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Spontaneous brain activity in patients with type 2 diabetes: linking serum neuroproteins to cognitive ability

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Abstract

Background Cognitive impairment is a complication of type 2 diabetes with high prevalence and serious harm. This study aims to identify serum and imaging biomarkers of type 2 diabetes with mild cognitive impairment (MCI) and to study whether spontaneous brain activities evaluated by functional MRI mediate the relationships between these serum neuroprotein biomarkers and cognitive ability.

Methods According to Montreal Cognitive Assessment (MoCA) scores, 38 patients with MCI and 32 patients with normal cognition in type 2 diabetes were recruited. Serum neuroproteins were determined with Olink Proteomics. Brain activities were evaluated by functional MRI. Logistic regression were performed to determine the biomarkers of type 2 diabetes with MCI. Mediation analysis were performed to examine the potential causal chain between the neuroproteins and cognitive ability.

Results The levels of Epiregulin (EREG), Abhydrolase domain containing 14B (ABHD14B) and Inositol monophosphatase 1 (IMPA1) decreased, while the level of Cysteine-rich intestinal protein 2 (CRIP2) increased in type 2 diabetic patients with MCI (FDR $p < 0.05$). Combination of EREG and CRIP2, a ROC curve was generated with an AUC of 0.85 and an accuracy of 70.0%, and a sensitivity of 86.7% for diagnosing type 2 diabetes with MCI. Brain activities in bilateral superior parietal gyrus, bilateral postcentral gyrus, left inferior frontal gyrus, right cerebellum 6, left cerebellum crus1 decreased, while increased in right hippocampus and left middle frontal gyrus in type 2 diabetic patients with MCI (FDR $p < 0.05$). The mALFF values in the left cerebellum crus-1 mediated 29.1% of the association between the EREG and MoCA scores ($p < 0.001$). The mALFF values in the right cerebellum-6, left cerebellum crus-1,

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right hippocampus, right superior parietal gyrus and right postcentral gyrus all significantly and partially mediated the association between ABHD14B levels and MoCA scores, with mediating effects of 21.7%, 27.1%, 25.8%, 20.7% and 19.2%, respectively (all p values < 0.001).

Conclusions Type 2 diabetic patients with MCI exhibit specific serum neuroprotein and functional MRI characteristics. The relationships between these neuroproteins and cognitive ability were mediated by spontaneous brain activities.

Keywords Type 2 diabetes mellitus, Cognitive dysfunction, Functional magnetic resonance imaging, Proteomics

Introduction

Both type 2 diabetes mellitus (T2DM) and dementia are age-related diseases with high prevalence [1, 2]. T2DM is one of the strong risk factors of Alzheimer's disease and related dementia [3]. The main manifestations of T2DM-related dementia are defects in learning, judgment, memory, information processing speed and execution ability [4]. It causes worse diabetes self-management, poorer blood sugar control, and more frequent hypoglycemia attacks, which leads to an increase in the risk of cardiovascular events and death. There are many commonalities in the pathophysiology of T2DM and AD, such as insulin resistance and altered insulin signaling, neuroinflammation, cerebral microvascular injury, and buildup of cerebral amyloid and tau proteins [5]. Although insulin resistance is considered as the core of AD, the effects of insulin sensitizer metformin on cognitive function are inconsistent [6]. Recently, efforts to treat AD with amyloidosis-targeted drugs have failed [7]. Simple hypoglycemic therapy can not effectively alleviate the progress of cognitive impairment, and there is a shortage of effective drugs to treat cognitive impairment of T2DM. Therefore, early and accurate diagnosis of T2DM-related cognitive impairment is vital for timely intervention and prevention of dementia.

Mild cognitive impairment (MCI) is a transitional stage from age-related cognitive decline to dementia [8]. MCI seems to begin as early as the insulin resistance stage of prediabetes, and diabetes accelerates the conversion rates of MCI to dementia [9]. Therefore, identifying biomarkers of T2DM with MCI (T2DM-MCI) could improve early diagnosis. Recent studies have found some biomarkers of AD [10]. However, very few studies have focused on specific biomarkers of cognitive impairment in T2DM. Olink proximity extension analysis (PEA), which is based on quantitative PCR amplification of DNA-labeled antibodies and paired hybridization DNA markers, can simultaneously measure a variety of customized proteins with high sensitivity and specificity [11]. It has been applied to identifying protein biomarkers associated with neurodegenerative diseases in recent years [12–15]. However, studies on T2DM-related cognitive impairment using this technology are still scarce.

In addition to blood biomarkers, imaging biomarkers play a crucial role in the early diagnosis of T2DM-MCI. While conventional MRI can detect structural abnormalities in T2DM patients, it cannot assess neural activity in the brain. Resting-state functional magnetic resonance imaging (rs-fMRI), however, serves as a powerful tool for evaluating spontaneous brain activity by utilizing the blood oxygen level–dependent (BOLD) contrast mechanism, which reflects the ratio of oxyhemoglobin to deoxyhemoglobin [16]. One key metric, the amplitude of low-frequency fluctuations (ALFF), provides insights into the intensity of spontaneous neural activity across different brain regions [17]. Accumulating evidence indicates that T2DM patients exhibit widespread ALFF alterations in multiple brain areas associated with cognitive function. Nevertheless, the relationship between these functional changes and blood biomarkers in T2DM-MCI remains unclear.

Recent studies did explore a number of candidate biomarkers related to cognitive function in type 2 diabetes. However, there is a lack of biomarkers specifically associated with spontaneous brain activity in T2DM-associated cognitive impairment. This study aims to assess cognitive impairment in T2DM by integrating PEA technology and ALFF analysis to provide a new perspective for screening novel candidate biomarkers for cognitive function in type 2 diabetes. Using PEA technology, we analyzed a comprehensive panel of neuroproteins to identify potential peripheral biomarkers for distinguishing MCI in T2DM patients. Additionally, spontaneous brain activity was also assessed with ALFF. Mediation analyses were conducted to investigate whether associations between serum neuroprotein levels and cognitive performance were mediated by spontaneous brain activity. The findings of this study may contribute to the early diagnosis of cognitive impairment in T2DM patients and offer insights into its underlying mechanisms.

Methods

Participants

The discovery and validation samples were all enrolled from inpatients of the Endocrinology Department of Jinling Hospital between January 2021 and December 2022. The Discovery Cohort was designed to discover

differentially expressed proteins using Olink Proteomics. The Validation Cohort was used to verify discovered proteins via ELISA. The inclusion criteria for patients were as follows: (1) met the diagnostic criteria for T2DM published by the World Health Organization in 1999, (2) aged between 30 and 70 years, (3) had a diabetes duration > 1 year, (4) were right-handed, and (5) were native Chinese Mandarin speakers. The exclusion criteria were (1) less than 6 years of education; (2) a history of severe hypoglycemia, alcoholism, drug abuse, brain trauma, or neural and mental diseases that could result in cognitive impairment; (3) severe visual or hearing loss; and (4) MR contraindications.

This study was examined and approved by the Medical Research Ethics Committee of Jinling Hospital (2020NZKY-025-02). Written informed consent was provided by each participant.

Sample size calculation

The sample size was not calculated in the discovery cohort. In the validation cohort, the sample size was calculated by Gpower 3.1 with the following parameters: two-sided test,α = 0.05, power = 0.8, and effect size = 0.91. The standardized effect size (*d*) is computed as: $d = \frac{|\mu_1 - \mu_2|}{\sigma}$. Based on our preliminary experiment, the effect sizes and required sample sizes were calculated for two biomarkers. For EREG, comparing T2DM-MCI (mean ± SD: 33.87 ± 16.74 pg/ml) and T2DM-nMCI (64.14 ± 27.95 pg/ml) groups, the effect size was 1.08, requiring 15 participants per group. For CRIP2, with T2DM-MCI (1103.00 ± 448.38 pg/ml) versus T2DM-nMCI (695.74 ± 323.49 pg/ml), the effect size was 0.91, necessitating 21 participants per group. The calculations used the larger standard deviation of each paired comparison. We estimated a 30% dropout rate (non-response rate or missing data rate) and increased the sample size to 30 per group accordingly.

Cognitive ability assessment

Cognitive ability was assessed using the Montreal Cognitive Assessment (MoCA) test. According to the MoCA score, participants were divided into T2DM-MCI (score ≤ 26) and T2DM-nMCI (score > 26) groups.

Clinical and biochemical data collection

Demographic data, physical examination information and medical history were collected by two trained investigators. Hemoglobin A1c (HbA1c) was measured by high-performance liquid chromatography (HLC-723G8 Automatic Glycosylated Hemoglobin Analyzer, TOSOH, Japan). Biochemical indexes were determined by an automatic biochemical analyzer (Model 7600 Series Automatic Analyzer, Hitachi, Japan). C-peptide concentrations were tested by an Electrochemiluminescent Immunoassay (IMMULITE2000 XPi, Siemens, Germany). Insulin resistance was evaluated by improving Homa formula with fasting C-peptide instead of insulin [18].

Serum neuroprotein detection

Ninety-two neurology-related proteins were detected with PEA using the Neuro Exploratory panel (OLINK Proteomics, Uppsala, Sweden). Detailed information on the process of detection can be found on the website of Olink Proteomics. The raw data were converted to an arbitrary unit, normalized protein expression (NPX). Quality control (QC) was conducted using internal extension and interplate controls. Samples that did not pass QC were excluded from this experiment.

Serum neuroprotein validation

To further verify whether the neuroproteins detected by PEA can be used as biomarkers for diagnosing MCI in T2DM patients, we analyzed the main neuroproteins in the validation cohort by ELISA kits (RayBio).

MRI data acquisition

MRI data were collected on the same day as the cognitive assessment and blood collection. All the data were acquired with a 3.0-T MRI scanner (TIM Trio, Siemens Healthineers, Erlangen, Germany) with a standard 12-channel head coil. Rs-fMRI data were acquired using a single-shot, gradient-recalled echo planar imaging sequence with the following parameters: number of volumes = 250; repetition time (TR) = 2000 ms; echo time (TE) = 40 ms; flip angle = 90°; field of view (FOV) = 240 × 240 mm^2; slice number = 30; image matrix = 64 × 64; voxel size = 3.75 × 3.75 × 4mm^3; and slice thickness = 4 mm. Structural imaging (3D T1-weighted SFPGR sequence) data were collected with the following parameters: number of volumes = 176; TR/TE = 2300/2.98 ms; flip angle = 90°; FOV = 256 × 256 mm^2; acquisition matrix = 256 × 256; and slice thickness = 1 mm. The scan time lasted for 500 s. During MRI, subjects laid supine, fixed their heads with foam pads, plugged their ears with earplugs and closed their eyes but did not fall asleep or think about anything.

Image preprocessing

The toolkit of Data Processing & Analysis for Brain Imaging (DPABI) was used to perform image preprocessing [19]. The specific process was as follows: (1) Conversion of the DICOM data to NIFTI images; (2) removal of the first 10 volumes to avoid signal fluctuations at the beginning; (3) slice timing correction; (4) realign correction; (5) spatial normalization to the Montreal Neurological Institute (MNI) template (resampling voxel

size = 3 × 3 × 3mm^3); (6) spatial smoothing conducted with a full-width Gaussian kernel = 6 × 6 × 6 mm^3; and (7) linear regression performed to eliminate the influence of covariates, including Friston 24 motion, global average signal, white matter signal and cerebrospinal fluid signal.

ALFF analysis

The ALFF was calculated based on preprocessed images using the modules in the DPABI toolkit after filtering (0.01–0.08 Hz). For standardization purposes, the mean ALFF (mALFF) was used to reduce the errors caused by individual differences. To exclude the possible effect of structural differences among groups, total intracranial volume (TIV), grey matter volume (GMV) and white matter volume (WMV) were calculated via voxel-based morphometry analysis using the Statistical Parametric Mapping (SPM) and the Computational Anatomy Toolbox, version 12 (CAT12) toolbox.

Statistical analysis

Demographic and clinical characteristics analysis

Demographic and clinical data were analyzed using two-sample t tests for continuous variables with normal distribution, mann-whitney u-tests for non-normally distributed data and chi−square tests for categorical variables by R software version 4.3.3 (The R Foundation, Vienna, Austria) [20]. Two-sided p values < 0.05 were considered statistically significant.

Neuroprotein analysis

Since the data satisfied normality and equal variance assumptions, the expression of neuroproteins was analyzed using two-sample t tests with R software version 4.3.3 (The R Foundation, Vienna, Austria) [20]. Post hoc comparisons were adjusted for multiple testing using the Benjamini-Hochberg procedure, controlling the false discovery rate (FDR) at 0.05.

ALFF analysis

Two-sample t tests were performed using Statistical Parametric Mapping (SPM, http://www.fil.ion.ucl.ac.uk/spm) in a voxel wise way to compare the differences in the mALFF between groups after controlling for the covariates of age, sex, and education. Thresholds were set at a p value < 0.005, FDR adjusted p value < 0.05 and a minimum cluster size of 20 mm^3.

Logistic regression and ROC curve analyses

Binary logistic regression with forward selection was applied to determine the associations between serum neuroproteins and T2DM-MCI. The area under the ROC curve (AUC) was calculated to evaluate the diagnostic value of each neuroprotein combination.

Correlation and mediation analysis

Because MoCA score does not conform to normal distribution, spearman's correlation analysis was performed between neuroprotein levels, mALFF values and MoCA scores by R software version 4.3.3 (The R Foundation, Vienna, Austria) [20] with a significance level of two-sided p values < 0.05. Mediation analysis was performed using the PROCESS toolbox [21] (PROCESS v4.1 by Andrew F. Hayes) implemented in SPSS version 20 (Statistical Program for Social Sciences Inc., Chicago, IL, USA). Neuroprotein levels acted as the independent variables, MoCA scores were the dependent variables, and mediators were mALFF values associated with both neuroprotein levels and MoCA scores. Age, sex, and education were controlled as covariates.

Results

Demographic and clinical characteristics

The discovery cohort included 38 T2DM-MCI and 32 T2DM-nMCI participants, and the validation cohort included 30 T2DM-MCI and 30 T2DM-nMCI participants (Fig. 1). Table 1 shows the demographic and clinical characteristics of the two cohorts. There were no significant differences in age, sex, education, BMI, smoking and drinking ratios, HbA1c, duration of diabetes, homeostasis model assessment of insulin resistance (HOMA-IR), blood lipids, hypertension, diabetic nephropathy, diabetic retinopathy, or the use of insulin between groups in the two cohorts.

Differentially expressed neuroproteins between groups

Among the 92 proteins determined through PEA, 22 proteins were excluded from further analyses because more than five samples were below the lower limit of detection. After controlling for FDR, the levels of Epiregulin (EREG), Abhydrolase domain containing 14B (ABHD14B) and Inositol monophosphatase 1 (IMPA1) decreased, while the level of Cysteine-rich intestinal protein 2 (CRIP2) increased in T2DM-MCI patients (adjusted p < 0.05) (Fig. 2).

Neuroproteins in the diagnosis of T2DM-MCI

The above four differentially expressed neuroproteins were included in the multivariate binary logistic regression analysis. Finally, EREG (OR = 0.36, p = 0.002) and CRIP2 (OR = 7.56, p = 0.003) were retained in the model and further validated by ELISA in the validation cohort. Consistent with the PEA test results, the EREG level decreased and the CRIP2 level increased in T2DM-MCI patients (Fig. 3). By combining EREG and CRIP2, an ROC curve was generated with an AUC of 0.85, an accuracy of 70.0%, and a sensitivity of 86.7% (Fig. 4). The cutoff values for EREG and CRIP2 were 70.56 pg/ml and 1028.43 pg/ml, respectively.

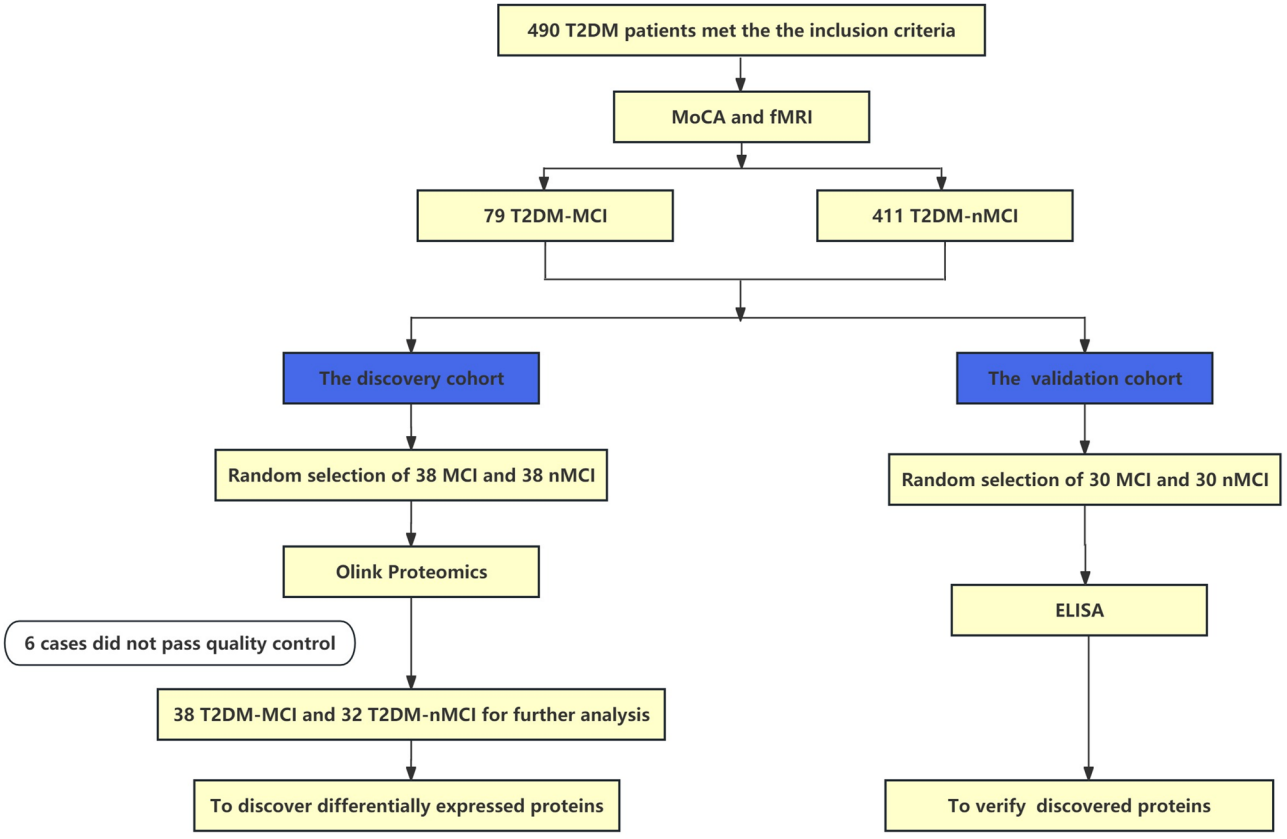

Fig. 1 Flowchart of participants selected

ALFF differences between groups

Compared with those in the T2DM-nMCI group, the mALFF values in the bilateral superior parietal gyrus, bilateral postcentral gyrus, left inferior frontal gyrus, right cerebellum-6 and left cerebellum crus-1 were decreased, while they were increased in the right hippocampus and left middle frontal gyrus in the T2DM-MCI group (Fig. 5; Table 2). Brain structure, such as the TBV, GMV and WMV, was not significantly different between the two groups (Fig. 6).

Correlation and mediation analysis

The correlations between neuroproteins and mALFF values in abnormal brain regions and MoCA scores are shown in Fig. 7. The MoCA score was positively correlated with the EREG, ABHD14B and IMPA1 levels but was negatively correlated with the CRIP2 level (all p values < 0.05). Moreover, positive correlations between MoCA scores and mALFF values were found in the bilateral superior parietal gyrus, bilateral postcentral gyrus, left inferior frontal gyrus, right cerebellum-6 and left cerebellum crus-1 (all p values < 0.05). Negative correlations between MoCA scores and mALFF values were found in the right hippocampus and left middle frontal gyrus (all p values < 0.05). Furthermore, EREG level was positively correlated with the mALFF in the left cerebellar crus 1. ABHD14B level was negatively correlated with the mALFF in right hippocampus and positively correlated with mALFF in right superior parietal gyrus, right postcentral gyrus, right cerebellum 6 and left cerebellum crus 1. CRIP2 was negatively correlated with the mALFF in the right superior parietal gyrus, left postcentral gyrus and left cerebellum crus1. IMPA1 was positively correlated with the mALFF in the right superior parietal gyrus and left postcentral gyrus (all p values < 0.05).

Table 3 shows the mediation effects between neuroproteins and MoCA scores via mALFF values in abnormal brain regions. The mALFF values in the left cerebellum crus-1 mediated 29.1% of the association between the EREG and MoCA scores (p < 0.001). The mALFF values in the right cerebellum-6, left cerebellum crus-1, right hippocampus, right superior parietal gyrus and right postcentral gyrus all significantly and partially mediated the association between ABHD14B levels and MoCA scores, with mediating effects of 21.7%, 27.1%, 25.8%, 20.7% and 19.2%, respectively (all p values < 0.001). No significant mediation effect was observed between the other two neuroprotein levels and the MoCA scores, with abnormal brain activity as a mediator.

Table 1 Information of T2DM-MCI and T2DM-nMCI patients

Characteristics	The discovery cohort			The validation cohort		
	T2DM-MCI (*n* = 38)	T2DM-nMCI (*n* = 32)	*P*- value	T2DM-MCI (*n* = 30)	T2DM-nMCI (*n* = 30)	*P*-value
Age (years)	54.03 ± 7.64	50.47 ± 7.36	0.052	53.30 ± 9.03	49.83 ± 7.50	0.11
Sex (F/M)	15/23	9/23	0.319	10/20	7/23	0.78
Education Primary school	5(13%)	5(16%)	0.181	5(17%)	7(23%)	0.72
Junior high school	17(45%)	6(19%)		14(46%)	10(34%)	
Senior high school	7(18%)	10(31%)		6(20%)	6(20%)	
University or above	9(24%)	11(34%)		5(17%)	7(23%)	
BMI (kg/m2)	25.17 ± 3.34	25.01 ± 3.58	0.855	24.20 ± 3.22	24.87 ± 3.71	0.46
Smoking(%)	8(21%)	10(31%)	0.331	10 (33%)	8 (27%)	0.78
Drinking(%)	7(18%)	6(19%)	0.972	6 (20%)	8 (27%)	0.76
HbA1c(%)	8.36 ± 1.84	9.00 ± 1.50	0.113	8.39 ± 2.17	9.27 ± 1.82	0.10
Diabetes duration(years)	7.13 ± 5.10	7.68 ± 6.07	0.683	5.90 ± 4.42	6.62 ± 5.61	0.58
HOMA-IR	2.87 ± 1.13	2.71 ± 0.99	0.55	2.74 ± 1.08	2.96 ± 1.13	0.43
Hypertension(%)	20(53%)	12(38%)	0.206	20 (67%)	12 (40%)	0.07
Dyslipidemia(%)	10(26%)	8(25%)	0.900	10 (33%)	7 (23%)	0.57
Insulin treatment(%)	16(42%)	11(34%)	0.508	14 (47%)	12 (40%)	0.80
Diabetic nephropathy(%)	14(37%)	6(19%)	0.095	8 (27%)	11 (37%)	0.58
Diabetic retinopathy(%)	13(34%)	5(16%)	0.076	13 (43%)	9 (30%)	0.42
MoCA	21.26 ± 2.83	26.69 ± 1.09	0**	22.67 ± 1.35	26.70 ± 1.18	0**

Abbreviations:

F: Female

M: Male

BMI: Body mass index

HbA1c: Hemoglobin A1c

HOMA-IR: Homeostasis model assessment of insulin resistance

MoCA: Montreal Cognitive Assessment

* p <0.05, ** p<0.01

Discussion

This study represents the first application of PEA technology to screen for serum neuroprotein biomarkers in T2DM-MCI patients. We further examined the relationships between these biomarkers and spontaneous brain activity. Our findings revealed significantly decreased expression of EREG, ABHD14B, and IMPA1, along with increased CRIP2 expression, in T2DM-MCI patients compared to controls. Notably, the combination of EREG and CRIP2 demonstrated moderate diagnostic efficacy in distinguishing T2DM-MCI from T2DM-nMCI patients. Mediation analysis indicated that the associations between these neuroproteins and cognitive performance were partially mediated by mALFF values in specific brain regions. These findings provide valuable insights into potential serum biomarkers and molecular mechanisms underlying cognitive impairment in T2DM.

As a member of the epidermal growth factor family, EREG promotes cell proliferation, differentiation and survival [22]. Emerging evidence has highlighted its significance in the central nervous system. Notably, cortical EREG levels were found to be significantly elevated during cerebral ischemia, with recombinant EREG demonstrating neuroprotective effects against endoplasmic reticulum stress-induced neuronal apoptosis [23]. In our study, the reduction in EREG may be related to a deficiency in endoplasmic reticulum stress and subsequent neuronal cell apoptosis in T2DM-MCI patients.

CRIP2 belongs to the cysteine-rich intestinal protein family, which can inhibit the expression of proangiogenic cytokines mediated by nuclear factor κB and inhibit angiogenesis. CRIP2 has been identified as a heart vascular marker, and an increase in its level reflects the impairment of vascular endothelial function [24]. Consequently, the increase in serum CRIP2 in T2DM-MCI patients may reflect damage to the cerebral vascular endothelium in our study.

Although not included in the regression equation, ABHD14B and IMPAI levels were significantly correlated with MoCA scores in patients with T2DM. ABHD14B functions as a lysine deacetylase that catalyzes the transfer of protein lysine residues to coenzyme A, generating acetyl-CoA and playing a pivotal role in glucose metabolism regulation. Animal experiments have shown that ABHD14B deficiency leads to systemic glucose metabolism deficiency and hyperglycemia [25]. Whether ABHD14B influences cognitive function in T2DM through glucose metabolism regulation requires further

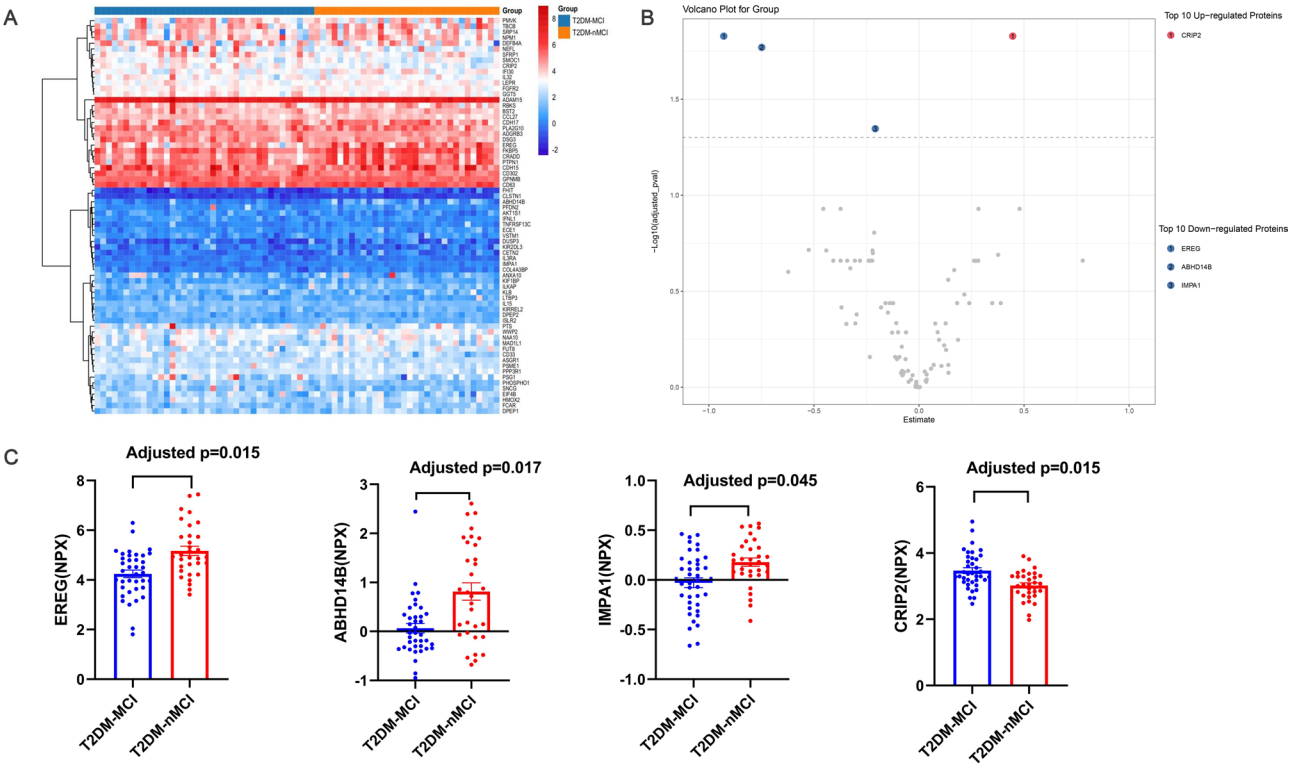

Fig. 2 Differentially expressed neuroproteins. **A**: Heatmap of protein expression; **B**: Volcano map; **C**: Boxplots of differentially expressed neuroproteins

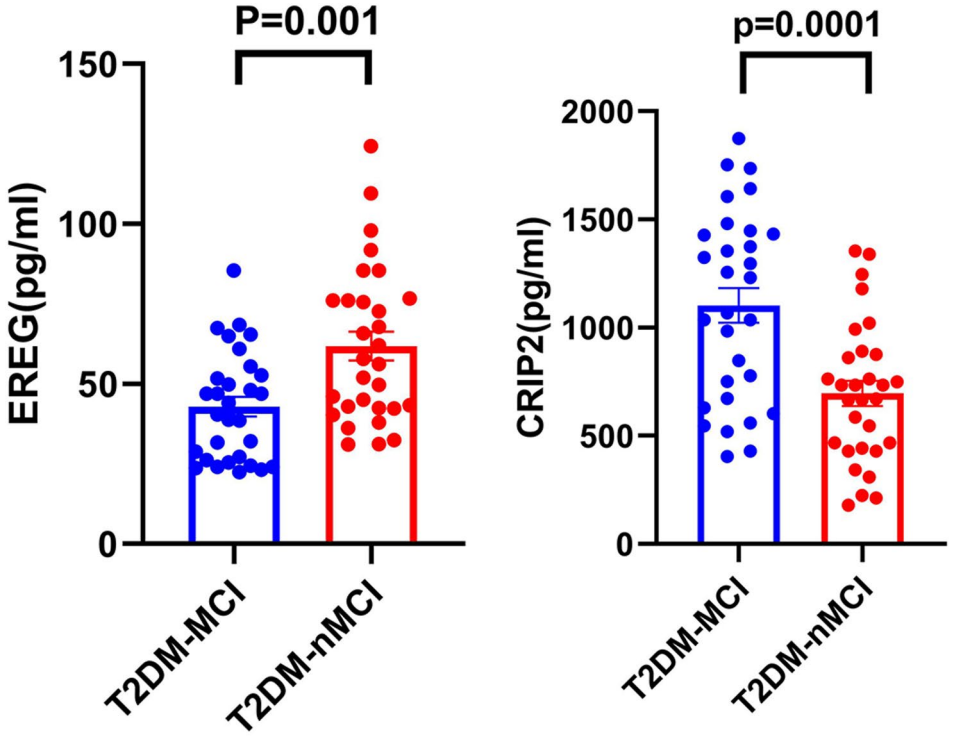

Fig. 3 Expression of EREG and CRIP2 in the validation cohort

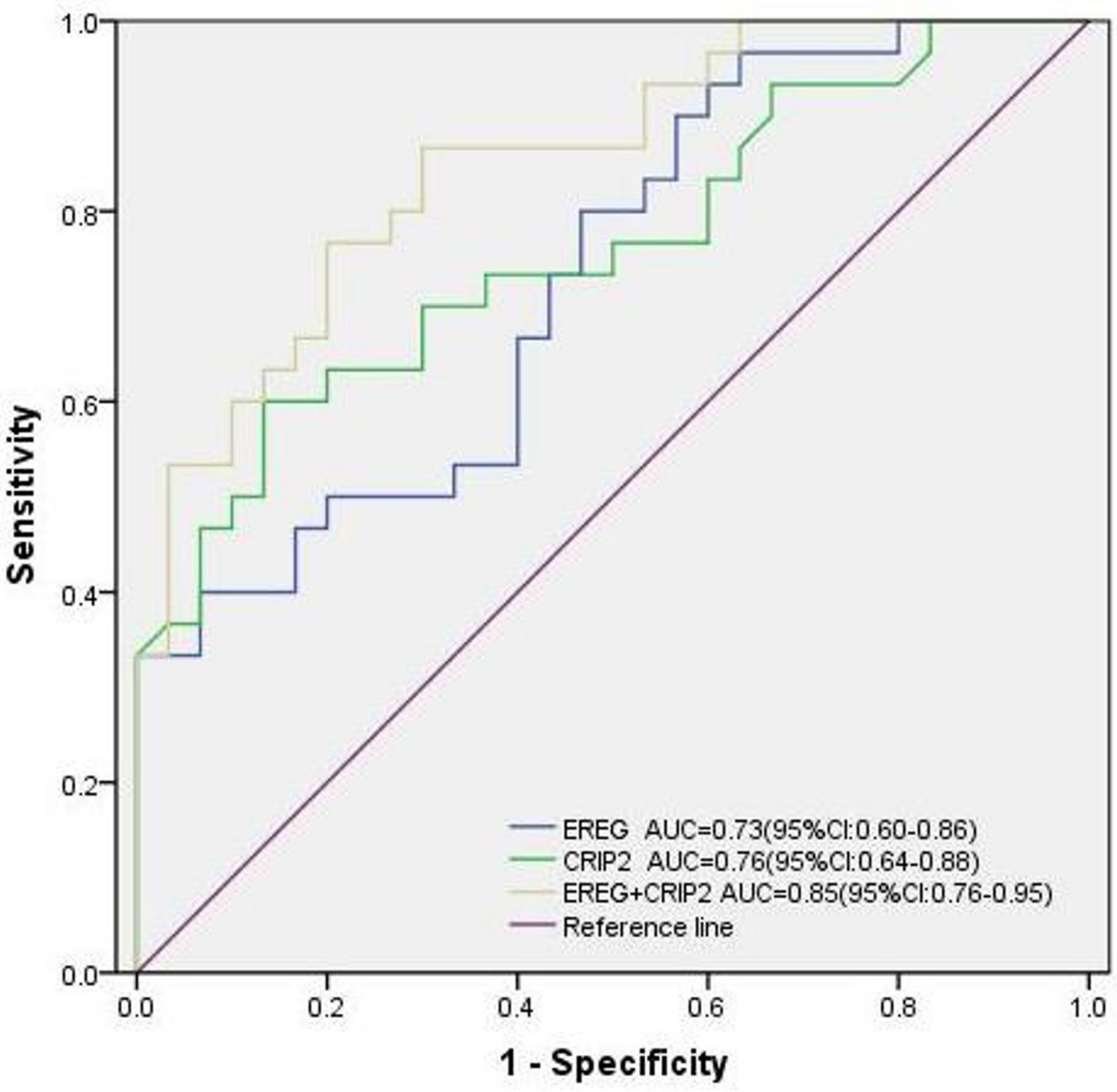

Fig. 4 ROC curve of neuroprotein biomarkers for diagnosing T2DM-MCI

investigation. IMPA1 catalyzes the hydrolysis of inositol phosphate to inositol and is the key enzyme that regulates the first step of inositol biosynthesis. Dysregulated inositol metabolism is relevant to insulin resistance and chronic complications of diabetes [26]. Accumulating evidence highlights IMPA1's critical roles in central nervous system function, including synaptic vesicle exo- and endocytosis, neuronal migration, cell death, and nuclear translocation [27]. These PEA-screened proteins likely represent either core pathological processes or cerebrovascular endothelial damage in T2DM-MCI.

In addition to serum biomarkers, neuroimaging, especially rs-fMRI, is helpful for revealing early brain dysfunction in patients with T2DM. Although studies have shown that T2DM patients exhibit extensive abnormal spontaneous brain activity in brain regions [28], relatively little research has compared spontaneous brain activity between T2DM-MCI patients and T2DM-nMCI patients. The abnormal brain regions identified in this study are partly consistent with those identified in previous studies. Qian et al. [29] reported that compared with T2DM patients with normal cognition, T2DM patients with cognitive impairment had decreased mALFF in the

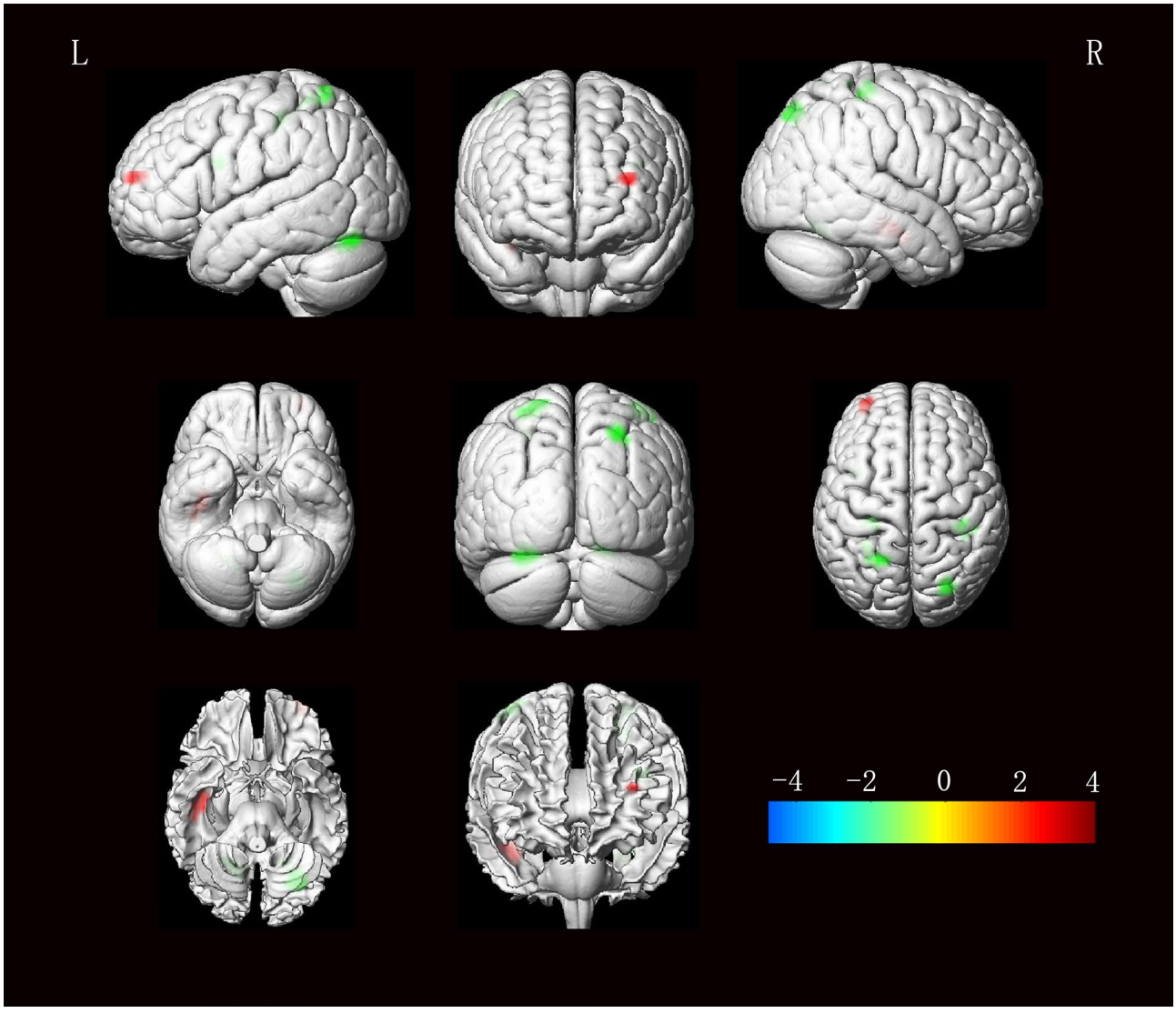

Fig. 5 Brain regions with different mALFF values. Thresholds: *p* value < 0.005, FDR *p* value < 0.05 and a minimum cluster size of 20 mm^3

frontal lobe, parietal lobule, inferior parietal lobule, postcentral gyrus, precuneus, cingulate gyrus and occipital lobe. Differences in comorbidities and parameters among different studies and small sample sizes may explain these inconsistent findings.

In the correlation analysis, we found that the mALFF values in abnormal brain regions were all significantly correlated with MoCA scores. The main function of the parietal gyrus is vigilance and orientation, which plays a guiding role in the process of attention [30]. In this study, the decreased mALFF in the bilateral parietal gyrus can partly explain the mechanism of decreased orientation and attention in patients with T2DM. The inferior frontal gyrus is related to language and semantic processing. Similarly, Xue et al. [31] also reported a negative correlation between the inferior frontal gyrus and cognition, including executive and memory function in MCI patients. In addition, recent evidence has shown that the cerebellum plays a key role in cognitive processing [32]. The cerebellum was found to exhibit abnormal spontaneous neural activity in T2DM [33], which was also confirmed in the present study.

However, we found that the mALFF values in two brain regions increased in the T2DM-MCI group. The hippocampus is one of the main components of the limbic system and is closely related to advanced cognitive function. Dickerson et al. [34] found that MCI patients showed greater hippocampal activation, but AD patients showed inactivation of the hippocampus. The middle frontal gyrus is located mainly in the lateral prefrontal cortex and plays a vital role in executive function and selective attention [35]. Similarly, a previous study also found amnestic MCI patients showed increased mALFF values in left middle frontal gyrus [36]. The enhanced mALFF

Table 2 Brain regions showing significant differences on mALFF between groups

Brain regions	Peak MNI Coordinates			voxels	T-values	*P*-value
	x	y	z			
Hippocampus.R	42	-18	-18	57	3.6254	<0.005
Frontal.Mid.L	-33	57	18	36	3.9635	<0.005
Frontal.Inf.Oper.L	-36	9	27	21	-3.5828	<0.005
Postcentral.L	-27	-30	51	32	-3.8297	<0.005
Parietal.Sup.R	27	-78	54	51	-4.1637	<0.005
Parietal.Sup.L	-21	-57	69	59	-4.1423	<0.005
Postcentral.R	39	-30	63	35	-3.1619	<0.005
Cerebelum.6.R	21	-57	-18	49	-3.2574	<0.005
Cerebelum.Crus1.L	-33	-75	-21	103	-4.7386	<0.005

Abbreviations:

Hippocampus.R: right hippocampus

Frontal.Mid.L: left middle frontal gyrus

Frontal.Inf.Oper.L: left inferior frontal gyrus

Postcentral.L: left postcentral gyrus

Postcentral.R: right postcentral gyrus

Parietal.Sup.R: right superior parietal gyrus

Parietal.Sup.L: left superior parietal gyrus

Cerebellum.6.R: right cerebellum 6

Cerebellum.Crus1.L: left cerebellum crus1

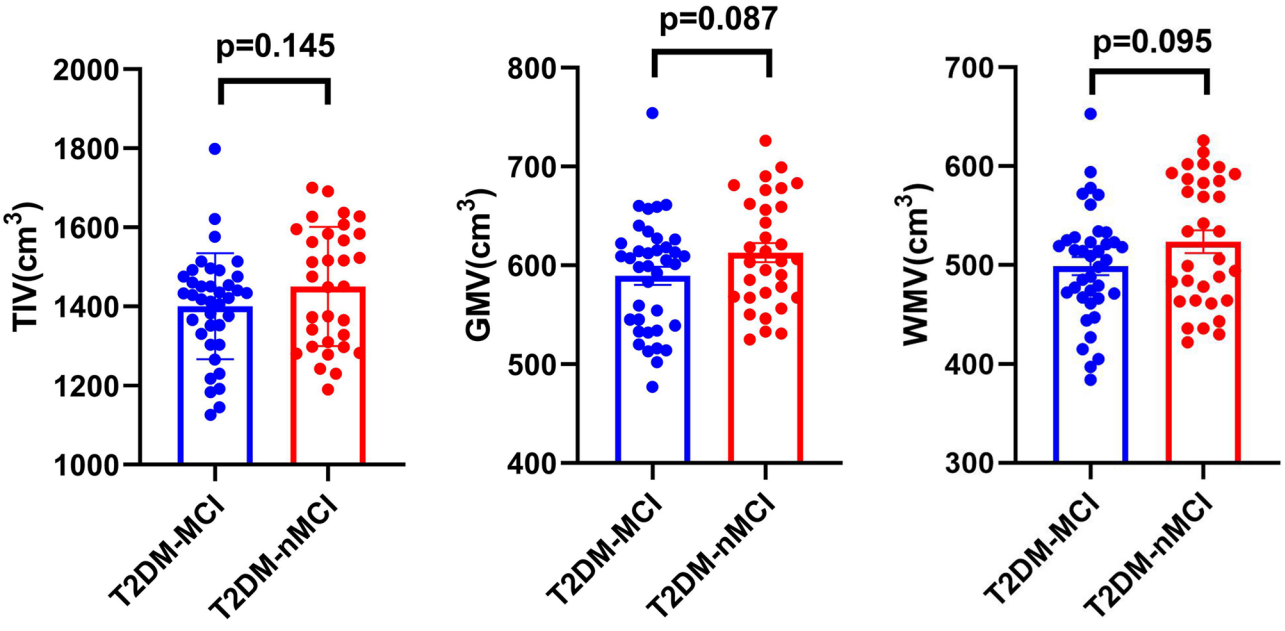

Fig. 6 Brain structure of T2DM-MCI and T2DM-nMCI patients. Abbreviations: TIV: total intracranial volume; GMV: gray matter volume; WMV: white matter volume

values of the right hippocampus and left middle frontal gyrus may be interpreted as an adaptive compensation for cognitive impairment in T2DM-MCI patients.

Given that cognitive behavior fundamentally arises from brain activity, we hypothesize that circulating neuroproteins may influence cognitive function through modulation of neural activity. Mediation analysis is beneficial for determining causal mechanisms or effect pathways. With mALFF values in abnormal brain regions as mediators, there were partial mediating effects on the associations between serum EREG /ABHD14B levels and MoCA scores after adjusting for potential confounding factors, indicating that these neuroproteins may affect cognitive ability by regulating spontaneous brain activities to a certain degree. However, further experimental studies are required to establish causality and elucidate the underlying molecular mechanisms.

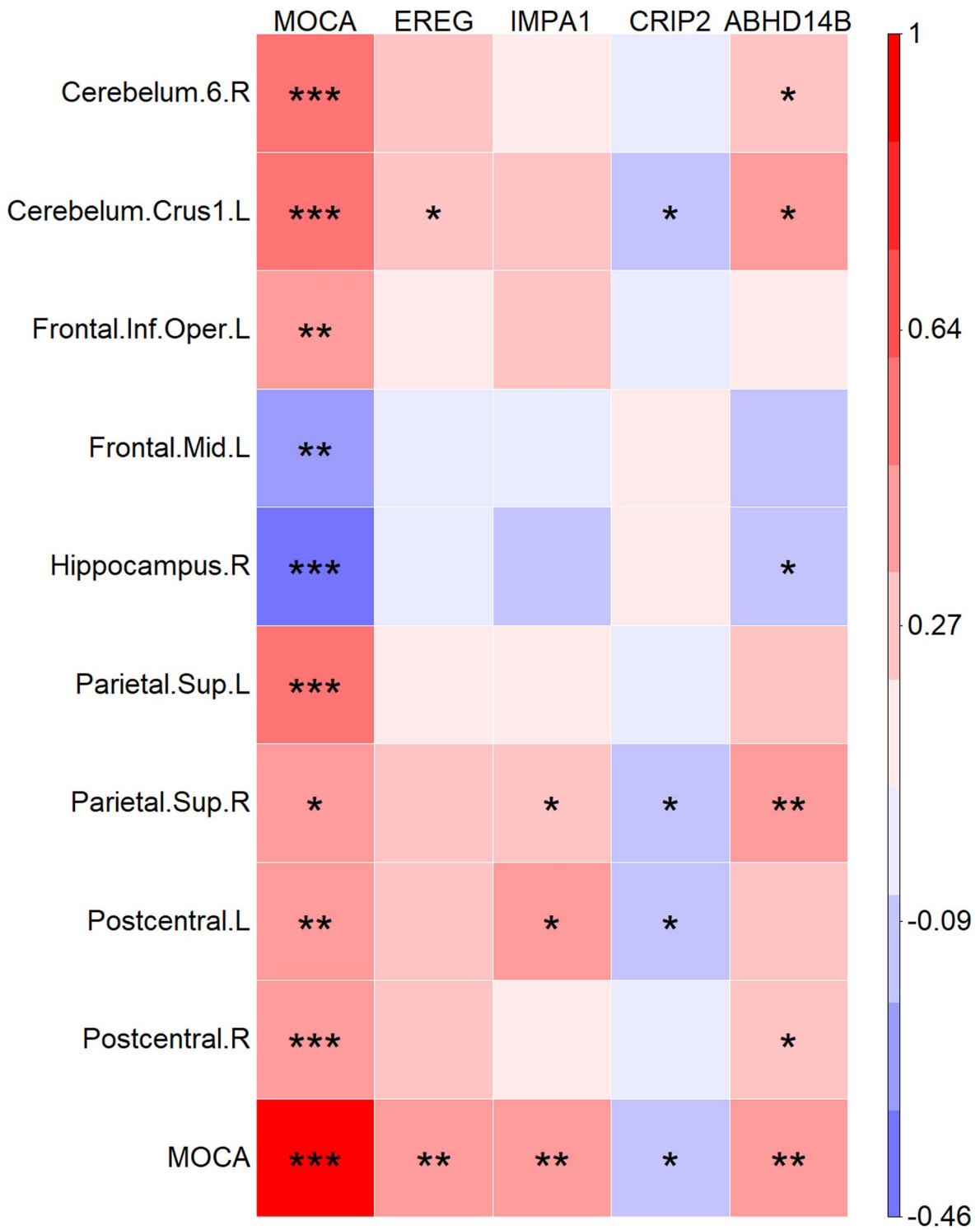

Fig. 7 Heatmaps of Spearman correlations between neuroproteins, MoCA scores and mALFF values in abnormal brain regions. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Limitations

As a preliminary study, this study has several limitations. First, it was a cross-sectional design and could not have sufficient power to determine a causal relationship. Second, the sample size of the discovery cohort was not calculated a priori based on preliminary effect size estimates, which may impact the statistical power of the findings. Third, the neuropsychological tests performed

Table 3 Mediation analysis for the neuroproteins and MoCA scores via the mALFF values in abnormal brain regions

Independent	Mediator	Total effect	Mediation effect	Direct effect	PM%	*P*-value of PM
EREG	Cerebellum.Crus1.L	1.339(0.648–2.030)	0.390(0.035–0.227)	0.949(0.253–1.645)	29.1	<0.001
ABHD14B	Cerebellum.6.R	1.507(0.641–2.373)	0.326(0.006–0.181)	1.181(0.352–2.009)	21.7	<0.001
	Cerebellum.Crus1.L		0.409(0.005–0.221)	1.099(0.260–1.937)	27.1	<0.001
	Hippocampus.R		0.388(0.040–0.181)	1.119(0.268–1.970)	25.8	<0.001
	Parietal.Sup.R		0.311(0.009–0.171)	1.196(0.306–2.085)	20.7	<0.001
	Postcentral.R		0.289(0.004–0.151)	1.218(0.357–2.078)	19.2	<0.001

Abbreviations:

PM: propotion mediate

Adjusting variables: age, sex and education

in this study were not comprehensive enough. Finally, rs-fMRI analyses using SPM in this study might have led to false positive results, which need to be interpreted carefully.

Conclusion

In conclusion, our integrated analysis of serum and imaging biomarkers using PEA and rs-fMRI revealed that spontaneous brain activity mediates the relationship between neuroprotein expression and cognitive function in T2DM-MCI. Future longitudinal studies are warranted to validate the predictive value of these neuroproteins for T2DM-related cognitive impairment. Additionally, it is necessary to explore the mechanism of these associations through animal experiments.

Abbreviations

T2DM	Type 2 diabetes mellitus
AD	Alzheimer's disease
MCI	Mild cognitive impairment
PEA	Proximity extension analysis
rs-fMRI	Resting-state functional MRI
ALFF	Amplitude of low-frequency fluctuations
MoCA	Montreal Cognitive Assessment
HbA1c	Hemoglobin A1c
NPX	Normalized protein expression
QC	Quality control
TR	Repetition time
TE	Echo time
FOV	Field of view
MNI	Montreal Neurological Institute
DPABI	Data Processing & Analysis for Brain Imaging
TIV	Total intracranial volume
GMV	Gray matter volume
WMV	White matter volume
CAT12	Computational Anatomy Toolbox, version 12
FDR	False discovery rate
AUC	Area under the ROC curve
HOMA-IR	Homeostasis model assessment of insulin resistance
EREG	Epiregulin
ABHD14B	Abhydrolase domain containing 14B
IMPA1	Inositol monophosphatase 1
CRIP2	Cysteine-rich intestinal protein 2

Author contributions

Zhen Zhang: Writing original draft, data curation and formal analysis. Wei Wang: Investigation and methodology. Xinyi Yang: Review and editing. Yuting Yang: Collecting patient information. Bingjie Yang: Statistical analysis. Yunchuan Ding: Review and editing. Yunxiao Zhang: Collecting patient information. Xiang Lin: Project administration. Ping Gu: Conceptualization and supervision. Fangping Li: Financial and technical support. Jiaqing Shao: Financial and technical support; Review and editing.

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Data availability

The data generated during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was examined and approved by the Medical Research Ethics Committee of Jinling Hospital (2020NZKY-025-02).

Competing interests

The authors declare no competing interests.

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