

Cognitive Impairment in MASLD is associated with Amygdala-Related Connectivity Dysfunction in the Prefrontal and Sensory Cortex

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Abstract

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a common metabolism-related multisystem clinical disorder, often accompanied by a high comorbidity of mild cognitive impairment (MCI). Increasing evidence suggests that the amygdala is crucial in cognitive processing during metabolic dysfunction. Nevertheless, the role of the amygdala in the neural mechanisms of MASLD with MCI (MCI_MASLD) remains unclear. **Methods:** A total of 74 MASLD patients (43 with MCI_MASLD and 31 without MCI [nonMCI_MASLD]) and 62 demographic-matched healthy controls (HC) were enrolled. All participants underwent resting-state functional magnetic resonance imaging scans and psychological scale assessments. Liver fat content and blood index measurements were performed on the patients. Using the bilateral amygdala as seeds, the seed-based functional connectivity (FC) maps were calculated and one-way analysis of covariance with post hoc tests was performed to investigate the difference among the three groups. **Results:** Compared to nonMCI_MASLD patients, MCI_MASLD patients demonstrated enhanced FC between the right amygdala and the medial prefrontal cortex (mPFC), while reduced FC between the left amygdala and the left supplementary motor area (SMA). Interestingly, the FC values of the mPFC were correlated with the Montreal Cognitive Assessment Scale (MoCA) scores and liver controlled attenuation parameters, and the FC values of the SMA were also correlated with the MoCA scores. Furthermore, the FC values between the bilateral amygdala and regions within the frontal-limbic-mesencephalic circuits were higher in MASLD patients when compared to HC. **Conclusions:** Aberrant FC of the amygdala can provide potential neuroimaging markers for MCI in MASLD, which is associated with amygdala-related connectivity disturbances in areas related to cognition and sensory processing. Moreover, visceral fat accumulation may exacerbate brain dysfunction.

Keywords: metabolic dysfunction-associated steatotic liver disease; non-alcoholic fatty liver disease; amygdala; mild cognitive impairment; functional connectivity

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a clinical metabolic syndrome characterized by hepatic steatosis with cardiometabolic risk factors, formerly known as Non-alcoholic fatty liver disease (NAFLD), which affects about 32% of the global population [1,2]. MASLD has been identified as a multisystem disease that can involve extrahepatic organs, of which the cardiovascular system is considered to be the primary organ affected by metabolic syndrome over time [3]. More recently, brain health issues related to MASLD have attracted much attention. In addition to an increased risk of stroke, patients with MASLD may have moderate or early cognitive impairment involving aspects of memory, language, and executive attention [4,5]. A growing amount of evidence has demonstrated that MASLD can be used as an independent risk factor for impaired cognitive performance and cerebral function, and depression (independent of cardiometabolic disorders) [6], which seriously affects the quality of life of patients.

Recently, advanced neuroimaging techniques have been applied to investigate the neurophysiological mechanisms of MASLD. Structural imaging studies have shown that patients with MASLD have reduced total brain volume, particularly in regions involved in cognitive functions [7–9]. It is well known that changes in neuronal activity precede structural changes, but little is known about spontaneous brain activity patterns in MASLD patients. Resting-state functional magnetic resonance imaging (rs-fMRI), is a powerful technique for exploring spontaneous neuron activity at baseline, has been increasingly used to study the neural mechanisms of abnormal liver-brain interaction, such as hepatic encephalopathy [10].

Previous studies have suggested that patients with MASLD have abnormal functional connectivity (FC) between regions within the salience network (SN) and the default mode network (DMN), which are associated with cognitive decline [11,12]. The amygdala, serving as a core component of the SN, exhibits extensive connections with diverse regions including the prefrontal, temporal and sen-

sory cortex, which play pivotal roles in cognitive processing, emotion regulation and reward anticipation [13]. Recent study has proposed that the amygdala is a vital region significantly affected by metabolic disorders, and modulating its activity may be a novel treatment for diverse neurological problems in metabolic disease-related dementia [14]. It is of great clinical value to elucidate the relationship between amygdala connectivity and neuropsychiatric problems in patients with metabolic cognitive impairment. However, the role of the amygdala and its connectivity in the neural mechanisms of cognitive impairment in MASLD remains ambiguous.

In this study, for the first time, MASLD patients were divided into patients with mild cognitive impairment (MCI_MASLD) and patients without MCI (non-MCI_MASLD) according to the Montreal Cognitive Assessment (MoCA) score. rs-fMRI techniques and seed-based FC analysis were used to explore FC changes in the bilateral amygdala and the whole brain in MASLD patients, as well as correlations between abnormal patterns and clinical scales. We hypothesized that patients with MASLD have abnormal FC between the amygdala and cognition-related areas, and that patients with mild cognitive impairment (MCI) may have even more severe connectivity disturbance.

2. Materials and Methods

2.1 Subjects

This study was carried out in accordance with the guidelines of the Declaration of Helsinki and approved by the ethics committee of Affiliated Hospital of Hangzhou Normal University (approval number: [2019 (E2) -HS-02]) and all patients or their families/legal guardians signed written informed consent prior to the study. A total of 74 patients with MASLD (male/female: 54/20, age: 37.03 ± 11.40 years) were enrolled from the fatty liver clinic of the Affiliated Hospital of Hangzhou Normal University, Hangzhou, China. Meanwhile, 62 age- and gender-matched healthy controls (HC) (male/female: 38/24, age: 38.47 ± 10.52 years) were recruited. All participants were enrolled according to the following criteria: age between 18 and 60 years; Mini-Mental State Examination (MMSE) score ≥ 24 (Chinese versions); no history or current diagnosis of psychiatric disorders, hepatic encephalopathy, chronic liver disease, diabetes, cerebrovascular accidents, epilepsy, brain trauma and surgery, or hypertension; right-handed as defined by the criteria of the Edinburgh Handedness Questionnaire [15]. All patients with NAFLD were simultaneously diagnosed by two senior hepatologists based on the criteria announced by the Chinese Society of Hepatology in 2018. The term MASLD was proposed to replace the NAFLD in 2023, and all patients in the study met the diagnostic criteria of MASLD based on the updated criteria [1]. The degree of hepatic steatosis in patients with MASLD was assessed using magnetic resonance imaging-derived proton density fat

fraction (MRI-PDFF) [16]. According to the MoCA score, MASLD patients were further divided into two groups: 43 MCI_MASLD and 31 nonMCI_MASLD. MoCA scores less than or equal to 26 points is defined as MCI_MASLD [17]. All HC underwent abdominal B-ultrasonography or computed tomography (CT)/MRI to exclude fatty liver, and the other exclusion criteria were the same as for the patients. The assessment of liver fat was conducted on the same day as diagnosed by two senior hepatologists, which was beneficial for the diagnosis of MASLD. The neurocognitive assessment and rs-fMRI scan were completed on the same day within one week after diagnosis, thus enabling a stronger matching between the two indicators.

2.2 Biological Indicators

Fasting venous blood samples were collected from all patients on the day of brain MRI scanning, and fasting blood glucose, glycosylated hemoglobin (HbA1c), fasting insulin, and liver function indexes (including alanine aminotransferase and aspartate aminotransferase liver enzyme indexes) were measured. Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) formula (fasting blood glucose $\times$ fasting insulin level/22.5). Weight, height and abdominal circumference were measured and recorded simultaneously for all subjects. All patients underwent a liver quantitative MRI (qMRI) scan based on the six-echo Dixon technique one week prior to the head MRI.

2.3 Neurocognitive Assessment

The cognitive functions of all subjects were assessed by the MoCA, digit span test (DST), and trail-making test (TMT), which related to global cognition, auditory memory, attention, and working memory. MoCA is a sensitive screening tool for MCI detection, which primarily examines visuospatial ability, executive ability, attention, working memory, language, and orientation. DST is a neuropsychological test that measures working memory and auditory processing. A higher score indicates a better attention function. TMT, which includes TMT-A and TMT-B, is a widely used neuropsychological assessment that can quickly and easily evaluate a variety of executive functions, including attention, processing speed, working memory, visuospatial ability, and set switching ability.

2.4 Liver Fat Assessment

The degree of liver steatosis in patients with MASLD was assessed by MRI-PDFF and ultrasound-based controlled attenuation parameter (CAP).

Abdominal MRI image data sets were collected by a 1.5T MRI scanner (Magnetom Avanto, Siemens Medical Solutions, Erlangen, Germany) and an 8-channel phased-array surface coil. MRI sequences included conventional MRI and 3D Six-echo Dixon sequence. The parameters of 3D Six-echo Dixon sequence were: field of view (FOV) = 320 mm $\times$ 320 mm; repetition time (TR) = 15.60 ms;

echo time (TE) = 2.38 ms, 4.76 ms, 7.14 ms, 9.52 ms, 11.90 ms, and 13.90 ms; flip angle (FA) = 5°; slice thickness = 4.0 mm; and slice gap = 0.8 mm; whole-liver data were collected within a single breath hold. MRI-PDFF values were measured by a senior radiologist who was unaware of the subject information. The MRI workstation software was used to measure the MRI-PDFF value. Specifically, the regions of interest (ROI) were placed in the VI segment of the liver PDFF image, avoiding the adjacent structures and large vessels.

FibroScan was performed on each patient by an experienced registered operator. FibroScan-502 with an M-probe (Echosens, Paris, France) was used to capture CAP values in the liver. Fasting patients were instructed to lie in a supine position with their heads in their hands, which facilitates adequate expansion of the intercostal space. The tip probe of the sensor was placed on the skin of the right intercostal space between the seventh and ninth ribs. A reliable CAP was defined as more than 10 consecutive effective monitoring sessions with a detection success rate of >60% and an interquartile range of <30% [18].

2.5 Rs-fMRI Acquisition

Brain MRI examination was performed using a 3T MRI scanner (MR-750, GE Medical Systems, Milwaukee, WI, USA) equipped with an 8-channel head coil. Subjects were instructed to keep their eyes closed, remain awake, lie quietly, 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: TR = 2000 ms, TE = 30 ms, FA = 90°, FOV = 192 mm × 192 mm, matrix size = 64 × 64, thickness/gap = 4/0 mm. Anatomical images were acquired using the three-dimensional spoiled gradient recalled echo sequence with the following parameters: TR = 8.16 ms, TE = 3.18 ms, FOV = 256 mm × 256 mm, matrix size = 256 × 256, FA = 8°, and slice thickness = 1 mm. The scanning range included the whole brain, with a total of 176 layers. In addition, T1-weighted imaging (T1WI) and T2 fluid-attenuated inversion recovery sequences were acquired to exclude abnormal anatomical structures and organic lesions.

2.6 Seed-Based Functional Connectivity Analysis

Based on our priori hypotheses, we defined the bilateral amygdala as a ROI respectively, the mask of the bilateral amygdala was created on the automated anatomical labelling (AAL) template (Fig. 1). The average time series of each ROI was extracted, the Pearson correlation coefficient between the average time series of each ROI and the time series of other voxels in the whole brain was calculated, and the correlation graph was generated. Finally, Fisher's r -to- z transformation is performed to improve the normality of the correlation coefficients.

2.7 Statistical Analysis

The SPSS24.0 software (IBM Corp., Chicago, IL, USA) was utilized for conducting statistical analysis on demographic data, clinical indicators, and neuropsychological scale scores. Kruskal-Wallis H test and Chi-squared test were employed to compare the demographic data and scale scores among the three groups. A two-sample t -test or Mann-Whitney U-test was applied to assess differences in biochemical indicators between the two groups. Statistical significance was considered at a p -value of less than 0.05.

Based on the prior assumptions, the FC differences associated with the amygdala among the three groups were calculated using a seed-based FC analysis in Data Processing and Analysis for Brain Imaging (DPABI) soft (version v6.2) under MATLAB (Mathworks, Natick, MA, USA) platform, using a one-way analysis of covariance (ANCOVA) and post-hoc analysis. Age, sex, and education level were set as covariates. We then separately extracted analysis of variance (ANOVA) results for the left and right amygdala associated FC as the corresponding masks. A post-hoc two-sample t -test was used to compare the pairwise FC differences between the three groups based on the ANCOVA brain mask. Gaussian random field (GRF) was applied to multiple comparison correction. The significance threshold was set as voxel-level $p < 0.005$, cluster-level $p < 0.05$, and cluster size > 10 voxels. Functional connectivity values (z -values) were extracted from ROI defined as a spherical region with a radius of 5 mm, that showed significant differences in connectivity to the left/right amygdala between the MCI_MASLD and nonMCI_MASLD groups. Partial correlation analysis was performed between abnormal FC values and neuropsychological scores and biochemical indicators in MASLD patients. Age, sex and education were used as covariates.

3. Results

3.1 Demographic and Clinical Data

The clinical characteristics and demographic information of the subjects are summarized in Table 1. There were no statistical differences in sex, age, and education level among the three groups ($p > 0.05$). Compared with HC, both MASLD patients had significantly higher body mass index (BMI), waist-to-hip ratio, Hamilton Anxiety and Depression Rating Scale (HAD) and lower MoCA scores. Moreover, MCI_MASLD patients also showed lower DST scores and higher TMT-B scores than the HC group. There were no significant differences in TMT-A scores among the three groups ($p > 0.05$). Compared with nonMCI_MASLD patients, the MCI_MASLD group showed the MRI-PDFF ($p = 0.002$) values were statistically higher. However, no significant differences in the CAP ($p > 0.05$) values between the patient groups. In neuropsychological data, the MCI_MASLD group showed significantly lower MoCA ($p < 0.001$) and DST ($p < 0.001$) scores, as well as significantly higher TMT-B ($p = 0.002$)

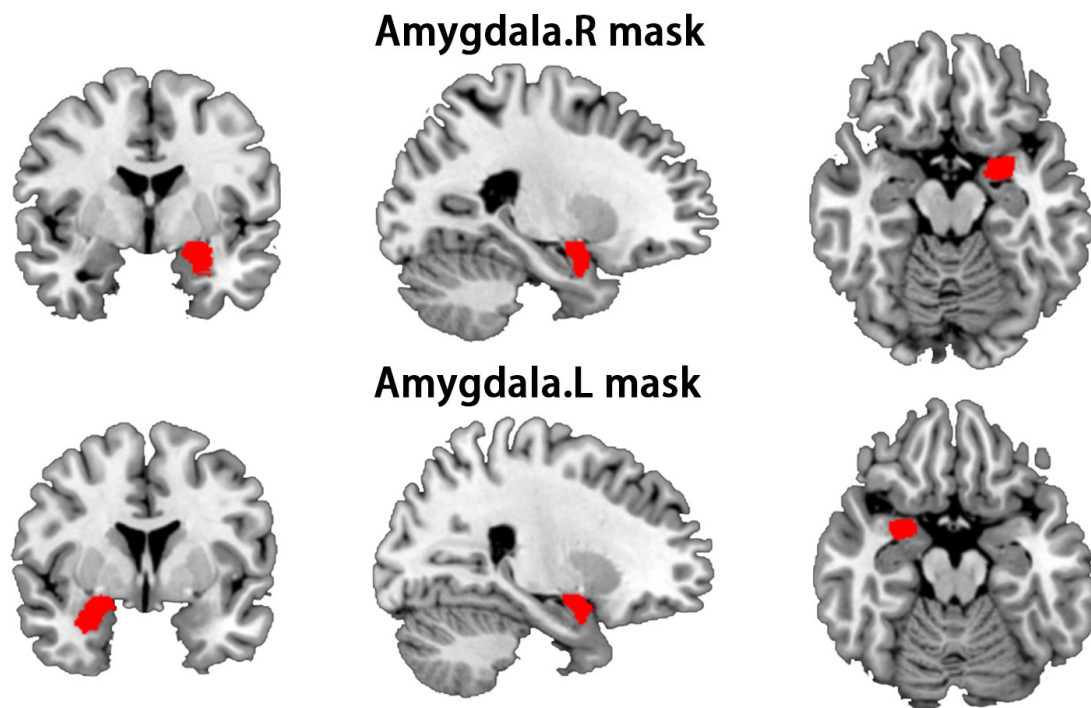


Fig. 1. Bilateral amygdala was created based on the automated anatomical labelling (AAL) template.

scores than nonMCI_MASLD patients. In the emotional data, The HAD ($p = 0.002$) scores of MCI_MASLD patients were significantly higher than nonMCI_MASLD patients. In terms of biochemical data, the MCI_MASLD patients had significantly higher insulin ($p = 0.003$), HbA1c ($p = 0.003$) and HOMA-IR ($p = 0.03$) levels, compared to nonMCI_MASLD patients. No significant difference in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and fasting blood glucose (FBG) levels was observed between the two patient groups. None of the patients met the diagnostic criteria for diabetes.

3.2 FC Analysis

One-way ANCOVA results (GRF-corrected, cluster size >10 , voxel-level: $p < 0.005$, cluster-level: $p < 0.05$, Fig. 2A) showed that the regions with differences in the right amygdala-related FC values among the 3 groups included the right insular and the right medial prefrontal cortex (mPFC). Post hoc 2-sample t -test comparison results (Fig. 2B–D) showed that compared with the non-MCI_MASLD patients, the MCI_MASLD patients exhibited increased FC between the right amygdala and the right mPFC (Table 2 and Fig. 2B). Compared with HC, both patient groups displayed increased right amygdala-related FC in the right insula (Table 2 and Fig. 2C,D).

One-way ANCOVA results (GRF-corrected, cluster size >10 , voxel-level: $p < 0.005$, cluster-level: $p < 0.05$, Table 3 and Fig. 3A) showed that the regions with differences in the bilateral insular, the midbrain, the left middle temporal gyrus, the left postcentral gyrus. Post hoc

two-sample t -test comparison results (Fig. 3B–D) showed that compared with the nonMCI_MASLD patients, the MCI_MASLD patients showed decreased FC between the left amygdala and the left supplementary motor area (SMA) (Table 3 and Fig. 3B). Compared with HC, both patient groups displayed increased left amygdala-related FC in the bilateral insula and the left postcentral gyrus. In addition, MCI_MASLD patients also showed enhanced FC between the left amygdala and the midbrain, while non-MCI_MASLD patients exhibited enhanced FC between the left amygdala and the left middle temporal gyrus (Table 3 and Fig. 3C,D).

3.3 Correlation Analysis

In MASLD patients, the right amygdala-related FC values in the right mPFC were negatively correlated with MoCA scores and positively correlated with the CAP ($r = -0.383$, $p = 0.001$; $r = 0.294$, $p = 0.023$). While the left amygdala-related FC values in the left SMA were positively correlated with MoCA scores ($r = 0.417$, $p < 0.001$) (Fig. 4A–C). No significant association was found between abnormal amygdala-related FC and HAD score, BMI, or biochemical markers.

4. Discussion

To the best of our knowledge, this study was the first to perform a whole-brain seed-to-voxel analysis focusing on amygdala-based FC in MCI_MASLD patients. The main findings were as follows: (1) compared with the nonMCI_MASLD group, the MCI_MASLD group had in-

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

Variable	MCI_MASLD	nonMCI_MASLD	HC	<i>p</i> values
	(<i>n</i> = 43)	(<i>n</i> = 31)	(<i>n</i> = 62)	
Sex (male/female)	31/12	23/8	38/24	0.343 ^a
Age (years)	35 (18~59)	34 (18~59)	39 (21~59)	0.212 ^b
Education (years)	15 (6~19)	16 (9~19)	16 (6~19)	0.114 ^b
BMI (kg/m ²)	29.70 (22.05~50.70) ^{†§}	26.17 (20.57~33.63) [‡]	23.58 (17.58~28.40)	0.000 ^b
Waist-hip ratio	0.93 (0.86~1.09) [†]	0.93 (0.86~1.04) [‡]	0.83 (0.69~0.92)	0.000 ^b
DST	10 (5~14) ^{†§}	14 (8~18)	13.5 (6~19)	0.000 ^b
MoCA	23 (18~25) ^{†§}	27 (26~29) [‡]	29 (26~30)	0.000 ^b
TMT-A (seconds)	40 (18~125)	32 (13~69)	37 (16~78)	0.151 ^b
TMT-B (seconds)	96 (41~230) ^{†§}	75 (38~145)	78 (28~132)	0.001 ^b
HAD	9 (2~17) ^{†§}	7 (3~11) [‡]	0 (0~5)	0.000 ^b
MRI-PDFF (%)	19.60 ± 7.81 [§]	14.05 ± 7.00		0.002 ^c
CAP (mmol/L)	326.59 ± 36.13	314.79 ± 41.46		0.232 ^c
ALT (mmol/L)	93 (18~268)	77.5 (20~445)		0.805 ^d
AST (mmol/L)	52 (15~203)	50 (18~186)		0.785 ^d
HbA1c (mmol/L)	5.70 (4.90~9.90) [§]	5.40 (4.80~6.70)		0.030 ^d
FBG (mmol/L)	5.44 (4.35~8.25)	5.28 (4.74~7.65)		0.158 ^d
Insulin (pmol/L)	112.30 (30.40~548.03) [§]	76.50 (48.98~199.66)		0.003 ^d
HOMA-IR (mmol/L)	3.60 (1.73~23.36) [§]	2.62 (1.57~6.36)		0.003 ^d

Data are shown as mean ± standard deviation; median (min-max range). ^a The *p*-value was obtained by the Chi-square test. ^b The *p*-value was obtained by Kruskal-Wallis test (MCI_MASLD vs HC, [†]*p* < 0.05; MCI_MASLD vs nonMCI_MASLD, [§]*p* < 0.05; nonMCI_MASLD vs HC, [‡]*p* < 0.05). ^c The *p*-value was obtained by the two-sample *t*-test. ^d The *p*-value was obtained by the Mann-Whitney U test. MASLD, metabolic dysfunction-associated steatotic liver disease; MCI_MASLD, MASLD with mild cognitive impairment patients; nonMCI_MASLD, MASLD without mild cognitive impairment patients; HC, healthy controls; BMI, body mass index; DST, digit span test; MoCA, Montreal Cognitive Assessment; TMT-A, trail making test A; TMT-B, trail making test B; HAD, Hamilton depressive and anxiety scales; MRI-PDFF, magnetic resonance imaging-derived proton density fat fraction; CAP, controlled attenuation parameter; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HbA1c, hemoglobin A1c; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance.

Table 2. Results of post hoc two-sample t-test comparison of right amygdala-related FC in three groups.

	Anatomical region	MNI coordinates of Peak voxel			Cluster size	<i>T</i> -value
		x	y	z		
MCI_MASLD vs HC	INS.R	45	0	0	55	3.999
nonMCI_MASLD vs HC	INS.R	36	-6	-9	100	4.873
MCI_MASLD vs nonMCI_MASLD	mPFC.R	15	54	6	11	4.659

MNI, Montreal Neurological Institute; (GRF-corrected, cluster size >10, voxel-level: *p* < 0.005, cluster-level: *p* < 0.05); INS, insula; mPFC, medial prefrontal cortex; L/R, left/right; FC, functional connectivity; GRF, gaussian random field.

creased FC between the right amygdala and the right mPFC, while reduced FC between the left amygdala and the left SMA; (2) The bilateral amygdala-related FC values in the insular and postcentral gyrus in both MASLD groups were significantly higher than those in the HC group. In addition, MCI_MASLD and nonMCI_MASLD patients also had enhanced left amygdala-related FC in the midbrain and left middle temporal gyrus, respectively; (3) the right amygdala-related FC in the right mPFC was negatively correlated with MoCA score and positively correlated with the

CAP values, and the left amygdala-related FC in the left SMA was positively correlated with MoCA scores in the MASLD patients.

As an important part of the default mode network (DMN), the mPFC regulates cognitive function through glutamatergic interactions with the basolateral amygdala and hippocampus [19]. In particular, glutamatergic excitatory neurons can project to the nucleus accumbens to regulate the reward system, thus playing a vital role in cognitive decision-making and emotional processing [20]. Increased

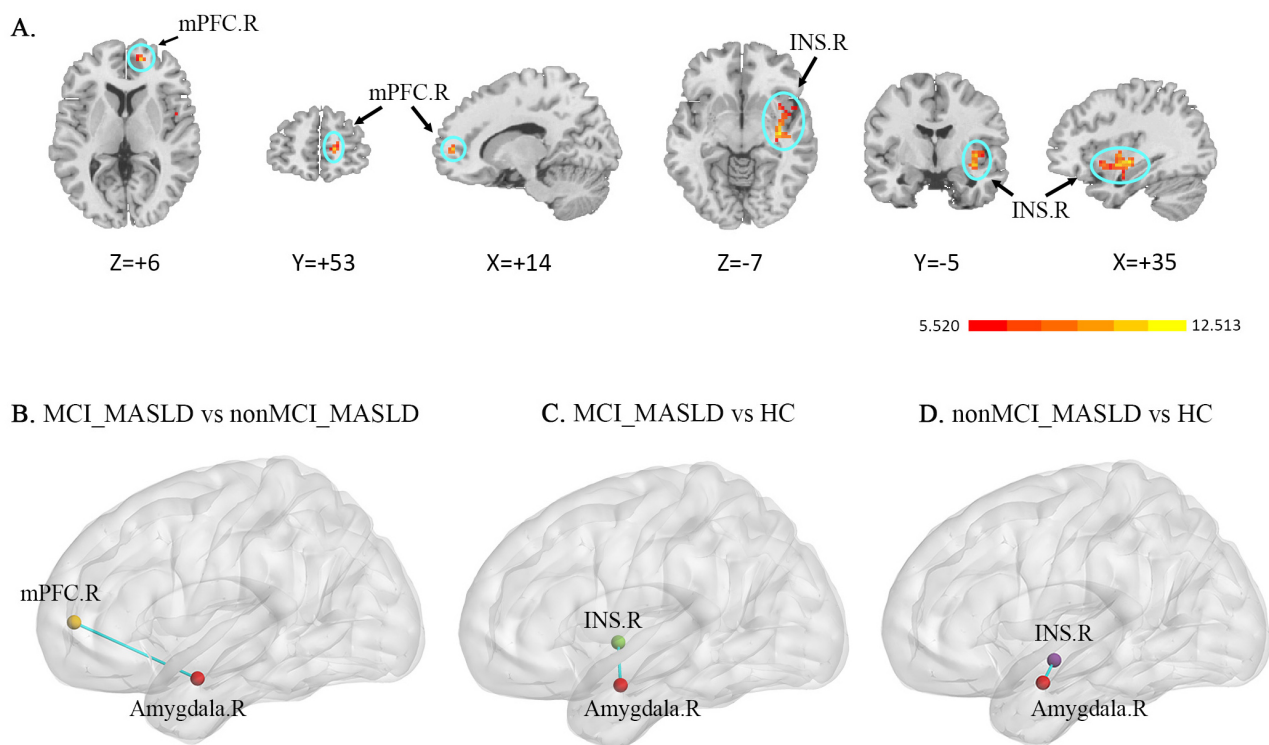


Fig. 2. Brain regions with significant differences in functional connectivity of the right amygdala among groups. (A) The one-way analysis of covariance (ANCOVA) results of the right amygdala-related FC among MCI_MASLD patients, nonMCI_MASLD patients, and HC. (B) The post-hoc two-sample *t*-test results of the right amygdala-related FC between MCI_MASLD patients and nonMCI_MASLD patients, (C) between MCI_MASLD patients and HC, (D) between nonMCI_MASLD patients and HC. INS, insula; mPFC, medial prefrontal cortex; L/R, left/right.

Table 3. Results of post hoc two-sample *t*-test comparison of left amygdala-related FC in three groups.

	Anatomical regions	MNI coordinates of Peak voxel			Cluster size	<i>T</i> -value
		x	y	z		
MCI_MASLD vs HC	INS.L	-27	15	-3	76	4.718
	Midbrain	18	-12	-12	13	4.369
	INS.R	30	-12	-3	76	3.929
	PoCG.L	-54	-3	39	11	3.258
nonMCI_MASLD vs HC	INS.R	36	-3	-9	121	5.034
	INS.L	-36	-3	-3	38	4.270
	MTG.L	-45	-51	0	22	4.527
	PoCG.L	-54	-3	45	12	4.255
MCI_MASLD vs nonMCI_MASLD	SMA.L	-24	0	66	21	-4.657

MNI, Montreal Neurological Institute; (GRF-corrected, cluster size >10, voxel-level: $p < 0.005$, cluster-level: $p < 0.05$);

INS, insula; PoCG, postcentral gyrus; MTG, middle temporal gyrus; SMA, supplementary motor area; L/R, left/right.

functional connectivity between the mPFC and the amygdala and decreased white matter connectivity were identified as predictors of impaired decision-making [21]. Cognitive performance in MCI patients was found to be associated with impaired white matter connectivity between mPFC and amygdala, and mediated by functional connectivity between these two regions [22]. Abnormalities in functional connectivity of prefrontal-limbic circuits have been widely reported in a variety of metabolic diseases [23]. The present study found that MCI_MASLD patients had in-

creased functional connectivity between the right amygdala and the mPFC, which was correlated with the MoCA scores and the CAP values. It is well known that CAP value can accurately reflect the degree of liver steatosis, especially for mild to moderate steatosis [24]. Compared with the increase of subcutaneous fat, the accumulation of visceral fat has been proved to be more related to cognitive decline [25]. Therefore, we propose that abnormal connectivity of the prefrontal-limbic circuit is a pivotal neural mechanism of cognitive impairment in MASLD patients, and that the

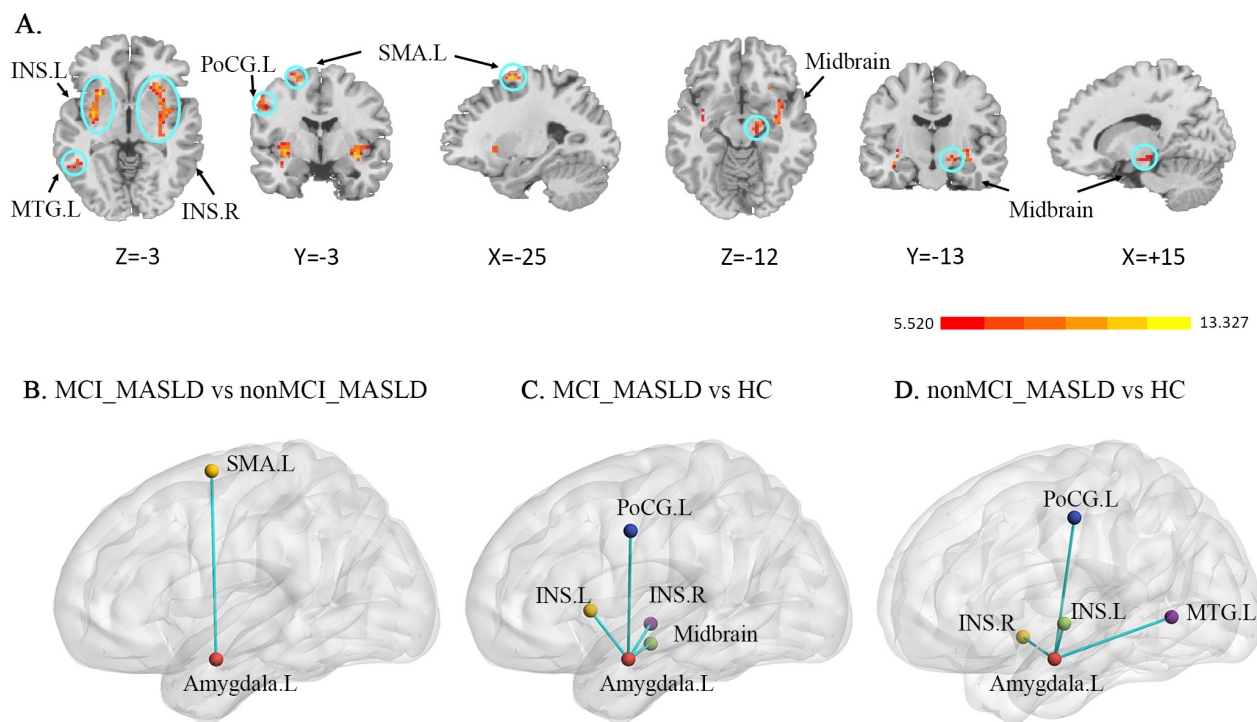


Fig. 3. Brain regions with significant differences in functional connectivity of the left amygdala among groups. (A) The one-way analysis of covariance (ANCOVA) results of the left amygdala-related FC among MCI_MASLD patients, nonMCI_MASLD patients, and HC. (B) The post-hoc two-sample *t*-test results of the left amygdala-related FC between MCI_MASLD patients and nonMCI_MASLD patients, (C) between MCI_MASLD patients and HC, (D) between nonMCI_MASLD patients and HC. INS, insula; PoCG, postcentral gyrus; MTG, middle temporal gyrus; SMA, supplementary motor area; L/R, left/right.

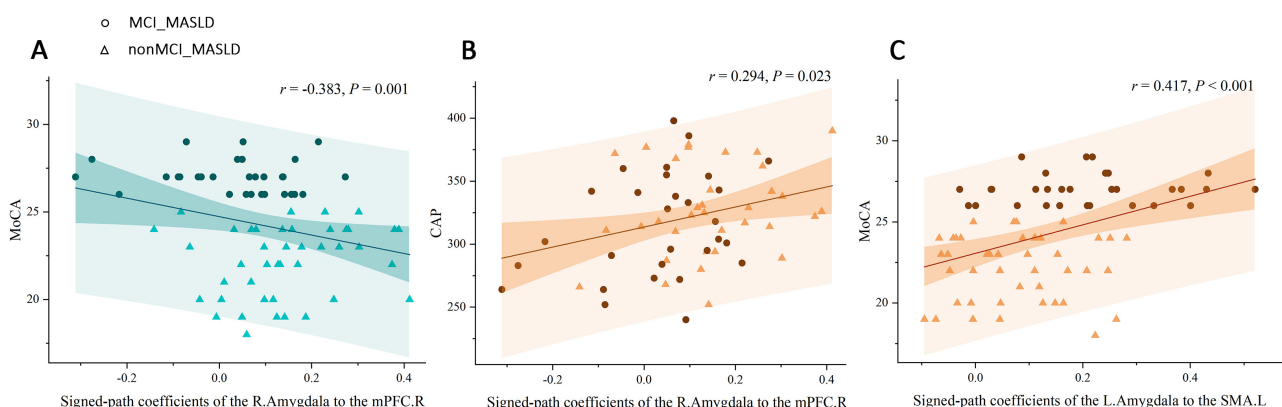


Fig. 4. Scatter plots depicting the correlation between the abnormal FC values of the right/left amygdala and MoCA scores and CAP scores in MASLD patients. (A) Scatter plots depicting the correlation between right amygdala-related FC values in the right mPFC and the MoCA scores and (B) the CAP scores; (C) Between left amygdala-related FC values in the left SMA and the MoCA scores in the MASLD group. Abbreviations: MoCA, Montreal Cognitive Assessment; CAP, controlled attenuation parameter; mPFC, medial prefrontal cortex; SMA, supplementary motor area; FC, functional connectivity; L/R, left/right. Circles represent the MCI groups, triangles represent the non-MCI groups.

accumulation of visceral fat exacerbates the dysfunction of this circuit and thus further contributes to cognitive deterioration.

The SMA and postcentral gyrus are located in the frontoparietal cortex and belong to the sensorimotor network (SMN). In addition to its role in motor preparation and ex-

ecution, the SMN has recently been found to potentially contribute to cognitive function [26]. White matter fiber tracts in the basolateral subregion of the amygdala were found to be connected to motor-related areas (SMA and postcentral gyrus), the SMA is known to be responsible for linking sensory information to appropriate behavioral

choices and cognitive control [27]. Impaired appetite control has been found to be associated with abnormal activity of the SMA and disturbed functional connectivity between the SMA and executive control brain areas [28–30]. The postcentral gyrus, which includes the somatosensory cortex, works in conjunction with the amygdala to integrate somatosensory information and emotional input, and to link the perception of emotional stimuli to action [31]. One study has shown that obese people have increased functional connectivity between the amygdala and sensorimotor areas, which is more pronounced in the female group [32]. The somatosensory cortex is associated with process palatability and metabolism in this region is related to striatal D2 receptor availability, which is considered to be a potential reward mechanism related to sensory information processing and modulation in obese people [33]. In this study, we found that FC between the left amygdala and the left SMA decreased in MCI_MASLD patients compared to nonMCI_MASLD patients and was positively correlated with MoCA score, which may be related to poorer executive control of eating in these patients. In addition, patients with MASLD had enhanced FC between the left amygdala and the postcentral gyrus, which may be associated with impaired integration of sensory information, motor behavior generation and control in patients with this obesity-related metabolic disorder.

The amygdala, insula, and midbrain are regulated by the dopamine (DA) system and receive input from the limbic system and the prefrontal cortex, which functions are closely related to the control of food intake, reward and motivation [34,35]. The insula is a key region of the reward circuit and the salience network (SN), and it plays a crucial role in visceral sensorimotor function and taste processing [36]. Previous studies have proposed that hyperactivity and hyperconnectivity within regions of the SN and reward circuit (including the insula) may explain the neural mechanism of food craving in obese patients [37,38]. As is widely known, visceral obesity has a significant correlation with the pathological characteristics of MASLD [39]. Recent study has found that the accumulation of visceral fat is related to disrupted FC of the insula and mediates the relationship between abnormal insula connectivity and food craving [40]. Therefore, we believe that the enhanced FC between the amygdala and the insula demonstrated in this study may be related to the accumulation of visceral fat in MASLD patients, affecting insula coding related to body homeostasis signals and thus potentially increasing externally driven food craving. In particular, dysfunctional connectivity between the amygdala and midbrain has also been found in patients with MCI_MASLD, which may be related to disturbances in the neural mechanisms of cognitive decline involving the dopamine pathway [41]. Abnormal spontaneous activity in the midbrain is considered to be a prospective indicator for identifying MCI comorbidities of psychiatric disorders [42], and the midbrain may also be a key pathological brain region for metabolic disease-

associated MCI. The medial cortex of the temporal lobe is involved in semantic cognition, contextual integration and updating [43]. We suggest that enhancement of left amygdala related-FC in the left medial temporal gyrus in non-MCI_MASLD patients may be a compensatory mechanism to maintain cognitive behavior [44].

This study presents several limitations. Firstly, the sample size of this research is comparatively small. Secondly, hepatic steatosis was not confirmed by biopsy in the vast majority of MASLD patients in our study. Non-invasive imaging measures (liver MRI-PDFF and CAP values) were used in this study, which are generally thought to be highly correlated with liver biopsy results. Thirdly, although patients with diagnosed conditions such as diabetes and hypertension are excluded in this study, which provides more compelling evidence for the correlation between liver steatosis and brain dysfunction. However, it diminishes clinical universality. Additionally, the majority of patients are those with mild to moderate hepatic steatosis. In the future, patients with severe hepatic steatosis and those with metabolic syndrome should be included for subgroup comparison. Finally, this is a cross-sectional study, and further longitudinal investigations are required to elucidate the causal relationship between FC changes and cognitive impairment in MASLD.

5. Conclusions

Aberrant FC of the amygdala can provide potential neuroimaging markers for MCI in MASLD, which is associated with amygdala-related connectivity disturbances in areas related to cognition and sensory processing. Moreover, visceral fat accumulation may exacerbate brain dysfunction.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available due to data privacy and intellectual property issues but are available from the corresponding author on reasonable request.

Author Contributions

JL, YHJ and JPS designed the research study. RYT, LQW and YXZ performed the research. XFC, KHX provided help and advice on the analysis of the resting-state fMRI data. YHJ, XFC and JL analyzed the data. YHJ wrote the manuscript. JL revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was carried out in accordance with the guidelines of the Declaration of Helsinki and approved by the ethics committee of Affiliated Hospital of Hangzhou

Normal University (approval number: [2019 (E2) -HS-02]) and all patients or their families/legal guardians signed written informed consent prior to the study.

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Conflict of Interest

The authors declare no conflict of interest.

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