REGULAR RESEARCH ARTICLE

Brain Activity of Benzoate, a D-Amino Acid Oxidase Inhibitor, in Patients With Mild Cognitive Impairment in a Randomized, Double-Blind, Placebo Controlled Clinical Trial

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The trial was registered on the ClinicalTrials.gov website (NCTNCT02239003): https://clinicaltrials.gov/ct2/show/NCT02239003

Abstract

Background: Current anti-dementia drugs cannot benefit mild cognitive impairment (MCI). Sodium benzoate (a D-amino acid oxidase [DAO] inhibitor) has been found to improve the cognitive function of patients with early-phase Alzheimer's disease (mild Alzheimer's disease or MCI). However, its effect on brain function remains unknown. This study aimed to evaluate the influence of benzoate on functional magnetic resonance imaging in patients with amnestic MCI.
Methods: This was a 24-week, randomized, double-blind, placebo-controlled trial that enrolled 21 patients with amnestic MCI and allocated them randomly to either of 2 treatment groups: (1) benzoate group (250–1500 mg/d), or (2) placebo group. We assessed the patients' working memory, verbal learning and memory, and resting-state functional magnetic resonance imaging and regional homogeneity (ReHo) maps at baseline and endpoint.
Results: Resting-state ReHo decreased in right orbitofrontal cortex after benzoate treatment but did not change after placebo. Moreover, after benzoate treatment, the change in working memory was positively correlated with the change in ReHo in right precentral gyrus and right middle occipital gyrus; and the change in verbal learning and memory was positively correlated with the change in ReHo in left precuneus. In contrast, after placebo treatment, the change in working memory or in verbal learning and memory was not correlated with the change in ReHo in any brain region.
Conclusion: The current study is the first to our knowledge to demonstrate that a DAO inhibitor, sodium benzoate herein, can alter brain activity as well as cognitive functions in individuals with MCI. The preliminary finding lends supports for DAO inhibition as a novel approach for early dementing processes.

Significance Statement

This is the first study to our knowledge exploring the effect of an N-methyl-D-aspartate receptor (NMDAR) enhancer (sodium benzoate) on brain activity in the individuals with mild cognitive impairment (MCI). The benzoate treatment decreased regional homogeneity (ReHo) in the right orbitofrontal cortex, while placebo treatment did not alter ReHo. These results may indicate the possible brain mechanisms for the treatment of benzoate on MCI patients. The results may contribute to the elucidation of the pathophysiology of MCI and the development of novel therapy to enhance NMDAR.

Introduction

Mild cognitive impairment (MCI) is a slight cognitive impairment that is accompanied by mostly normal function in processes that control the performance of daily activities (Levey et al., 2006). The concept of MCI was developed in an attempt to recognize dementia in its earliest clinically expressed form (Bowen et al., 1997; Boeve, 2012). MCI, particularly amnestic MCI (aMCI), is a risk factor or a prodromal stage of Alzheimer's disease (AD).

Acetylcholinesterase inhibitors (AChEIs) have been developed for treating AD, especially mild-moderate AD (Birks, 2006; Burns et al., 2006). However, AChEIs are not recommended for the treatment of MCI because of the weak beneficial effects (Fellgiebel, 2007). Memantine, an N-methyl-D-aspartate (NMDA) receptor (NMDAR) antagonist, has been used to treat moderate-severe AD based on the "glutamate excitotoxicity theory" (Reisberg et al., 2003; Scarpini et al., 2003) but not for mild AD (Schneider et al., 2011). Moreover, MCI is not effectively treated by memantine either (O'Brien et al., 2011). The poor efficacy of AChEIs and memantine for MCI implies that there should be other mechanism(s) underlying the pathogenesis of MCI.

NMDAR, a subtype of ionotropic glutamate receptor, plays an important role in synaptic plasticity, learning, memory, and cognition (Wu et al., 2007; Amano and Maruyama, 2011). The NMDAR density decreases with age (Segovia et al., 2001). In AD patients, glutamate levels declined in cerebrospinal fluid (Martinez et al., 1993) and brain (Lowe et al., 1990), the number of glutamate terminals decreased in the hippocampus (Cowburn et al., 1988), and D-serine (an NMDAR agonist) levels decreased in the serum (Hashimoto et al., 2004). Therefore, dysfunction in the NMDAR neurotransmission may contribute substantially to the pathophysiology of AD.

The classical way for activation of NMDAR is applying agonists, such as D-serine (Esposito et al., 2012) or D-cycloserine (Pitkanen et al., 1995a, 1995b). However, D-cycloserine has failed in the treatment of patients with AD (Laake and Oeksengaard, 2002). A novel way to activate NMDAR is inhibiting the activity of D-amino acid oxidase (DAO) (Lin et al., 2012), which is responsible for degrading D-serine (Vanoni et al., 1997; Sasabe et al., 2012). DAO levels have been found to play a role in early-phase AD (Lin et al., 2017). Sodium benzoate, a pivotal DAO inhibitor, is generally recognized as safe and widely used as food preservatives in many countries (Joint FAO/WHO Expert Committee on Food Additives, 1973). Sodium benzoate is also used for the treatment of urea cycle enzymopathies, with the therapeutic dose in the range of 250–500 mg/kg body weight (15 000–30 000 mg for a 60-kg patient) per day (Tremblay and Qureshi, 1993; Feillet and Leonard, 1998). In a 24-week, randomized, double-blind, placebo-controlled clinical trial (Lin et al., 2014) on aMCI or mild AD, benzoate significantly improved cognitive functions (including working memory and verbal learning and memory tests). The elderly patients tolerated sodium benzoate 250–1500 mg/d very well.

Resting-state functional magnetic resonance imaging (rfMRI) is a non-invasive way to investigate the spontaneous brain activity that may reflect the brain dynamics in local cortical tissues or large-scale brain networks (Jiang and Zuo, 2016). A recent study suggested that the changes of local functional connectivity (FC) may affect whole-brain dynamics via the change of the local excitation-inhibition ratio (Deco et al., 2014). With the treatment of NMDAR antagonist MK801, increased brain responses to olfactory stimuli have been observed in primates using pharmacological fMRI (Zhao et al., 2018). The increased brain responses may be underpinned by the change of the local excitation-inhibition ratio with blockade of NMDAR on local inhibitory interneurons and then disinhibit principal neurons. Moreover, long-term exposure of ketamine, another NMDAR antagonist, has been associated the altered local FC in human (Liao et al., 2012). Hence, with treatment of sodium benzoate, the local excitation-inhibition ratio may be changed in terms of the changes in local FC. Regional homogeneity (ReHo) is a reliable method to characterized the local FC (Zang et al., 2004). In the present study, we conducted the rfMRI scans and ReHo analysis to investigate the benzoate treatment-associated local FC changes, which may contribute to the improvement of cognitive function in early-phase AD.

Methods

This study enrolled patients with aMCI from the outpatient clinic at the Department of Psychiatry, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung in a 24-week clinical trial. The institutional review board of the hospital approved the study in accordance with the current revision of the Declaration of Helsinki.

The trial was registered on the ClinicalTrials.gov website (NCTNCT02239003): https://clinicaltrials.gov/ct2/show/NCT02239003.

Patients

After a description of the study to the patients, written informed consent was obtained. Patients were evaluated by the research psychiatrist after a thorough medical and neurological workup.

Patients were enrolled in this study if they were aged 50–90; (2) satisfied the criteria for aMCI (McKhann et al., 1984) of a presumably degenerative nature defined as subjective memory complaint corroborated by an informant and insufficient global

cognitive and functional impairment to meet NINCDS-ADRDA criteria, and had a Clinical Dementia Rating (Morris, 1993) score of 0.5; (3) were physically healthy and had all laboratory assessments (including urine/blood routine, biochemical tests, and electrocardiograph) within normal limits; and (4) had sufficient education to communicate effectively and were capable of completing the assessments of the study. For patients who had already been on AChEIs therapy, AChEIs had to be continued for at least 3 months before enrollment and AChEIs doses had to be kept unchanged during the study duration. For patients who had not yet been on AChEI therapy, AChEIs or other anti-dementia medications were forbidden during the study duration.

Exclusion criteria included history of significant cerebrovascular disease; Hachinski Ischemic Score >4; major neurological, psychiatric, or medical conditions other than MCI; substance (including alcohol) abuse or dependence; delusion, hallucination, or delirium symptoms; severe visual or hearing loss; and inability to follow the protocol.

Treatments

Eligible patients continued their originally ongoing psychotropic drugs (if any) throughout the study period and were randomly assigned to either of 2 treatment groups in a double-blind manner: sodium benzoate (250–1500 mg/d) or placebo for 24 weeks. Sodium benzoate (250 mg/capsule) was purchased from Excelsior Biopharma Inc.

To ensure concealment of the randomization assignment, study medication was provided in coded containers with supply of identical-appearing capsules of placebo or benzoate. Patients were randomized through a computer-generated randomization table to receive placebo or benzoate treatment in a 1:1 ratio. Non-blinded pharmacists dispensed appropriate medication for treatment according to the randomization table. Benzoate was initiated at 250–500 mg/d. According to the clinical condition, cognitive function assessment, and the patients' tolerance, the dose was adjusted every 8 weeks in each group. If cognition did not improve, the dose could be titrated by 250–500 mg/d from the ninth week and another 250–500 mg/d from the 17th week of the study. The total dose range was 250–1500 mg/d. The dosing strategy of benzoate was based on the doses in our aforementioned pilot study (Lin et al., 2014), where benzoate was effective and safe after 24 weeks of treatment in the elderly patients.

During the study period, limited use of benzodiazepines (up to 4 mg/d lorazepam or equivalent) was allowed as concomitant medication for anxiety or insomnia. No other centrally acting drugs or cytochrome P450 inducers (or inhibitors) were permitted (Lane and Chang, 1998).

Patients, caregivers, and investigators, except the investigational pharmacist, were all blinded to the assignment. Patient medical adherence and safety were closely monitored by caregivers and research physicians, and pill-counting was monitored by the study staff.

Evaluation of Cognitive Function and Side Effects

Cognitive functions were measured by working memory (Wechsler Memory Scale–Third Edition [WMS-III], Spatial Span) (Wechsler, 1997; Silver et al., 2003) and verbal learning and memory tests (WMS-III, Word Listing) (Wechsler, 1997) at week 0 and week 24, and the Alzheimer's disease assessment scale-cognitive subscale (ADAS-cog) (Rosen et al., 1984) at weeks 0, 8, 16, and 24.

Clinical ratings were performed by a research psychiatrist (C.H.L.), who was trained and experienced in the rating scales.

Side effect assessments were examined every 8 weeks during the drug treatment period by routine physical and neurological examinations and the Udvalg for Kliniske Undersogelser Side-effects Rating Scale (Lingjaerde et al., 1987). Routine laboratory tests, including CBC and biochemistry, were checked at baseline (week 0), week 8, week 16, and endpoint of the drug treatment (week 24).

Methods for Image Study

Image Acquisition

Resting-state fMRI was measured at baseline (week 0) and endpoint (week 24). Images were acquired with an 8-channel head coil in a 3.0 Tesla MRI scanner (Signa Excite, GE). The rfMRI sessions were continuously scanned with ascending echo-planar imaging sequence for whole-brain scanning (repetition time = 2000 ms; echo time = 30 ms; flip angle = 80°; matrix = 64×64; field of view = 240×240 mm^2; slice number = 32; slice thickness = 4 mm) after 10-second blank scans for stabilized the signal. All scans were acquired within a dim-light shielding room. Before scanning, patients were instructed to remain relaxed and awakened but not to move head or focus on any specific matter.

Preprocessing of Resting-State fMRI Data

The preprocessing protocol of rfMRI data was reported elsewhere (Wu et al., 2016). In brief, the rfMRI images (without first 10-second blank scans) were preprocessed using Data Processing Assistant for Resting-State fMRI 4.3 (DPARSF 4.3, State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, China). Images were corrected for different slice acquisition times, realigned to correct the head motions occurring alone the session, normalized into Montreal Neurological Institute reference space with echo-planar image template, and resampled with the voxel size $2 \times 2 \times 2$ mm^3. The time-series activities in each voxel were then linearly detrended, band-pass filtered (0.01–0.08 Hz), and regressed out the confounding variables as 6 head movement parameters, global mean signal, mean signal of white matter, and mean signal of cerebral spinal fluid. The ReHo maps were generated by calculating the Kendall's coefficient of concordance voxel by voxel on preprocessed time-series activities between a given voxel and its nearest neighbors (26 voxels). The ReHo maps were spatially smoothed using a 3D Gaussian kernel of 8 mm full-width at half-maximum and then standardized by divided with its own global mean.

Data Analysis

Chi-square test (or Fisher's exact test) was used to compare differences of categorical variables, and Student 2-sample t test (or Mann-Whitney U test if the distribution was not normal) was used for continuous variables between 2 treatment groups.

The statistical analysis of standardized ReHo maps was conducted by statistical parametric mapping 12 (SPM12, Wellcome center for Human Neuroimaging, University College, London, UK). The 2-sample t test and paired t tests were performed to probe the possible difference between different groups at baseline and different time points (i.e., before and after treatment) in each group, respectively. The correlation analysis was also conducted between change of cognitive measurements and change of ReHo maps in each group. As an exploratory study, a less

Table 1. Demographic and Clinical Characteristics of Patients With aMCI

	Treatment groups		
	Sodium benzoate (n=9)	Placebo (n=12)	P value
Demographics			
Gender, female, n (%)	7 (77.8)	5 (41.7)	.184[a]
Age, year, mean (SD)	66.1 (3.2)	69.2 (3.6)	.058[b]
Age at illness onset, y, mean (SD)	65.4 (3.6)	68.9 (3.6)	.042[b]
Education, y, mean (SD)	5.0 (1.8)	8.8 (2.7)	.003[c]
BMI, mean (SD)	22.7 (5.8)	24.0 (2.4)	.466[b]
No. of patients using anti-dementia drugs			
Total	0	1	1.000[a]
Donepezil (dose, mean ± SD)	0	1 (5.0±0.0)	1.000[a]

Abbreviations: aMCI, amnestic mild cognitive impairment; BMI, body mass index.
[a]Fisher's exact test.
[b]independent t test.
[c]Mann-Whitney U test if the distribution was not normal.

stringent significant threshold (uncorrected $P<.005$ at voxel-level with the cluster size >50, corresponding to the uncorrected cluster level $P<.080$) was applied in the present study to reduce the chance of Type II error (Cremers et al., 2017).

Results

A total of 24 patients with aMCI were enrolled into this study. Among them, 3 patients were excluded (1 alcohol use and poor medical adherence, 1 comorbid with delusional disorder, 1 diagnosed as Parkinson disease). The remaining 21 patients completed the 24-week clinical trial and brain MRI measurement.

The demographic and clinical characteristics are shown in Table 1. Patients in the sodium benzoate group appeared younger ($P=.042$) and had a lower education level ($P=.003$) than patients in the placebo group.

At week 0 (baseline), patients in the benzoate group had a higher ADAS-cog score than the placebo group (15.1 ± 3.9 vs 10.8 ± 3.6, $P=.023$, Mann-Whitney U test). At endpoint, there was no significant difference between the 2 groups in ADAS-cog (9.1 ± 3.5 vs 6.8 ± 2.7, $P=.113$, t test). There was no significant difference in ADAS-cog score change from baseline to endpoint between the 2 groups (6.0 ± 2.2 vs 4.0 ± 4.0, $P=.196$, t test).

ReHo at Baseline and After Treatment

At baseline, the benzoate group showed higher ReHo than in the placebo group in right middle frontal gyrus (recognized as part of orbitofrontal cortex [OFC]) and bilateral medial frontal gyrus (recognized as supplementary motor area) (supplemental Table 1).

After benzoate treatment, decreased ReHo was found in right middle frontal gyrus, while no increased ReHo was found. After placebo treatment, no significant changes of ReHo were found (Figure 1; Table 2).

Correlations Between Cognitive Changes and ReHo Alterations

Regarding the relationship between the change in working memory (assessed by WMS-III, Spatial Span) and the change in ReHo, a positive correlation was found in right precentral gyrus (recognized as primary motor cortex) and right middle occipital
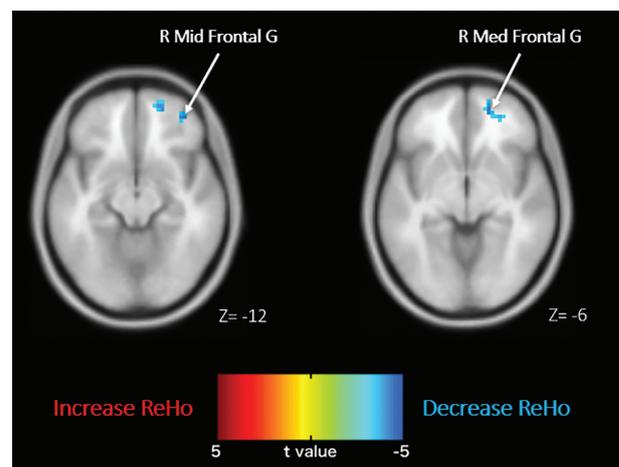
Figure 1. The changes of regional homogeneity after benzoate treatment in schizophrenia patients. After 24 weeks benzoate treatment, decreased regional homogeneity (ReHo) was found in right middle and medial frontal gyrus that belong to right orbitofrontal cortex. The warm and cold colors denote increased and decreased ReHo, respectively. G, gyrus; Med, medial; Mid, middle; R, right.

gyrus (recognized as primary visual cortex), but no negative correlation was detected in any brain region in the benzoate group. There was neither positive nor negative correlation observed in any brain region in the placebo group (Figure 2; Table 3).

Regarding the relationship between the change in verbal learning and memory (measured by WMS-III, Word Listing) and the change in ReHo, a positive correlation was shown in left precuneus, but no negative correlation was revealed in any brain region in the benzoate group. There was neither positive nor negative correlation discovered in any brain region in the placebo group (Figure 2; Table 3).

Safety

Both sodium benzoate and placebo were well-tolerated. The side effect was mild and did not warrant medical treatment. All 21 patients completed the trial without dropout. The routine blood cell count and chemistry were all within the normal ranges and remained unchanged after treatment (data not shown).

Table 2. Changes of ReHo After Treatment With Benzoate or Placebo

Increased ReHo							Decreased ReHo						
				Coordinates (mm)							Coordinates (mm)		
Anatomic area	BA	Size	t Score	x	y	Z	Anatomic area	BA	Size	t Score	x	y	z
Benzoate													
No significant cluster							R Mid Frontal G	11	61	4.46	39	48	−12
							R Med Frontal G	10		4.45	18	54	−6
Placebo													
No significant cluster							No significant cluster						

Abbreviations: BA, Brodmann area; G, gyrus; Med, medial; Mid, middle; R, right; ReHo, regional homogeneity; Size, number of voxels in the cluster.
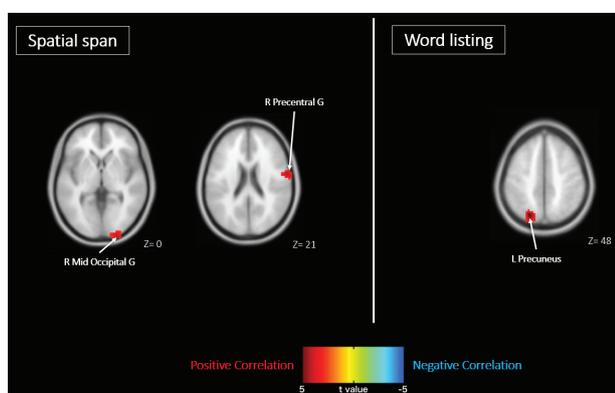
Figure 2. Correlation between the changes of regional homogeneity and the changes of memory tests with benzoate treatment in schizophrenia patients. The changes of regional homogeneity were positively correlated with (left) the changes of working memory in right precentral gyrus and right middle occipital gyrus and with (right) the changes of verbal learning and memory in left precuneus. The warm and cold colors denote positive and negative correlation, respectively. G, gyrus; Mid, middle; R, right.

Discussion

To our knowledge, this is the first study exploring the effect of an NMDAR enhancer on brain activity in individuals with MCI or dementia. The benzoate treatment decreased ReHo in the right OFC (which consists of Brodmann area 10, 11, and 47) (Figure 1), while placebo treatment did not alter ReHo (Table 2). These results may indicate the possible brain mechanisms for the treatment of benzoate on MCI patients.

Anatomically, OFC is interconnected with parahippocampus (Haber and Behrens, 2014). It has been indicated that increased parahippocampal-prefrontal functional connectivity is predictive of impaired episodic memory in aMCI (Zhang et al., 2016). Further, the MCI patients with increased ReHo in the orbital part of the inferior frontal gyrus (which is cytoarchitectonically most closely represented by Brodmann area 47) showed a trend of deteriorating into AD or remaining in MCI rather than reverting to a cognitively intact state (Cai et al., 2018). Accordingly, in the present study, at baseline, the benzoate group also displayed greater ReHo in the right OFC and more cognitive impairment (as shown by the higher ADAS-cog score) than the placebo group.

After treatment, the mean ADAS-cog score decreased by 6.0 ± 2.2 in the benzoate group and 4.0 ± 4.0 in the placebo group; however, the group difference was insignificant in this small-sized study ($P = .196$). Of interest, as aforementioned, after benzoate treatment, decreased ReHo was found in right middle frontal gyrus, while, after placebo treatment, no significant ReHo change was found (Figure 1; Table 2). Therefore, decreased ReHo in OFC with benzoate treatment may be potentially beneficial for the outcome of the cognitive aging process. In accordance, transcranial direct current stimulation, with its potential to enhance NMDAR-related neurotransmission and neuroplasticity (Chang et al., 2018), significantly reduced prefrontal hyperactivity and resulted in "normalization" of abnormal network configuration during fMRI (Meinzer et al., 2015).

Furthermore, after benzoate treatment, the change in nonverbal (spatial) working memory was positively correlated with the change in ReHo in right precentral gyrus and right middle occipital gyrus; and the change in verbal learning and memory was positively correlated with the change in ReHo in left precuneus (Figure 2). On the other hand, after placebo treatment, the change in nonverbal working memory or in verbal learning and memory was not correlated with the change in ReHo in any brain region (Table 3). In accordance, previous study has shown that, in the nonverbal working memory, regions of activation included the precentral gyrus and precuneate gyrus in the left parietal lobe and the occipital cortex in the right hemisphere (Binder and Urbanik, 2006). In addition, in the MCI patients, perfusion in precunei, parietal cortex, and left hippocampus was correlated with verbal memory (Nobili et al., 2008).

Dysregulation of NMDAR is implicated in the pathogenesis of AD (Lin et al., 2019a, 2019b; Chang et al., 2020). DAO can regulate the NMDAR function. In a recent study in 397 individuals (including aMCI, mild AD, moderate to severe AD, and healthy elderly), DAO levels in the serum increased with the severity of the cognitive deficits (Lin et al., 2017); this is the first study indicating that the peripheral DAO levels may increase with age-related cognitive decline. The finding supports the hypofunction of NMDAR hypothesis in AD (Lin et al., 2020). It is critical to identify and treat AD as early as possible, potentially to arrest its progression (Hsu et al., 2018). Following the pilot study on sodium benzoate for the treatment of cognitive function of early-phase AD (Lin et al., 2014), the current study further supports that DAO could serve as a novel target of drug development for early stages of cognitive decline.

This study is limited by its small sample size. In addition, whether the finding in Han Taiwanese can be extrapolated to other populations is unclear.

In conclusion, the preliminary results may contribute to the elucidation of the pathophysiology of MCI and the development of novel therapy to enhance NMDAR. Future larger-sized studies in patients of various ethnicities are warranted. In addition,

Table 3. Correlation Between the Changes of Cognitive Tests and the Changes of ReHo With Benzoate or Placebo Treatment

				Positive correlation							Negative correlation		
					Coordinate (mm)							Coordinate (mm)	
Anatomic area	BA	Size	t Score	x	y	z	Anatomic area	BA	Size	t Score	x	y	z
Wechsler Memory Scale-Third Edition, spatial span													
Benzoate													
R Precentral G	6	99	4.19	63	–3	21	No significant cluster						
R mid-occipital G	18	93	4.10	24	–102	0							
Placebo							No significant cluster						
No significant cluster													
Wechsler Memory Scale-Third Edition, word listing													
Benzoate													
L Precuneus	7	103	4.80	–21	–75	48	No Significant Cluster						
Placebo							No significant cluster						
No significant cluster													

Abbreviations: B, bilateral; BA, Brodmann area; EC, Entorhinal cortex; G, gyrus; Inf, inferior; L, left; Mid, middle; N, nucleus; PCC, posterior cingulate cortex; R, right; ReHo, regional homogeneity; Size, number of voxels in the cluster; Sup, superior.

whether neuroimaging biomarkers, as shown in the current study, can be applied for early detection and individualized medicine also deserves further investigation.

Acknowledgments

Statement of Interest

All authors declare that they have no conflicts of interest.

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