Altered baseline brain activity in type 2 diabetes: A resting-state fMRI study

Wenqing Xia a,1,*, Shaohua Wang a,1,*, Zilin Sun a, Feng Bai b, Yi Zhou c, Yue Yang c, Pin Wang c, Yan Huang c, Yang Yuan a

a Department of Endocrinology, Affiliated Zhongda Hospital of Southeast University, No. 87 Dingjiaqiao Road, Nanjing 210009, PR China
b Department of Neurology, Affiliated Zhongda Hospital of Southeast University, No. 87 Dingjiaqiao Road, Nanjing 210009, PR China
c Medical School of Southeast University, Nanjing, China

Received 19 February 2013; received in revised form 6 May 2013; accepted 20 May 2013

KEYWORDS
TZDM; Cognitive impairment; Amplitude of low-frequency fluctuations; Resting-state fMRI

Summary
Purpose: This study aims to investigate whether altered baseline brain activity exists in type 2 diabetes mellitus (TZDM) patients using resting-state functional magnetic resonance imaging (rs-fMRI) and whether abnormal neural activity in the middle temporal gyrus (MTG) is correlated with cognitive function.
Methods: TZDM patients (n = 28) were compared with nondiabetic age-, sex-, and education-matched control subjects (n = 29) using rs-fMRI. We computed the amplitude of low-frequency fluctuations (ALFF) of fMRI signals to measure spontaneous neuronal activity and detect the relationship between rs-fMRI information and clinical data.
Results: Compared with healthy controls, TZDM patients had significantly decreased ALFF values in the bilateral middle temporal gyrus, left fusiform gyrus, left middle occipital gyrus, right inferior occipital gyrus; and increased ALFF values in both the bilateral cerebellum posterior lobe and right cerebellum culmen. Moreover, we found an inverse correlation between the ALFF values in the MTG and both the HbA1c (r = −0.451, p = 0.016) and the score of Trail Making Test-B (r = −0.420, p = 0.026) in the patient group. On the other hand, C-peptide level and pancreatic β-cell function had a positive correlation (r = 0.429, p = 0.023; r = 0.453, p = 0.016, respectively) with the ALFF value in the middle temporal gyrus.
Conclusion: The present study confirms that TZDM patients have altered ALFF in many brain regions, which is associated with poor neurocognitive performances, severity of consistent hyperglycemic state and impaired β-cell function. ALFF disturbance in MTG may play a central role in cognitive decline associated with TZDM and serve as reference for future clinical diagnosis.

© 2013 Elsevier Ltd. All rights reserved.

* Corresponding authors. Tel.: +86 25 83272261; fax: +86 25 83285132.
E-mail address: gyjwsh@gmail.com (S. Wang).
1 Both authors contributed equally to this work.
1. Introduction

Diabetes mellitus is associated with a 1.5–2-fold increased risk of Alzheimer’s disease (AD) and a 2–2.5-fold increased risk of vascular dementia (Biessels et al., 2008). Type 2 diabetes mellitus (T2DM) is related to decrements in cognition, particularly learning and memory deficits (McCrimmon et al., 2012). Accumulating studies supported the fact that T2DM and AD share several same pathogenesis in the brain (Biessels et al., 2008; Vagelatos and Eslíck, 2013), such as insulin deficits, glucose-mediated toxicity and Aβ accumulation. However, T2DM-related cognitive impairment is not comparable to the cognitive impairment found in mild cognitive impairment (MCI) or AD patients. For instance, recent longitudinal studies showed moderate decrements in cognitive function in the information-processing speed, attention and executive functioning domains, compared with individuals without diabetes (Van Den Berg et al., 2010; Spauwen et al., 2012). Particularly, to date, the hippocampus is largely proven to be specifically affected by T2DM (Gold et al., 2007; Bruehl et al., 2009), both structurally and functionally. Medial temporal lobe atrophy (MTA) is likewise observed in the brains of T2DM patients by brain magnetic resonance imaging (MRI) studies (Den Heijer et al., 2003; van Harten et al., 2006, 2007). However, the exact neuropathophysiological mechanism of cognitive impairment related with T2DM has not yet been fully elucidated.

In recent years, functional neuroimaging, especially resting-state functional MRI (rs-fMRI), with high security, spatial resolution and easy application (He et al., 2007), has become a novel and widely used technique to investigate the pathogenesis of various neuropsychiatric disorder diseases. Musen and her colleagues showed reduced functional connectivity in the default mode network (DMN) of T2DM patients compared with control subjects, which indicated abnormal connectivity among several brain areas (Musen et al., 2012). DMN, consisting of several brain areas, such as the middle temporal gyrus (MTG), the posterior cingulate cortex (PCC), the anterior cingulate cortex (ACC), the middle frontal gyrus (MFG), and the inferior parietal lobe, is active at rest and suspended during cognitive activity (Raichle, 1996). Zhou et al. (2010) observed that the hippocampus displays decreased functional connectivity bilaterally to widespread regions in T2DM patients, which may predict cognitive dysfunction. Meanwhile, several studies focused on the structural changes in the brain of diabetes patients. Chen et al. (2011) demonstrated that T2DM patients showed gray and white matter atrophy in the right temporal lobes using voxel-based morphometry (VBM). Hsu et al. (2012) also found microstructural abnormalities in various white matter pathways using diffusion tensor imaging (DTI) in T2DM patients. Nevertheless, other MRI techniques, such as VBM and DTI, merely observe the atrophy or abnormal white matter changes. Besides, previous studies using rs-fMRI all focus on the abnormal functional connectivity between two remote areas. We still do not know which area is abnormal, and we cannot observe the spontaneous neuronal activity over the entire brain of T2DM patients from such an examination.

The amplitude of low frequency fluctuation (ALFF) of the blood oxygen level dependent (BOLD) signal, one of the rs-fMRI analysis algorithms, was highly coherent among motor cortices during resting state and likely reflects spontaneous neuronal activity (Biswal et al., 1995; Kiviniemi et al., 2000; Fransson, 2005). This technique has also been used as an effective method in evaluating the spontaneous neuronal activity of diseases, such as AD, MCI (Wang et al., 2011), schizophrenia (Hoptman et al., 2010), and hepatic encephalopathy (Chen et al., 2012). However, this method has never been applied in cognitive dysfunction related with diabetes. The ALFF is reported higher in gray matter than in white matter (Biswal et al., 1995) and is associated with intrinsic, regional brain responses in local brain regions. When comparing with functional connectivity analyses, which focus on the changes among different regions, ALFF can be used as an index to evaluate changes in brain function and to measure the amplitude of regional activity and physiological states.

On the basis of the conclusion of previous studies that T2DM increases the risk of cognitive dysfunction, accompanied by MTA and the high sensitivity of the ALFF method, we aim to investigate whether T2DM patients show different ALFF in selected brain areas compared with control subjects and whether ALFF in the MTG is correlated with the performance of some cognitive functions and some clinical characteristics. This investigation might contribute to provide revelations to early diagnosis and prevention of cognitive decline induced by diabetes such that timely and effective treatment can be received.

2. Experimental procedures

2.1. Subjects and study design

The current study was conducted from June 2012 to February 2013. The protocol and informed consent document were approved by the Research Ethics Committee of the Affiliated Zhongda Hospital of Southeast University. All individuals provided written informed consent before their participation in the study protocol.

Sixty subjects (all right handed, educated for at least 6 years), with 30 diabetic patients and 30 age-matched healthy subjects were recruited through community health screening or newspaper advertisements. Two of the patients and one of the healthy control subjects were subsequently excluded because the limits for head motion were exceeded during the imaging processing. All patients met the diagnosis of T2DM according to the World Health Organization 1999 criteria (Alberti and Zimmet, 1998) and did not use insulin secreting drugs, such as sulfonylureas and repaglinide. The patients were aged between 45 and 70 years (average age = 58.7 ± 8.1 years), with disease duration of 3–20 years (mean = 9.8 ± 5.5 years) and BMI index of 19.3–32.1 (mean = 25.4 ± 3.0). The normal controls were recruited during the same period from the community and were matched for sex, age, BMI, and education. The fasting glucose levels and postprandial glucose levels were measured, and individuals with fasting glucose > 6.1 mmol/l or postprandial glucose > 7.8 mmol/l were excluded.

Participants with a past history of known stroke, alcoholism, head injury, Parkinson’s disease, epilepsy, major depression (excluded by self-rating depression scale) or other neurological or psychiatric illness (excluded by clinical assessment and case history), major medical illness (e.g.,
cancer, anemia and thyroid dysfunction), and severe visual or hearing loss were excluded from the study.

2.2. Clinical data and neuropsychological test information

Clinical data were collected, including weight and height, blood pressure, BMI = (weight in kg)/(height in m)$^2$ and waist:hip ratio. Blood samples were obtained twice by venepuncture at 8 A.M. after overnight fasting and 10 A.M. after drinking a 75 g glucose solution to assess the levels of fasting blood glucose, fasting serum C-peptide, HbA1c, triglyceride, total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), postprandial glucose and postprandial C-peptide.

A battery of neuropsychological tests, which consist of Mini Mental State Exam (MMSE), Montreal Cognitive Assessment (MoCA), Auditory Verbal Learning Test (AVLT), Rey-Osterreith Complex Figure Test (CFT), Digit Span Test (DST), Trail Making Test-A and B (TMT-A and TMT-B), Clock Drawing Test (CDT), and Verbal Fluency Test (VFT) were administrated to all subjects to evaluate each individual’s neuropsychological status, including the general cognitive function, the function of episodic memory regarding both verbal and visual information, semantic memory, attention, psychomotor speed, executive function, and visuospatial skills respectively. Approximately 50 min were used to complete all the tests in a fixed order. An experienced neuropsychiatrist facilitated this process, and a single-blind method was used. In other words, the neurologist did not know which group each subject belonged to. In addition, none of the participants displayed audiovisual or motor coordination impairment that would affect the neuropsychological tests.

2.3. Magnetic resonance imaging procedures

All subjects were scanned using a 3.0T MRI scanner (Siemens MAGNETOM Trio) with a birdcage head coil. Subjects lay supine with their head fixed by foam pads and a belt to minimize head motion. Earplugs were used to reduce scanner noise for the subjects. The subjects were instructed to lie quietly with their eyes closed but not to fall asleep, not to think of anything in particular, and to avoid head motion during the functional MR imaging.

Functional images were collected axially by using an echo-planar imaging (EPI) sequence as follows: repetition time (TR) = 2000 ms; echo time (TE) = 25 ms; slices = 36; thickness = 4 mm; gap = 0 mm; field of view (FOV) = 240 mm × 240 mm; acquisition matrix = 64 × 64; and flip angle (FA) = 90°. High-resolution 3D T1-weighted axial images covering the whole brain were acquired using the following parameters: TR = 1900 ms; TE = 2.48 ms; slices = 176; thickness = 1 mm; gap = 0 mm; FA = 90°; acquisition matrix = 256 × 256; FOV = 250 mm × 250 mm. The total process lasted for 12 min and 24 s.

2.4. Image preprocessing

Analyses were conducted with Data Processing Assistant for rs-fMRI (DPARSF) programs (Chao-Gan and Yu-Feng, 2010), which are based on statistical parametric mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm) and rs-fMRI data analysis toolkits (REST, http://www.restfmri.net). The ALFF values were calculated using the RSET software.

Preprocessing was performed as follows. The first 10 volumes were discarded, taking into account factors that signal equilibrium of the initial MR signals and the adaptation of the subjects to the circumstances. Then the remaining 230 consecutive volumes were used for data analysis. Afterward, slice-timing adjustment, realignment for head-motion correction, spatial normalization to the Montreal Neurological Institute (MNI) template (resampling voxel size = 3 mm × 3 mm × 3 mm) and smoothing with an isotropic Gaussian kernel (FWHM = 4 mm), detrend and filtering (0.01–0.08 Hz) were performed in order. Participants with head movement exceeding 2.0 mm of maximum translation in any of the x, y, and z directions or 2.0° of maximum rotation about the three axes were excluded from this study to minimize movement artifacts. ALFF were finally calculated through the procedure described in previous studies (Yu-Feng et al., 2007; Zhang et al., 2010). Briefly, time courses were first converted to the frequency domain using Fast Fourier Transform. The square root of the power spectrum was computed and then averaged squared across 0.01–0.08 Hz at each voxel. This averaged square root was taken as the ALFF (Yu-Feng et al., 2007). For standardization purpose, the ALFF of each voxel was divided by the global mean ALFF value. The ALFF computations and further analyses were performed within a gray matter mask (Wang et al., 2011).

2.5. Statistical analysis

2.5.1. Demographic and clinical characteristics analysis

Differences in demographic and cognitive data between patients and healthy controls were analyzed using between-group t-test for means and χ²-test for proportions. The analyses were adjusted for age, sex, and education levels. All tests were conducted using a two-sided α-level of 0.05.

Homeostasis model assessment version 2 (HOMA2) was used to assess pancreatic β-cell function (HOMA2-β%) from FPG and fasting C-peptide, which was completed by using the HOMA Calculator version 2.2, available from the Diabetes Trials Unit website (http://www.dtu.ox.ac.uk) (Levy et al., 1998; Wallace et al., 2004).

2.5.2. Within-group ALFF analysis

For within-group whole brain ALFF patterns, one-sample t tests were performed on the individual ALFF maps in a voxel-wise way for the patient and healthy control group. Thresholds were set at a corrected p < 0.05, with multiple comparison correction using the AlphaSim program (http://afni.nih.gov/afni/docpdf/AlphaSim.pdf) determined by Monte Carlo simulation. The parameters were: single voxel p value = 0.05, a minimum cluster size of 85 mm³, FWHM = 4 mm, within a gray matter mask corresponding to the AAL atlas (Forman et al., 1995). Group-level ALFF maps were then visualized with the REST Slice Viewer in the REST software.

2.5.3. Between-groups ALFF analysis

To detect the validation of ALFF that indicates spontaneous brain activity among groups, two-sample t-tests were
conducted. Age, sex and BMI were included as nuisance covariates to control for the possible influences of these three factors on the results. The result was also determined by Monte Carlo simulation (parameters: single voxel $p$ value = 0.05, a minimum cluster size of 83 mm$^3$, FWHM = 4 mm, within a gray matter mask corresponding to the AAL atlas), and $p < 0.05$ was considered statistically significant.

2.5.4. Correlation analysis
To investigate the relationship between ALFF and neurocognitive performances in T2DM patients, the mean ALFF values in the bilateral MTG were extracted. Then, the Pearson’s correlation coefficients between ALFF and the results of each neuropsychiatric test were analyzed by SPSS software (version 17.0), $p < 0.05$ was considered statistically significant. The correlations were also corrected for age and sex. Then Bonferroni corrections were used for multiple comparisons.

3. Results

3.1. Clinical and neuropsychological data

Table 1 reveals the demographic, clinical and neuropsychological tests characteristics of the T2DM patients and the healthy controls. No significant differences were observed between the groups in terms of age, sex, level of education, BMI, blood lipids and blood pressure. Compared with healthy controls, T2DM patients had higher HbA1c, fasting glucose, postprandial glucose but lower HOMA2-% index (all $p < 0.001$). Besides, postprandial C-peptides showed significant decreases ($p = 0.014$) in T2DM patients. Moreover, when compared with healthy controls, T2DM patients performed poorer on CFT, TMT-A, TMT-B and CDT ($p < 0.05$), whereas the other neuropsychological tests showed slight decreases in cognitive performance. However, the differences were not significant ($p > 0.05$).

3.2. ALFF data

A one-sample $t$-test showed that functional patterns was associated with the selected regions, including the ACC, PCC, precuneus, MFG and temporal lobes, which were within DMN. Besides, several other regions, such as cerebellum anterior lobe, superior frontal gyrus, cuneus, inferior parietal lobe, postcentral gyrus, insula lobes can also be observed to have similar changes (Fig. 1).

When using two-sample $t$-test analysis, T2DM patients had significantly decreased ALFF values in the bilateral MTG, left fusiform gyrus, left middle occipital gyrus, and right inferior occipital gyrus; whereas increased ALFF values were observed in both the bilateral cerebellum posterior lobe and the right cerebellum culmen (Fig. 2 and Table 2).

### Table 1: Demographic, clinical, and cognitive characteristics.

<table>
<thead>
<tr>
<th>Items</th>
<th>TZDM patients ($n = 28$)</th>
<th>Healthy controls ($n = 29$)</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age (year)</td>
<td>58.7</td>
<td>8.1</td>
<td>57.7</td>
</tr>
<tr>
<td>Gender (male:female)</td>
<td>15:13</td>
<td></td>
<td>13:16</td>
</tr>
<tr>
<td>Education levels (years)</td>
<td>9.9</td>
<td>3.7</td>
<td>11.0</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>9.8</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>25.4</td>
<td>3.0</td>
<td>24.9</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>132.6</td>
<td>14.0</td>
<td>134.4</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81.0</td>
<td>9.1</td>
<td>84.8</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.9</td>
<td>1.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>7.9</td>
<td>2.0</td>
<td>5.6</td>
</tr>
<tr>
<td>Postprandial glucose (mmol/L)</td>
<td>15.4</td>
<td>5.3</td>
<td>7.1</td>
</tr>
<tr>
<td>Fasting C-peptide (ng/ml)</td>
<td>2.0</td>
<td>1.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Postprandial C-peptide (ng/ml)</td>
<td>6.3</td>
<td>2.5</td>
<td>8.7</td>
</tr>
<tr>
<td>HOMA2-%β</td>
<td>57.9</td>
<td>25.6</td>
<td>99.2</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.6</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.7</td>
<td>0.8</td>
<td>5.5</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.5</td>
<td>0.6</td>
<td>3.4</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.4</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>MoCA</td>
<td>23.2</td>
<td>3.1</td>
<td>24.1</td>
</tr>
<tr>
<td>Auditory verbal learning test (AVLT)</td>
<td>32.5</td>
<td>8.3</td>
<td>36.1</td>
</tr>
<tr>
<td>Rey-Osterrieth Complex Figure Test (CFT)</td>
<td>34.5</td>
<td>2.2</td>
<td>35.7</td>
</tr>
<tr>
<td>Rey-Osterrieth Complex Figure Test (CFT) - delayed recall</td>
<td>13.8</td>
<td>5.6</td>
<td>18.7</td>
</tr>
<tr>
<td>Trail making test-A</td>
<td>74.7</td>
<td>23.4</td>
<td>62.3</td>
</tr>
<tr>
<td>Trail making test-B</td>
<td>194.0</td>
<td>71.8</td>
<td>150.0</td>
</tr>
<tr>
<td>Clock drawing test</td>
<td>3.1</td>
<td>0.6</td>
<td>3.5</td>
</tr>
<tr>
<td>Digit span test</td>
<td>11.1</td>
<td>1.3</td>
<td>12.3</td>
</tr>
<tr>
<td>Verbal fluency test</td>
<td>17.6</td>
<td>3.4</td>
<td>19.4</td>
</tr>
</tbody>
</table>

* $p < 0.05$; HOMA2-%β, homeostasis model assessment for β-cell function.
Figure 1  Changes in whole brain ALFF using one sample t-test in T2DM patients (a) and healthy controls (b). Thresholds were set at a corrected $p < 0.05$, determined by Monte Carlo simulation. Note that the left side corresponds to the right hemisphere.

Figure 2  Validation of ALFF in T2DM group compared with control group. The regions that showed significantly decreased ALFF values were the bilateral MTG, left fusiform gyrus, left middle occipital gyrus, and right inferior occipital gyrus; increased ALFF values were found in both the bilateral cerebellum posterior lobe and the right cerebellum culmen. Thresholds were set at a corrected $p < 0.05$, determined by Monte Carlo simulation. Note that the left side corresponds to the right hemisphere.

Table 2  Regions showing significant differences on ALFF of patients group compared with healthy controls.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>BA</th>
<th>Peak MNI coordinates x, y, z (mm)</th>
<th>Peak t score</th>
<th>Cluster size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(I) Decreased in T2DM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L middle temporal gyrus</td>
<td>21</td>
<td>−54, −63, 9</td>
<td>−4.4338</td>
<td>155</td>
</tr>
<tr>
<td>R middle temporal gyrus</td>
<td>21</td>
<td>39, −72, 12</td>
<td>−3.5097</td>
<td>114</td>
</tr>
<tr>
<td>L fusiform gyrus</td>
<td>37</td>
<td>−48, −54, −24</td>
<td>−4.4577</td>
<td>167</td>
</tr>
<tr>
<td>R inferior occipital gyrus</td>
<td>18</td>
<td>27, −87, −15</td>
<td>−3.8761</td>
<td>85</td>
</tr>
<tr>
<td>L middle occipital gyrus</td>
<td>19</td>
<td>−24, −94, 3</td>
<td>−4.2171</td>
<td>166</td>
</tr>
<tr>
<td><strong>(II) Increased in T2DM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B cerebellum posterior lobe</td>
<td>–</td>
<td>9, −69, −48</td>
<td>3.96964</td>
<td>111</td>
</tr>
<tr>
<td>R cerebellum culmen</td>
<td>–</td>
<td>15, −48, −24</td>
<td>4.2251</td>
<td>606</td>
</tr>
</tbody>
</table>

A corrected threshold of $p < 0.05$ determined by Monte Carlo simulation was taken, as meaning that there was a significant difference between groups. BA: Brodmann’s area; MNI: Montreal Neurological Institute; L: left; R: right; B: bilateral; cluster size is in mm$^3$. 
Figure 3  (a) Correlation between HbA1c and mean ALFF value in bilateral middle temporal gyrus ($r = -0.451$, $p = 0.016$). (b) Correlation between neuropsychological score and mean ALFF value in bilateral middle temporal gyrus ($r = -0.420$, $p = 0.026$). TMT-B, Trail Making Test-B. (c) Correlation between C-peptide level and mean ALFF value in bilateral middle temporal gyrus ($r = 0.429$, $p = 0.023$). (d) Correlation between HOMA-β and mean ALFF value in bilateral middle temporal gyrus ($r = 0.453$, $p = 0.016$).

4. Correlation analysis results

The ALFF value in the bilateral MTG was found to be correlated inversely with both HbA1c ($r = -0.451$, $p = 0.016$) and the score of the TMT-B ($r = -0.420$, $p = 0.026$) among the T2DM patients (Fig. 3a and b). C-peptide level and HOMA2-β had positive correlations ($r = 0.429$, $p = 0.023$; $r = 0.453$, $p = 0.016$, respectively) with the ALFF value in the bilateral MTG (Fig. 3c and d). Table 3 shows the correlations before and after having been corrected for age and sex. However, when multiple comparisons were corrected, no significance was found.

5. Discussion

To the best of our knowledge, the current study was the first to examine resting-state ALFF values to investigate cognitive dysfunction in T2DM. Meanwhile, we also focused on the correlation between the rs-fMRI information and clinical data, suggesting that abnormal alterations in MTG may play a central role in T2DM-related cognitive decline to serve as a guide for clinical diagnosis.

In this study, global cognitive function was evaluated by using MMSE and MoCA. MMSE, including five different cognitive domains, orientation, immediate memory, delayed memory, attention/calculation, and linguistic capacity, is the most widely used test for cognitive function. MoCA was developed as a brief screening instrument for MCI, and AD and was considered more sensitive than MMSE (Whitney et al., 2012; Yu et al., 2012). However, both failed to show significant decrease between patients and healthy controls. As the participants were relatively younger and their disease durations were not long enough, the cognitive decline degree seemed not so severe that it could affect their normal life at this age and disease stage. Nevertheless, several neuropsychological tests, including CFT, TMT-A, TMT-B, and CDT showed significant decreasing trends. These changes reveal that T2DM patients indeed suffer cognitive decline in multidimensional aspects.
We found increased ALFF within DMN in both T2DM patients and healthy controls through one-sample t-tests, which corresponded well with the consensus that DMN is involved in the maintenance of baseline brain activities (Raichle et al., 2001). This study also demonstrated disrupted ALFF in multiple brain regions in T2DM patients, with decreased amplitude distributed over the left fusiform gyrus, left middle occipital gyrus, right inferior occipital gyrus, and especially the bilateral MTG.

We believe that the impaired MTG function in rs-fMRI might be a vital area to identify the existence of T2DM-related cognitive dysfunction. Den Heijer et al. (2003) demonstrated that T2DM was linked with hippocampal atrophy, which indicated MTA, regardless of vascular pathology. Numerous studies have demonstrated that dysfunction of the hippocampus is related to the severity of diabetes (Trudeau et al., 2004; Stranahan et al., 2008). Moreover, diabetes mellitus is associated with MTA, independent of the severity of white matter hyperintensities (Korf et al., 2007). Although there is little previous information concerning ALFF changes in T2DM patients, some scholars have reported decreased functional connectivity between the hippocampus and other regions in the brain (Zhou et al., 2010), which may support our above conclusion. Notably, in the present study, poor neurocognitive performances paralleled with ALFF measures were found in MTG, suggesting that ALFF changes probably reflect neurobehavioral deficits in T2DM patients. TMT-B, which is a commonly used neurocognitive test to define T2DM-related cognitive decline that is regarded as more sensitive than TMT-A, reveals the attention, psychomotor speed, and executive function. The deficits in these aspects are the most prominent characters in T2DM patients. The correlation between the TMT performances in the current research is also supported by previous researches (Bäckman et al., 1997; Zakzanis et al., 2005; Delmaire et al., 2012). Therefore, to some extent, ALFF values in MTG may imply the existence or degree of cognitive dysfunction in T2DM patients.

Remarkably, we also found that ALFF values in MTG have significant correlations with HbA1c, C-peptide levels, and HOMA-β before multiple comparison. HbA1c is a measure of long-term blood glucose control. Poor glucose control leads to hippocampus impairment (Fotuhi et al., 2012) and cognitive decline in T2DM rats and patients (Blaak et al., 2012). Conversely, decreased glucose level is accompanied by improved working memory ability (Ryan et al., 2006). In addition, C-peptide reflects the islet function. We also calculated the HOMA2-%β, which reveals the function of β-cells. The positive correlation between HOMA2-%β and ALFF corresponds to the results above related with C-peptide, increasing their credibility. Impaired β-cell function and lower C-peptide level indicate insulin deficit, which may also be involved in Alzheimer neuropathology in T2DM because insulin deficit has a significant role in developing AD (Rönnemaa et al., 2008). Based on the above results and previous studies, we suggest the interrupted ALFF in MTG as the early marker of the cognitive decline in T2DM. Significance was not observed after the multiple comparison correction, probably partly due to the relatively strict calculation. Nevertheless, we believe that our research is still meaningful in providing direction for future study in this field.

As a region of the visual network, the fusiform gyrus is frequently recruited during attention tasks (Langner et al., 2011) and memory processing (Bokde et al., 2006). Another neuroimaging study also identified a ‘visual word-form area’ (VWFA), showing increased hemodynamic activation to words compared with sensory controls, centered in the left posterior fusiform gyrus (Dehaene and Cohen, 2011). In addition, the left middle occipital gyrus and right inferior occipital gyrus are associated with the original stage of visual processing (Le Bihan et al., 1991). In T2DM patients, we found not only decreased ALFF located at those regions but even worse performance than control subjects in TMT, CFT and CDT, which may be specific indicators of attention, memory, and visual function (Stuss et al., 1987; Bernard et al., 1993; Takahashi et al., 2008) and features of brain dysfunction of T2DM (Elias et al., 1997). These rs-fMRI findings confirm the abnormal changes in T2DM patients, and may underline the mechanisms of neuropathology in T2DM. However, we failed to find any correlations between lower visual cortex ALFF and cognitive performance. This may be explained by the limitations of our research and remain to be confirmed by in-depth studies.

By contrast, two distributed brain regions, including the bilateral cerebellum posterior lobe and right cerebellum culmen showed enhanced ALFF values, indicating the enhancement of neuronal activity in specific regions at rest, which may be interpreted as a recruitment of additional neural resources to compensate for loss of cognitive function in other brain areas. This may be the reason why memory function is only partly damaged in diabetes patients. For example, the cerebellum plays an important role not only in motor function but also in advanced cognitive function (Marklund et al., 2007). The association of the posterior cerebellum with cognitive processing and emotion mediation has been proposed (Schmahmann and Caplan, 2006). Taken together, it seems that the hyperactivity in these regions may be involved in limiting the cognitive disability in the case of diabetes. However, the memory network is based on complicated neural functions involving multiple interconnected cortical and subcortical structures (Perani et al., 1993). Therefore, the details of the compensation mechanism require further research.

The current study has some limitations. First, as a preliminary study, the study had a relatively small patient cohort and was not a longitudinal research. Following up on these patients is of great significance to examine whether the disturbed ALFF values can be a biomarker for cognitive decrement in T2DM-related illness progression. Second, in terms of technique, several recent studies have used multi-modal MRI methods to improve the diagnosis accuracy of AD (Fan et al., 2007; Walhovd et al., 2010). These studies mainly combine MRI, PET, cerebral blood flow (CBF), and cerebrospinal fluid (CSF) techniques. Furthermore, the spatial resolution of fMRI is relatively lower compared with the electroencephalogram (EEG) and the magnetoencephalogram (MEG). Despite that the EEG and MEG are two main methods for assessing the neuronal activity, their activation profiles, which may not always provide a complete picture of the extent of activity, seem to be less sensitive than fMRI. In other words, EEG and MEG are weaker in localizing and showing the level of neuronal activity. Anyhow, a combination of such methods seems more convincing and is recommended for future studies. Third,
although participants were instructed to relax and not think of anything in particular, the resting state cannot be regarded as an absolute static condition. Spontaneous thoughts and random uncontrolled cognitive processing is difficult to avoid completely. Finally, microangiopathy has been reported to be related to cognitive and brain alterations, but we cannot afford the confounding because that given there are no “gold standards” to assess the existence of microangiopathy. Moreover, the risk factors, including ApoE ε4 genotype and aortic macrovascular disease as well as clinical CVD also play roles in developing dementia. The current study does not eliminate these interference factors. Therefore, more studies are required in this field to form more compelling conclusions.

In summary, we used the ALFF approach in the current investigation to localize the brain dysfunction in T2DM and provide additional data in understanding the disturbances in T2DM-related neural networks. This study may lead to a better understanding of the neurological pathophysiology in T2DM patients. Most importantly, results suggest that rs-fMRI may be able to track the very early progression of brain functional alterations associated with T2DM, prior to the onset of abnormal behavior performance. However, more comprehensive research should be performed to confirm these implications.

Role of the funding source
This work was partially supported by the following foundations: the National Nature Science Foundation of China (No. 31070638, Wang SH), the Social Development Project of Jiangsu Province (No. SBE201170735, Wang SH), the National Nature Science Youth Foundation of China (No. 81200635, Yuan Y) and the National Nature Science Foundation of Jiangsu Province (No. SBK201122155, Yuan Y).

Conflict of interest
There are no conflict of interest and commercial relationships with others.

Acknowledgments
We would like to express our heartfelt gratitude to the staff of the Department of Neurology and the Department of Radiology, affiliated Zhongda Hospital of Southeast University, especially Prof. Chuming Xie, Dr. Yuchen Chen and Dr. Ying Cui for their selfless help and valuable assistance. In addition, the authors thank the contribution of Prof. Jie Min and Prof. Hui Jin at School of Public Health of Southeast University. This work was partially supported by the following foundations: the National Nature Science Foundation of China (No. 31070638, Wang SH), the Social Development Project of Jiangsu Province (No. SBE201170735, Wang SH), the National Nature Science Youth Foundation of China (No. 81200635, Yuan Y) and the National Nature Science Foundation of Jiangsu Province (No. SBK201122155, Yuan Y).

References
fMRI reveals the altered baseline brain activity in type 2 diabetes


