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Original Research

Aberrant Spontaneous Brain Activity and its Association with Cognitive Function in Non-Obese Nonalcoholic Fatty Liver Disease: A Resting-State fMRI Study

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) has been proven to be associated with an increased risk of cognitive impairment and dementia, and this association is more significant in non-obese NAFLD populations, but its pathogenesis remains unclear. Our study aimed to explore the abnormalities of spontaneous brain activity in non-obese NAFLD patients by resting-state fMRI (RS-fMRI) and their relationship with cognitive function. **Methods**: 19 non-obese NAFLD, 25 obese NAFLD patients, and 20 healthy controls (HC) were enrolled. All subjects underwent RS-fMRI scan, psychological scale assessment, and biochemical examination. After RS-fMRI data were preprocessed, differences in low-frequency fluctuation amplitude (ALFF), regional homogeneity (ReHo) and functional connectivity (FC) were compared among the three groups. Furthermore, the relationship between RS-fMRI indicators and cognitive and clinical indicators were performed using correlation analysis. **Results**: The cognitive function was declined in both NAFLD groups. Compared with obese NAFLD patients, non-obese NAFLD patients showed increased ALFF and ReHo in the left middle temporal gyrus (MTG), increased ReHo in the sensorimotor cortex and reduced FC between left MTG and right inferior frontal gyrus (IFG). Compared with HC, non-obese NAFLD patients showed increased ALFF and ReHo in the left calcarine cortex and fusiform gyrus (FG), decreased ALFF in the bilateral cerebellum, and reduced FC between left FG and right IFG and left angular gyrus. In addition to the same results, obese patients showed increased activity in different regions of the bilateral cerebellum, while decreased ALFF in the right superior frontal gyrus and ReHo in the right orbitofrontal cortex (OFC). Correlation analysis showed that in non-obese patients, the ALFF values in the FG and the FC values between the left MTG and the right IFG were associated with cognitive decline, insulin resistance, and fasting glucose disorder. **Conclusions**: Non-obese NAFLD patients showed abnormal local spontaneous activity and FC in regions involved in the sensorimotor, temporo-occipital cortex, cerebellum, and reward system (such as OFC), some of which may be the potential neural mechanism difference from obese NAFLD patients. In addition, the temporo-occipital cortex may be a vulnerable target for cognitive decline in non-obese NAFLD patients.

Keywords: non-obese alcoholic fatty liver disease; cognition; amplitude of low frequency fluctuation; regional homogeneity; functional connectivity

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a metabolic stress-induced liver injury that is closely associated with insulin resistance and genetic susceptibility [1]. With the increasing incidence of obesity and metabolic syndrome, NAFLD has replaced chronic hepatitis B as the most common cause of chronic liver disease, affecting one-quarter of the general population worldwide [2]. Although NAFLD is often associated with obesity, it is increasingly being found in people of normal weight (body mass index [BMI] <25 kg/m^2). The incidence of non-obese NAFLD is increasing rapidly worldwide and accounts for a significant proportion of the burden of chronic liver disease [3].

Accumulating evidence suggests that the risk factors for NAFLD, such as type 2 diabetes, obesity, and other metabolic diseases are becoming high-risk factors for cog-

nitive decline and dementia [4,5]. The risk of cognitive decline is four times higher in NAFLD patients than in healthy people, involving memory, language, executive function, and attention [6,7]. A recent nationwide study found that NAFLD was associated with an increased risk of dementia, especially in non-obese NAFLD subjects [8]. Neuroimaging studies have demonstrated decreased cerebral perfusion, total brain volume, and blood flow in NAFLD patients, suggesting that NAFLD may be the cause of cognitive impairment independent of other components of the metabolic syndrome [9,10]. However, the underlying neural mechanisms of NAFLD-related cognitive decline remain unclear, and the differences between non-obese NAFLD and obese NAFLD need to be further explored.

Functional magnetic resonance imaging (fMRI) is a non-invasive technique based on blood oxygenation level-dependent (BOLD) to reveal the spontaneous activity of brain neurons [11]. Resting-state fMRI (RS-fMRI) can detect the spontaneous activities of brain neurons under a resting state, which is one of the effective methods to study the central mechanism related to cognition and behavior. The amplitude of low-frequency fluctuation (ALFF) [12] and regional homogeneity (ReHo) [13] are important RS-fMRI indicators that reflect the activity characteristics of brain regions, and their abnormalities can reflect the changes in brain activity under physiological states and pathological conditions. Previous studies have shown that obesity is not only associated with abnormalities in local brain regions, but also with impaired functional states of brain networks [14]. Functional connectivity (FC) is an RS-fMRI indicator that can reflect the correlation between brain regions and has been used in a large number of studies to explore the brain network mechanism of neurological and psychiatric diseases. In this study, we divided the patients with NAFLD into the non-obese group and the obese group for the first time, combined with ALFF, ReHo, and FC analysis to comprehensively explore the neural activity characteristics of non-obese NAFLD patients from the local and whole-brain aspects. In addition, we further examined the correlation between these differences and cognitive performance and clinical indicators, providing objective neurobiological markers for cognitive dysfunction in non-obese NAFLD.

2. Materials and Methods

2.1 Subjects

In this study, 44 patients with NAFLD and 20 age- and education-matched healthy controls (HC) were recruited from the metabolic center of Affiliated Hospital of Hangzhou Normal University (Hangzhou, China). NAFLD was diagnosed by MRI-derived proton density fat fraction (MRI-PDFF). As a non-invasive, quantitative and reproducible assessment method, MRI-PDFF can accurately measure liver fat, which is superior to liver ultrasound [15]. The enrolled NAFLD patients were further divided according to the BMI scores forming two groups: 19 non-obese NAFLD (BMI <25 kg/m^2) and 25 obese NAFLD (BMI >25 kg/m^2). All participants were right-handed. The exclusion criteria for both patients and control subjects were as follows: (1) substance abuse, including drugs, alcohol, and cigarettes; (2) contraindications to MRI; (3) any history or current diagnosis of psychiatric or neurological disorders, including hepatic encephalopathy, depression, head injury, etc; (4) hypertension; (5) unable to complete the cognitive scale independently. All NAFLD patients underwent liver MRI-PDFF and blood tests one week before brain MRI.

2.2 Cognitive Functioning Assessment

After the diagnostic procedures, all the subjects underwent psychological testing for cognitive impairments using the Montreal Cognitive Assessment test (MoCA), digit span test (DST), and trail-making test A (TMT-A). The MoCA is a simple-to-administer screening instrument to evaluate the participants' global cognitive function. The Chinese version of MoCA, with a total score of 30, mainly assesses visuospatial ability, executive ability, sustained attention, concentration, working memory, short-term memory recall, language and orientation. A score of less than 26 on the MoCA scale was defined as mild cognitive impairment (MCI) [16]. DST is a number memorization test used to assess working memory and attention to auditory stimuli. The subjects were told a series of numbers and asked to recall the sequence correctly, with each trial testing a longer sequence than the one before. The higher the score, the better the attentional function. Trail making test (TMT) is a timed neuropsychological test that measures attention maintenance and cognitive flexibility, with an emphasis on visual scanning, distraction, and psychomotor speed. These neuropsychological measures involved assessments of global cognition, auditory memory, attention span and working memory.

2.3 MRI Data Acquisition

MRI scanning was performed using a 3T MRI scanner (MR-750, GE Medical Systems, Milwaukee, WI, USA) equipped with an 8-channel head coil. All subjects were instructed to lie quietly, remain awake with eyes closed, and use foam padding and earplugs to avoid head movement and reduce noise. Functional images were obtained using an echo-planar imaging sequence with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, flip angle (FA) = 90°, matrix size = 64 $\times$ 64, thickness/gap = 4/0 mm. Anatomical images were acquired using a spoiled gradient-recalled pulse sequence with the following parameters: TR = 8.16 ms, TE = 3.18 ms, matrix = 256 $\times$ 256, FA = 8°, and slice thickness = 1 mm.

2.4 fMRI Data Analysis

The RS-fMRI data were preprocessed and analyzed using the toolkits of DPABI [17] and Statistical Parametric Mapping (SPM12) on a MATLAB 2013b platform. The preprocessing steps were applied including: image format conversion; removal of the first 10 volumes of the time series data; slice timing was performed; realignment for head movement compensation was applied; spatially normalized to Montreal Neurologic Institute (MNI) standard space; the resulting images were detrended, smoothed using a Gaussian filter with FWHM = 6 mm, and bandpass filtered (0.01–0.1 Hz) to remove high frequency physiological noise; a Friston-24 parameter was regressed out as nuisance covariates for the subsequent analysis finally.

2.5 ALFF, ReHo and FC Calculation

The power spectrum was obtained by transforming all voxels from the time domain to the frequency domain using a fast Fourier transform. Then, the average square root of the power spectrum for each voxel was calculated and regarded as ALFF [12]. ALFF maps were converted into *z*-maps by subtracting the global mean value and then dividing by the standard deviation. The statistical analysis and correlation analysis were based on standardized ALFF maps.

ReHo was calculated as Kendall's coefficient of concordance of the time course of a given voxel with those of its nearest neighbors. For standardization, the ReHo value of each voxel was divided by the global mean ReHo value [13].

Moreover, seed-based FC analysis was used to explore FC abnormalities in NAFLD. First, based on the comparison results of ALFF and ReHo between the patient group and the HC group, the regions associated with cognitive function were used as regions of interest (ROIs) to conduct FC analysis between the patient group and the HC group. Secondly, the regions with ALFF or ReHo differences between the two patient groups were used as ROIs to conduct FC analysis between the two patient groups. ROI was defined as a spherical region with a radius of 6 mm centered on the peak coordinates of MNI in the ALFF/ReHo map. The analysis step consisted of extracting the mean time series of the ROI for each participant and correlating it with each voxel in the whole brain to obtain the seed-based FC map, which was then converted to a *z*-map according to the Fisher *z*-transform.

2.6 Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Science version 25.0 (SPSS 25.0, IBM Corp., Chicago, IL, USA). In the comparison of demographic characteristics and cognitive function among the three groups, the Chi-square test was used to assess gender differences, and the remaining variables were analyzed by one-way analysis of covariance (ANCOVA) and post-hoc analysis. The differences in biochemical data between the two patient groups were analyzed by two-sample *t* test. $p < 0.05$ was considered statistically significant.

To examine differences in ALFF and ReHo among the three groups, one-way ANCOVA and subsequent post-hoc analysis were performed, with age, sex, and education level as covariates. Two-sample *t* test was used to analyze FC differences between the patient group and the HC group and between the two patient groups. Pearson correlation analysis was conducted between abnormal brain activity (including ReHo, ALFF and FC) and cognitive behavior score and biochemical data in the non-obese NAFLD group and obese NAFLD group.

3. Results

3.1 Demographic and Clinical Data

The demographic and clinical data for participants are shown in Table 1. No significant differences were observed in education or age among the three groups ($p > 0.05$). However, significant group differences in the MoCA ($p < 0.001$), DST ($p < 0.001$), and TMT-A ($p = 0.023$) scores among the three groups. As expected, the cognitive status was lower in NAFLD patients according to the cognitive scale assessment. There were no statistically significant differences in cognitive scale scores between non-obese and obese patients. The MRI-PDFF values of NAFLD patients suggested that the degree of fatty liver was moderate (within 10% to 25%), and there was no statistical difference between obese and non-obese patients ($p = 0.405$). In terms of biochemical data, there were statistically significant differences in alanine aminotransferase (ALT), aspartate aminotransferase (AST), insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) levels between the two patient groups, while there were no significant differences in white blood cell (WBC), fasting blood glucose (FBG) and gamma-glutamyltransferase (GGT) levels. These results suggest that obese NAFLD patients have more impaired liver function and insulin tolerance than non-obese NAFLD patients. None of the patients met the diagnostic criteria for diabetes. Four patients had impaired fasting glucose (one patient in the non-obese group had fasting glucose slightly higher than 7, and three patients in the obese group had fasting glucose slightly higher than 6).

3.2 ALFF and ReHo Analysis

The results of ANCOVA analysis showed the regions with significant differences in ALFF among the three groups, including the left fusiform cortex, the left calcarine, the right superior frontal gyrus, and the bilateral cerebellum ($p < 0.001$, cluster size >14, uncorrected). Compared with HC, non-obese NAFLD patients exhibited increased ALFF in the left temporal fusiform cortex and the left calcarine cortex and decreased ALFF in the bilateral cerebellum (Table 2, Fig. 1A); the obese NAFLD patient group showed increased ALFF in the left cerebellum and left fusiform cortex

Table 1. Demographic and clinical data of the three groups.

Variable	Obese NAFLD (n = 25)	Non-obese NAFLD (n = 19)	HC (n = 20)	*p*-values
Sex (male/female)	21/4	9/10	15/5	0.027 [a]
Age (years)	34.32 ± 8.33	38.05 ± 11.48	31.10 ± 7.96	0.072 [b]
Education (years)	13.56 ± 2.75	13.63 ± 2.98	14.85 ± 1.98	0.211 [b]
BMI (kg/m^2)	29.74 ± 3.10 [†§]	23.19 ± 1.37	23.53 ± 1.08	<0.001[b]
MoCA	24.60 ± 2.00 [†]	24.42 ± 1.80 [‡]	27.95 ± 0.94	0.000 [b]
DST	12.72 ± 2.59 [†]	12.32 ± 2.23 [‡]	15.85 ± 1.78	0.000 [b]
TMT-A (seconds)	29.52 ± 7.36 [†]	31.42 ± 8.87 [‡]	25.45 ± 2.03	0.023 [b]
MRI-PDFF (%)	15.92 ± 7.34	13.84 ± 9.06	-	0.405 [c]
WBC (×10^9/L)	6.89 ± 1.37	6.18 ± 1.41		0.123 [c]
ALT (mmol/L)	110.88 ± 58.03 *	59.84 ± 41.13	-	0.002 [c]
AST (mmol/L)	54.39 ± 22.09 *	34.68 ± 15.57	-	0.002 [c]
GGT (mmol/L)	72.48 ± 51.81	89.00 ± 65.63	-	0.367 [c]
FBG (mmol/L)	5.36 ± 0.55	5.52 ± 0.60		0.382 [c]
INS (pmol/L)	114.77 ± 52.81*	73.14 ± 31.21		0.004 [c]
HOMA-IR	3.87 ± 1.98 *	2.48 ± 1.00	-	0.007 [c]

Data are shown as mean ± standard deviation. [a] The *p*-value was obtained by Chi-square test. [b] The *p*-value was obtained by one-way analyses of variance (ANOVA) and post-hoc comparisons (Obese NAFLD vs HC, [†] $p < 0.05$; Obese NAFLD vs Non-obese NAFLD, [§] $p < 0.05$; Non-obese NAFLD vs HC, [‡] $p < 0.05$). [c] The *p*-value was obtained by two-sample *t*-test, * $p < 0.05$. BMI, body mass index; MoCA, Montreal Cognitive Assessment; DST, digit span test; TMT-A, trail making test A; MRI-PDFF, magnetic resonance imaging-derived proton density fat fraction; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; FBG, fasting blood glucose; INS, insulin; HOMA-IR, homeostasis model assessment of insulin resistance.

and decreased in the superior frontal gyrus and the bilateral cerebellum (Table 2, Fig. 1B).

The results of ANCOVA analysis showed that the regions with significant differences in ReHo among the three groups, including the left fusiform cortex, the right precentral gyrus, and the left cerebellum ($p < 0.01$, cluster size >45, uncorrected). Compared with HC, non-obese NAFLD patients exhibited increased ReHo in the left fusiform cortex and the left calcarine cortex (Table 3, Fig. 2A); obese NAFLD patients showed increased ReHo in the bilateral cerebellum and the left fusiform cortex and decreased in the right orbital frontal cortex (OFC) (Table 3, Fig. 2B).

Compared with obese NAFLD patients, non-obese NAFLD patients showed increased ALFF and ReHo in the left middle temporal gyrus, and increased ReHo in the right precentral gyrus and the left postcentral gyrus ($p < 0.01$, cluster voxels >60, uncorrected) (Tables 2,3, Figs. 1C,2C).

3.3 Correlation Analysis

We further extracted ALFF and ReHo values of abnormal brain regions in the two patient groups, and analyzed their relationship with cognitive behavioral scores and biochemical data by pearson correlation analysis. In non-obese NAFLD group, the ALFF values in the left fusiform cortex was negatively correlated with the DST score, MoCA score, and HOMA-IR ($r = –0.486$, $p = 0.035$; $r = –0.574$, $p = 0.010$; $r = –0.490$, $p = 0.033$, respectively), but positively correlated with the TMT-A score ($r = 0.500$, $p = 0.028$) (Fig. 3). In the obese NAFLD group, the ALFF values in the right superior frontal gyrus were negatively correlated with DST score ($r = –0.411$, $p = 0.041$).

3.4 FC Analysis

FC analysis showed significantly different FC between the non-obese NAFLD and HC and between the two patient groups were mapped to cortex surface and visualized with the BrainNet Viewer package [18], see Fig. 4. Compared with HC, non-obese NAFLD patients showed decreased FC of the left fusiform cortex to the right inferior frontal cortex (Brodmann's area [BA] 47; peak MNI: x = 24, y = 33, z = –3) and the left angular gyrus (BA 39; peak MNI: x = –30, y = –57, z = 30) ($p < 0.01$, cluster size >30, uncorrected) (Fig. 4A). Compared with HC, no abnormality of FC between right superior frontal gyrus and whole brain voxels was found in obese NAFLD patients. Compared with obese NAFLD patients, non-obese NAFLD patients showed decreased FC of the left middle temporal gyrus to the right inferior frontal gyrus (BA 47; peak MNI: x = 30, y = 36, z = 6) ($p < 0.01$, cluster size >30, uncorrected) and negatively correlated with the score of TMT-A and FBG level ($r = 0.460$, $p = 0.047$; $r = 0.469$, $p = 0.043$, respectively) (Fig. 4B,C).

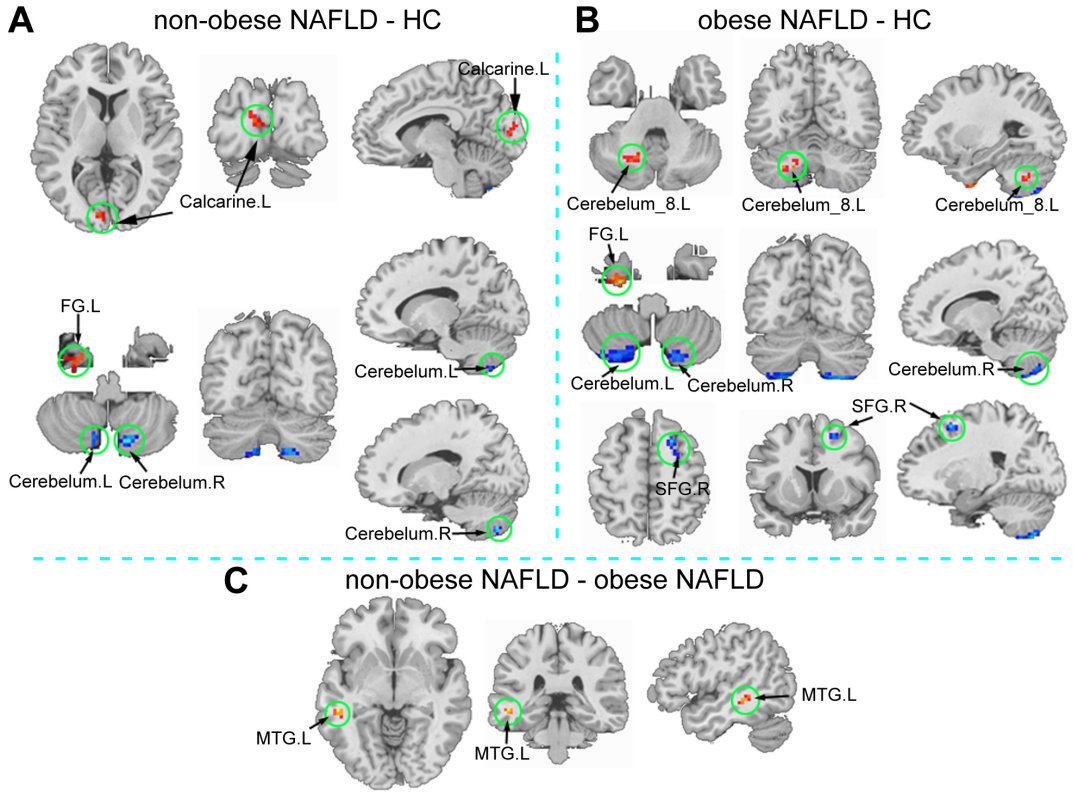

Fig. 1. Brain regions with abnormal ALFF among groups. (A) Differences in ALFF between non-obese NAFLD and HC. (B) Differences in ALFF between obese NAFLD and HC. (C) Differences in ALFF between non-obese NAFLD and obese NAFLD. Blue color denotes relatively lower ALFF values, red color denotes relatively higher ALFF values. FG, fusiform gyrus; SFG, superior frontal gyrus; MTG, middle temporal gyrus; L, left; R, right.

Table 2. Brain regions with abnormal ALFF among groups.

Brain regions	BA	L/R	MNI coordinates			Voxels	T-value
			X	Y	Z		
Non–obese NAFLD vs HC							
Calcarine	17	L	–6	–87	6	23	4.433
Fusiform cortex	37	L	–27	–18	–45	23	5.168
Cerebellum	/	L	–12	–66	–51	23	–4.334
Cerebellum	/	R	12	–72	–48	24	–5.368
Obese NAFLD vs HC							
Fusiform cortex	37	L	–27	–12	–48	56	5.731
Cerebellum	/	L	–18	–60	–39	26	4.458
Superior frontal gyrus	8	R	21	15	54	31	–5.076
Cerebellum	/	R	24	–69	–60	229	–5.660
Non–obese NAFLD vs Obese NAFLD							
Middle temporal gyrus	21	L	–51	–33	–6	141	5.245

BA, Brodmann's area; MNI, Montreal Neurological Institute; L/R, left/right.

4. Discussion

To the best of our knowledge, this study is the first to explore neural spontaneous activity abnormalities in non-obese patients combining ALFF, ReHo, and seed-based FC approach. The main findings were as follows: (1) compared with obese NAFLD patients, non-obese NAFLD patients showed increased ALFF and ReHo values in the left middle temporal gyrus, increased ReHo in the sensorimotor cortex, and reduced FC between the left middle temporal gyrus and right inferior frontal gyrus; (2) compared with HC, non-obese patients showed increased ALFF and ReHo in the left calcarine cortex and fusiform gyrus, and decreased ALFF in the bilateral cerebellum, and reduced FC between the left

Table 3. Brain regions with abnormal ReHo among groups.

Brain regions	BA	L/R	MNI coordinates			Voxels	T-value
			X	Y	Z		
Non-obese NAFLD vs HC							
Calcarine	17	L	–27	–18	–45	63	4.186
Fusiform cortex	37	L	–6	–87	6	119	5.115
Obese NAFLD vs HC							
Fusiform cortex	20	L	–27	–3	–48	114	6.074
Cerebelum	/	R	39	–45	–42	64	5.574
Cerebelum	/	L	–27	–60	–39	71	4.553
Orbitalfrontal cortex	38	R	51	27	–12	112	–4.655
Non-obese NAFLD vs Obese NAFLD							
Middle temporal gyrus	21	L	–51	–33	–6	36	4.115
Postcentral gyrus	3	L	–33	–33	–51	33	4.114
Precentral gyrus	3	R	18	–27	57	76	4.369

BA, Brodmann's area; MNI, Montreal Neurological Institute; L/R, left/right.

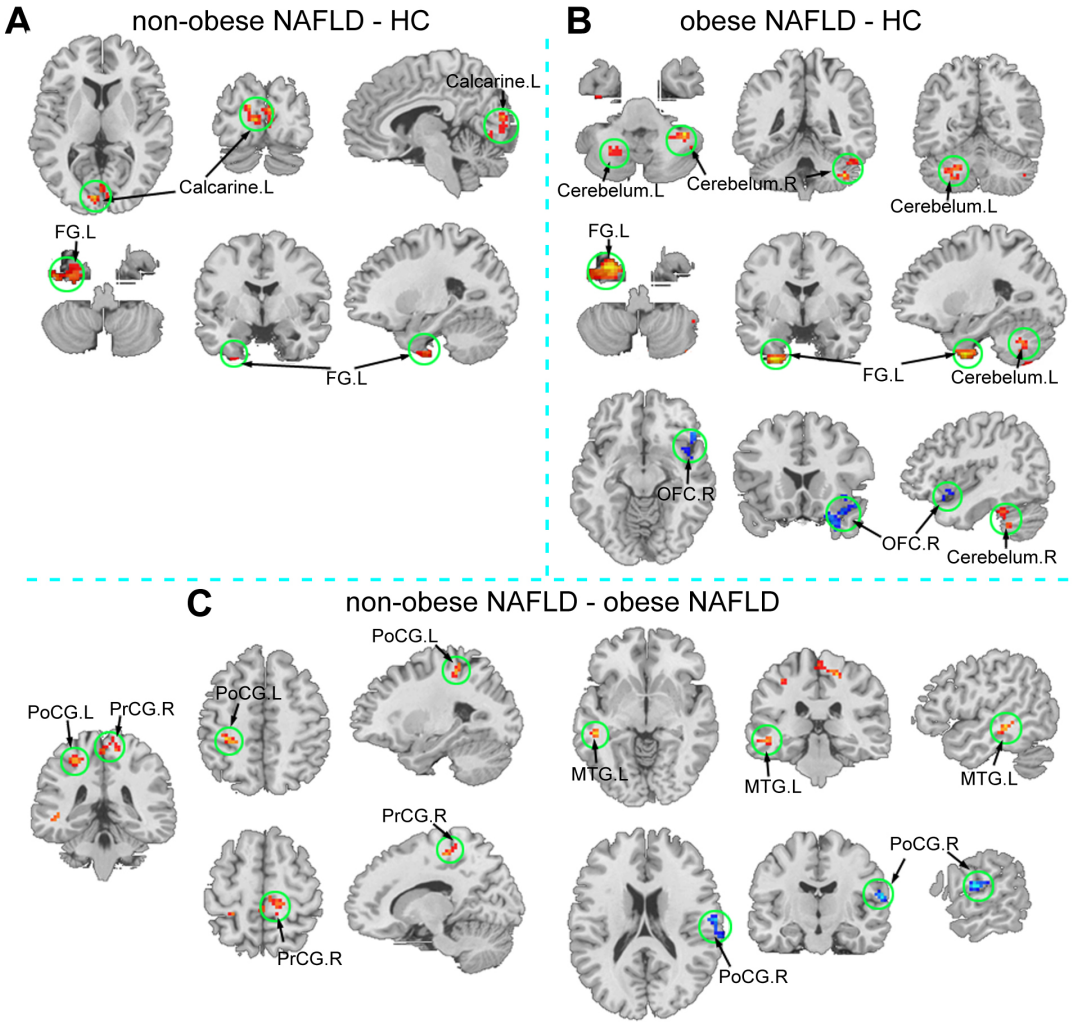

Fig. 2. Brain regions with abnormal ReHo among groups. (A) Differences in ReHo between non-obese NAFLD and HC. (B) Differences in ReHo between obese NAFLD and HC. (C) Differences in ReHo between non-obese NAFLD and obese NAFLD. Blue color denotes relatively lower ReHo values, red color denotes relatively higher ReHo values. FG, fusiform gyrus; OFC, orbitofrontal cortex; PoCG, postcentral gyrus; PrCG, precentral gyrus; MTG, middle temporal gyrus; L, left; R, right.

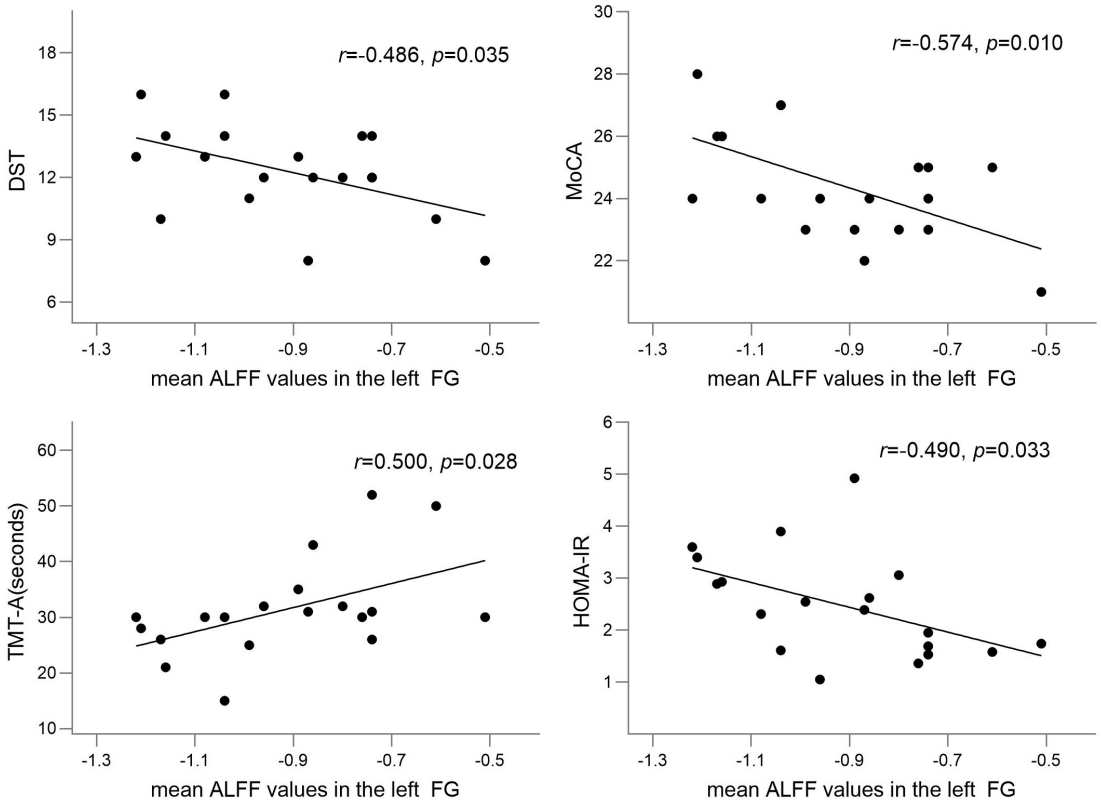

Fig. 3. Scatter plots depicting correlation between the ALFF values of the left fusiform cortex and the DST, MoCA, TMT-A scores, HOMA-IR for non-obese NAFLD patients. FG, fusiform gyrus; ALFF, amplitude of low-frequency fluctuation; DST, digit span test; MoCA, Montreal Cognitive Assessment; TMT-A, trail making test A; HOMA-IR, homeostasis model assessment of insulin resistance.

fusiform gyrus and right inferior frontal gyrus and left angular gyrus. In addition to the same findings, obese NAFLD patients showed increased activity in different regions of the bilateral cerebellum, while decreased ALFF in the right superior frontal gyrus and ReHo in the right OFC; (3) in non-obese patients, the ALFF values in the left fusiform gyrus and the FC values between the left middle temporal gyrus and the right inferior frontal gyrus were associated with cognitive decline, insulin resistance, and fasting glucose disorder were further observed.

The fusiform gyrus locates close to the inferior temporal gyrus on the lateral side and is connected with the parahippocampal gyrus on the medial side. It contains the critical fusiform face area and is considered to be responsible for facial recognition [19,20]. Some researchers have reported greater activation in the left fusiform gyrus when people are exposed to high-energy foods, and it is also thought that the fusiform gyrus helps process food-specific visual attributes [21,22]. Dysfunction of the fusiform gyrus in patients with MCI has also been confirmed in recent years [23,24]. The calcarine gyrus is located in the occipital cortex, which contains the anatomical area of the visual cortex and plays a key role in visual processing [25]. A recent meta-analysis showed that decreased ALFF and ReHo in MCI patients were primarily located in the posterior cingulate cortex, precuneus, bilateral frontal cortex, left occipitotemporal cortex, and parietal lobule [26]. Overactivation of the temporo-occipital cortex in patients with MCI is thought to be a compensatory expression for maintaining normal cognitive function [27]. Previous animal models with obesity and clinical trials of obese patients have found that HOMA-IR may promote beta-amyloid (Aβ) deposition and cognitive decline, which directly leads to cognitive impairment [28,29]. In this study, most obese NAFLD patients had increased HOMA-IR and insulin levels, which was consistent with the previous report [30]. Psychological studies have consistently found that obese people have reduced multi-dimensional cognitive function compared with non-obese people [31–33]. However, although the cognitive decline was observed in both obese and non-obese NAFLD patients in this study, there was no statistical difference between the two groups. We consider that in non-obese NAFLD people, even though HOMA-IR is normal, there is also a significant decline in cognitive function. Of course, this needs to be verified by a large sample. In the present study, we found that the abnormal brain activ-

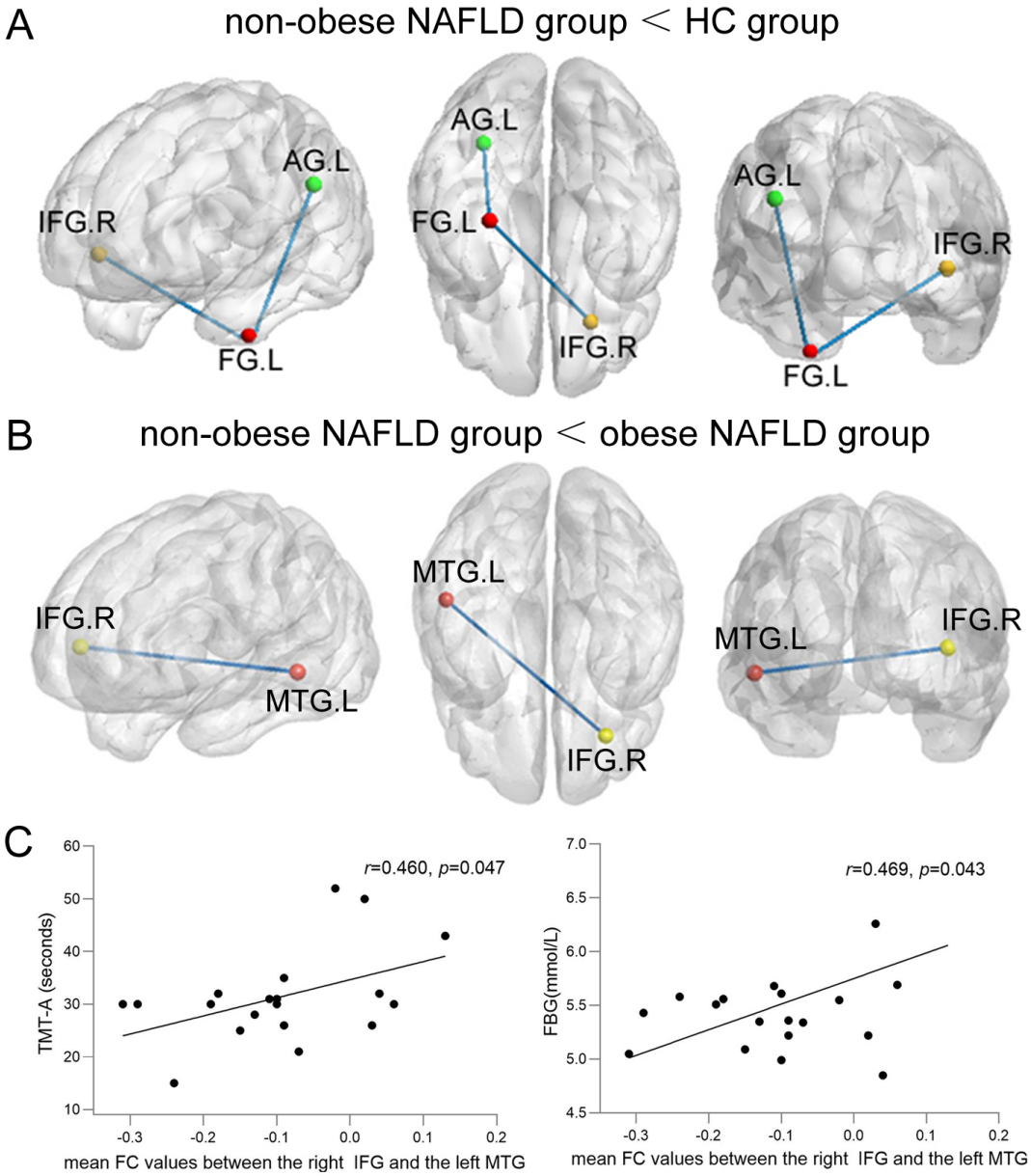

Fig. 4. The differences of functional connectivity analyses between the non-obese NAFLD group and the HC group and between the two patient groups. The results of left fusiform cortex seed functional connectivity analyses differences between non-obese NAFLD patients and HC (A). The results of left middle temporal gyrus seed functional connectivity analyses differences between non-obese NAFLD patients and obese NAFLD patients (B). Scatter plots depicting correlation between the left MTG-related functional connectivity in the right IFG and the TMT-A scores, FBG level for non-obese NAFLD patients (C). FG, fusiform gyrus; IFG, inferior frontal cortex; AG, angular gyrus; MTG, middle temporal gyrus; L, left; R, right. TMT-A, trail making test A; FBG, fasting blood glucose.

ity regions of NAFLD patients were predominantly in the fusiform gyrus and calcarine cortex, which may be significantly related to the decline of visual memory, information processing speed, and attention [34]. Correlation analysis showed that the ALFF value of the fusiform gyrus was significantly associated with cognitive function in non-obese NAFLD patients, further suggesting that this region could be used as a marker of cognitive impairment in non-obese NAFLD patients.

The OFC is a vital region of the reward circuit, receiving information from sensory processing (e.g., insula, fusiform gyrus), emotional processing (e.g., amygdala), and memory (e.g., hippocampus), and playing an important role in the integration of stimuli, encoding, and retrieving reward values [35]. The intensity of OFC activity is thought to be related to food/food cue pleasurable or taste rating [36–38]. Neuroimaging studies have reported reduced gray matter volume, abnormal diffusion of water

molecules, and low activity in the OFC in obese or morbid-obese patients, which are thought to be associated with enhanced food-related rewards that may lead to overweight or obesity [39–42]. Tuulari *et al*. [43] reported that the superior frontal gyrus is involved in cognitive appetite control in adults. Task-state fMRI found that performance on cognitive tasks correlated with BMI, with participants with higher BMI showing lower response inhibition [44]. The present study found reduced spontaneous activity of OFC and superior frontal gyrus in obese NAFLD patients, but not in non-obese NAFLD patients. Our results may further confirm that obesity interferes with OFC and may be the neural mechanism leading to altered food value representations [45].

Our results demonstrate the increased activities in the left middle temporal gyrus, the right precentral gyrus, and the left postcentral gyrus in the non-obese NAFLD patients compared with obese NAFLD patients. The left middle temporal gyrus is responsible for visual and semantic processing. One study showed greater fMRI activation in the left middle temporal gyrus when obese individuals visual presentations of actual foods vs size-matched nonfoods following an overnight fast [46]. Moreno-lopez *et al*. [47] found increased connectivity between the left middle temporal cortex and the reward system in overweight and obese individuals compared to normal-weight individuals. Consistent with these results, the present study found that the FC between the left middle temporal gyrus and the prefrontal cortex was decreased in non-obese patients when compared with obese patients. Some studies have confirmed the importance of the sensorimotor cortex and its close association with reward-related areas in obesity-related diseases. Differences in white matter connectivity in rewards-related areas and related sensorimotor networks could accurately classify individuals with high BMI from normal individuals [48]. In addition, task-state fMRI studies involving food images, food intake, and taste or olfactory cues have shown increased activity in the right precentral gyrus and left postcentral gyrus in the context of eating behavior and obesity [49]. We speculated that abnormal resting-state spontaneous activity in the regions involved in temporo-occipital and sensorimotor cortex may be the underlying neural mechanism differences between non-obese NAFLD and obese NAFLD.

Although the cerebellum is generally believed to be involved in the coordination of body balance and voluntary movement. However, there is increasing evidence that the cerebellum is also involved in memory and higher-level cognition [50–52]. In addition, the cerebellum plays an important role in feeding behavior and is an early marker of steatohepatitis-related brain damage [53]. This study found that both groups of NAFLD patients had decreased spontaneous activity in the bilateral cerebellum, which may be a noninvasive imaging marker of NAFLD-related cognitive impairment. Notably, in the present study, obese NAFLD patients also had increased spontaneous activity in other regions of the bilateral cerebellum. Cerebellar activation is thought to compensate for cortical dysfunction in patients with cognitive decline [54], which may explain the increased cerebellar activity in patients with obese NAFLD, possibly as compensatory activation for more frontal cortex dysfunction (including superior frontal gyrus and OFC).

It is widely considered that the processing of higher-order cognitive functions results from the interaction of distributed brain regions operating at the level of large-scale neural networks rather than the isolated brain region. We found decreased FCs from the left fusiform gyrus to the right inferior frontal cortex (orbit part) and left angular gyrus in non-obese NAFLD patients compared to HC. The orbital part of the inferior frontal cortex is a major part of the OFC and is considered responsible for the reappraisal of emotional stimuli, the evaluation of social cues, and the decision-making process [55,56]. The angular gyrus is a vital node belonging to the default mode network (DMN), which plays a critical role in monitoring the environment, emotional processing, self-introspection, and episodic memory retrieval [57,58]. Therefore, we speculated that the abnormal connection pattern between the fusiform gyrus and DMN and reward system might play a key role in the cognitive impairment of memory, attention, decision-making, and executive function in non-obese NAFLD patients.

This study has several shortcomings. First, the sample size of NAFLD is relatively small, and mostly young and middle-aged patients, which may affect the reliability and generalizability of the results. Second, this study lacked pathological grading of liver fatty or fibrosis in NAFLD patients. We quantified the degree of hepatic steatosis in patients by measuring the MRI-PDFF value, which is a non-invasive imaging indicator for accurately quantifying hepatic fat content and has a high correlation with the results of liver biopsy [59]. The average PDFF value of patients in this study indicated moderate fatty liver level, and there was no statistical difference between the two patient groups. Third, in addition to liver enzymes, inflammatory markers such as high-sensitivity C-reactive protein (CRP), tumor necrosis factor (TNF-α), and interleukin-6 (IL-6), are also closely associated with liver steatosis and cognitive decline [60,61]. Forth, there are differences in gender composition among the three groups, and the proportion of males in the group of obese NAFLD patients is high, which may cause some deviation in the results. However, gender was used as a covariable for regression in data processing and correlation analysis. In addition, insulin levels and insulin resistance are statistically different between obese and non-obese NAFLD patients. To exclude the contribution of this factor to the results, we need to further compare subgroups based on clinical indicators (such as insulin resistance, insulin, etc.). Finally, only a cross-sectional study design was used in this study. In the future, multi-center, large-sample,

interventional prospective studies can be further carried out and combined with liver pathology and other indicators to verify the results obtained in this study.

5. Conclusions

In summary, by combining ReHo, ALFF and FC analysis, we found that non-obese NAFLD patients had local spontaneous activity and FC abnormalities in regions involved in the sensorimotor, temporo-occipital cortex, cerebellum, and reward system when compared with obese NAFLD patients and HC, some of which may be the underlying differences in neural mechanisms between non-obese and obese NAFLD patient. In addition, the temporo-occipital cortex may be a vulnerable target for cognitive decline in non-obese NAFLD patients.

Abbreviations

NAFLD, nonalcoholic fatty liver disease; HC, healthy controls; RS-fMRI, resting-state functional magnetic resonance imaging; BOLD, blood oxygenation level-dependent; ReHo, regional homogeneity; ALFF, amplitude of low-frequency fluctuation; FC, functional connectivity; FG, fusiform gyrus; OFC, orbitofrontal cortex; MRI-PDFF, magnetic resonance imaging-derived proton density fat fraction; MoCA, montreal cognitive assessment test; DST, digit span test; TMT-A, trail making test A; MCI, mild cognitive impairment; MNI, Montreal Neurologic Institute; ROI, regions of interest; DMN, default mode network; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; HOMA-IR, homeostasis model assessment of insulin resistance; WBC, white blood cell; FBG, fasting blood glucose; CRP, C-reactive protein; TNF-α, tumor necrosis factor; IL-6, interleukin-6.

Author Contributions

JLX, JPG and JPS designed the research study. JLX, JPG, LYW, JL and NNY performed the research. QRZ, JL and LYW provided help and advice on the analysis of the resting-state fMRI data. JLX, JPG and JL analyzed the data. JLX, JPG and JL wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was approved by the Ethics Committee of Affiliated Hospital of Hangzhou Normal University (approval number: [2019(02)-HS-02]).

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Conflict of Interest

The authors declare no conflict of interest.

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