE studies showed decreased regional homogeneity in the cuneus and precuneus (11, 12) and weakened functional connectivity among the default mode network (14, 15) suggesting an abnormal connection among these regions. As members of the default mode network, the precuneus and MPFC are responsible for cognition. Decreased neuronal activity and consequent abnormal functional

### Table 2. Regions showing significant differences in ALFF between MHE patients and healthy controls

<table>
<thead>
<tr>
<th>Regions</th>
<th>BA</th>
<th>Voxels</th>
<th>MNI coordinates (x, y, z)</th>
<th>Peak t value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALFF increased in MHE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Insula</td>
<td>34</td>
<td>54</td>
<td>(-27, 3, -15)</td>
<td>4.0908</td>
</tr>
<tr>
<td>R-ITG</td>
<td>20</td>
<td>76</td>
<td>(54, -48, -27)</td>
<td>4.3602</td>
</tr>
<tr>
<td>R-Temporal pole/MTG</td>
<td>21</td>
<td>60</td>
<td>(39, 9, -39)</td>
<td>3.8904</td>
</tr>
<tr>
<td>R-PLC</td>
<td>58</td>
<td></td>
<td>(45, -45, -42)</td>
<td>3.6982</td>
</tr>
<tr>
<td><strong>ALFF decreased in MHE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Oper/IFG</td>
<td>48</td>
<td>77</td>
<td>(-42, 9, 21)</td>
<td>-4.6821</td>
</tr>
<tr>
<td>L-SFG</td>
<td>10</td>
<td>54</td>
<td>(-33, 63, 3)</td>
<td>-4.0135</td>
</tr>
<tr>
<td>L-Gyrus rectus</td>
<td>47</td>
<td>80</td>
<td>(-15, 15, -15)</td>
<td>-3.4602</td>
</tr>
<tr>
<td>R-PLC</td>
<td>54</td>
<td></td>
<td>(9, -90, -33)</td>
<td>-4.5152</td>
</tr>
<tr>
<td>R-PrCG</td>
<td>4</td>
<td>59</td>
<td>(36, -21, 51)</td>
<td>-3.4411</td>
</tr>
<tr>
<td>B-MPFC</td>
<td>6</td>
<td>121</td>
<td>(3, 33, 39)</td>
<td>-3.5014</td>
</tr>
<tr>
<td>B-Precuneus</td>
<td>7</td>
<td>71</td>
<td>(3, -42, 45)</td>
<td>-3.5021</td>
</tr>
</tbody>
</table>

ALFF, amplitude of low frequency fluctuation; MHE, minimal hepatic encephalopathy; BA, Brodmann’s area; MNI (x, y, z), Montreal Neurologic Institute (x, y, z); L, left; R, right; ITG, inferior temporal gyrus; MTG, middle temporal gyrus; PLC, posterior lobe of cerebellum; Oper/IFG, opercular part of inferior frontal gyrus; SFG, superior frontal gyrus; PrCG, precentral gyrus; B, bilateral; MPFC, medial prefrontal cortex.

### Discussion

In this study, ALFF and fALFF were used to detect the abnormal brain activity in MHE. We found that patients with MHE had decreased ALFF in the frontal lobes including the bilateral MPFC, left superior frontal gyrus, right precentral gyrus, left opercular part of inferior frontal gyrus, left gyrus rectus, bilateral precuneus, and the posterior lobe of right cerebellum; while they had decreased fALFF in the bilateral MPFC, right middle frontal gyrus, right superior temporal gyrus, and the posterior lobe of left cerebellum. These findings suggest the presence of abnormal brain spontaneous activity in MHE at the baseline.

Figure 1. a, b. Axial T1-weighted images (a, b) show minimal hepatic encephalopathy (MHE) group with decreased amplitude of low frequency fluctuation (ALFF) in bilateral precuneus, medial prefrontal cortex (MPFC) (a) and the posterior lobe of right cerebellum (R-PLC) (b) compared with the healthy controls. (REST1.8, http://www.restfmri.net)
connection cause cognitive dysfunction in MHE. We speculate that the dysfunction of prefrontal cortex and MPFC are manifestations of MHE, and changes in those two regions could provide a potential marker to detect MHE.

Previous studies suggested opposite patterns of changes in the cerebellum: decreased ALFF in the posterior lobe of left cerebellum in low-grade HE (20) and increased ALFF in the posterior lobe of left cerebellum in HE (21). Our results showed more complicated changes in the cerebellum in MHE (increased or decreased ALFF in different regions of the posterior lobe of right cerebellum, while increased and decreased fALFF in the anterior lobe and the posterior lobe of left cerebellum, respectively). As a regulator of the whole brain, the cerebellum influences several regions of prefrontal cortex via the thalamus (32). The anterior lobe of cerebellum is responsible for communication between cerebral and spinal motor systems, while the posterior lobe attends the cognitive control and emotion regulation (33, 34). Our complicat-ed findings in the cerebellum may be interpreted as impairments and compensations happening in different regions of the cerebellum at the same time. Further study is needed to clarify these findings.

Interestingly, we detected increased ALFF in the right inferior temporal gyrus, right temporal pole/middle temporal gyrus, and left insula and increased fALFF in the right insula, right lentiform nucleus, and left pallidum in MHE, suggesting an increased spontaneous neuronal activity in those regions. We speculate that this may reflect the compensation of brain dysfunction in MHE. This study has some limitations. First, although we instructed all participants to keep their head still and eyes closed during the scan, we cannot completely exclude uncontrollable factors such as the noise during the scanning affecting our results. Second, our sample size was small. Although we found significant differences in ALFF and fALFF in our study, further research with larger sample size is needed to confirm our findings.

In conclusion, we found ALFF and fALFF changes in many brain regions in MHE, demonstrating abnormality of the spontaneous neuronal activity. Especially, impairment of the right prefrontal cortex and left MPFC may represent the underlying manifestations in MHE. ALFF and fALFF can be used as potential markers for MHE.

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Conflict of interest disclosure

The authors declared no conflicts of interest.

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