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Resting-state functional reorganization in Alzheimer's disease and amnestic mild cognitive impairment: protocol for a systematic review and activation likelihood estimation meta-analysis

Diyang Lyu[1], Taoran Li[1], Xuanxin Lyu[2*]

1 Capital Medical University, Beijing, 100069, China
2 Neurological Rehabilitation Center, Beijing Rehabilitation Hospital, The Affiliated Hospital of Capital Medical University, Beijing, 100144, China

*Correspondence to: Dr. Xuanxin Lyu, lvxvanxin@163.com

Abstract

Introduction: The population of Alzheimer's disease (AD) is increasing rapidly, causing a growing health and economic burden worldwide. Previous clinical trials have failed in the past decade, and there is still a lack of satisfactory treatment. Scientists point out that early intervention for dementia is a possible factor. The cognitive decline in AD occurs continuously over a long period, however, there is still a lack of simple, rapid, and accurate diagnostic approaches for amnestic mild cognitive impairment (aMCI) or subjective cognitive decline (SCD) to help doctors, especially non-experts identify patients. Resting-state functional magnetic resonance imaging (rs-fMRI) can present the functional activities of human brain noninvasively. Amplitude of low frequency fluctuation (ALFF), fractional ALFF (fALFF) and regional homogeneity (ReHo) are rs-fMRI indicators with good repeatability. They have been studied in the early diagnosis of other diseases, and may be promising early diagnostic imaging markers of AD.

Methods and Analysis: Following electronic literature databases will be searched from inception to December 2021: Medline-Ovid, Medline-PubMed, EMBase-Ovid, Cochrane Central, and the ClinicalTrials.gov platform. Two independent reviewers will select studies with eligible criteria, extract data and assess the quality of original studies with our quality assessment tool individually. Missing data will be obtained through sending e-mails to corresponding authors or multiple imputation. Brain regions will be presented for ALFF/fALFF and ReHo by performing activation likelihood estimation (ALE) with the Seed-based d Mapping- Permutation of subject images (SDM-PSI) 6.21 software, respectively. Meta-regression will be performed to determine the potential brain regions that may have a strong correlation with cognitive decline progression. Subgroup analysis, funnel plot, Egger's test and sensitivity analysis will be conducted to detect and explain potential heterogeneity.

Ethics and Dissemination: This study does not require formal ethical approval. The findings will be submitted to a peer-review journal.

PROSPERO registration number: CRD42021229009.

Key words: Alzheimer's disease, amnestic mild cognitive impairment, systematic review, meta-analysis, rs-fMRI, ALFF, ReHo

Strengths and limitations of this study

- This systematic review and meta-analysis investigates ALFF/fALFF and ReHo together in AD and aMCI patients.
- Subjective cognitive decline patients may not be included if there are not enough high-quality original studies.

- We established a modified quality assessment tool to assess the quality of original studies for rs-fMRI systematic review and meta-analysis.
- Meta-regression will be used to identify brain regions that may be used as indicators of cognitive decline.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease that is causing a growing health and economic burden[1]. AD will reportedly affect 131 million people worldwide in 2050 and will cause over $2 trillion in economic losses in 2030[2]. However, there has never been a satisfactory breakthrough in the field of AD treatment. Since the approval of memantine in 2003[3], clinical trials of new drugs developed for different pathogenesis of AD all have failed[4]. Failure drugs can reduce the pathological products of AD in human body, but are not effective in improving the patient's ability of daily living and cognitive function, especially memory[5]. Some scientists have suggested that this current situation may be due to the fact that most of the study participants are patients with moderate or severe AD. These patients have too severe pathological changes in the brain to reverse or stop disease progression and have miss the best time for treatment[6 7]. Thus, early treatment for patients with AD may be one of the keys. In the Alzheimer's continuum, for patients in the predementia stages, such as mild cognitive impairment (MCI) or subjective cognitive decline (SCD), their clinical symptoms are not obvious, and it is difficult to identify them from cognitively healthy individuals efficiently and accurately through clinical information and neuropsychological scales, especially for clinicians not in the AD field[8]. Therefore, there is a highly demand of convenient and reliable markers for diagnosis currently[9].

The accurate diagnosis of AD depends on autopsy, while in the recent decade, with the development of detection technology, scientists can directly detect biomarkers in vivo to identify patients with AD[9-11]. More specifically, these biomarkers include amyloid-β (Aβ) and hyperphosphorylated tau in plasma and cerebrospinal fluid[6], and positron emission tomography (PET) imaging[12]. However, interlaboratory variations leads to a lack of robustness of these biomarkers for early diagnosis, and although many other biomarkers have been proposed, the repeated validation is still needed to prove their reliability[13 14], what's more, most of the hospital laboratories are unable to detect these biomarkers, especially for those non-tertiary hospital settings, similarly, the PET is difficult to be widely used. In addition, these examinations can cause harm to the body through vascular puncture, lumbar puncture, or administration of radioactive substances. In contrast, magnetic resonance imaging (MRI) can harmlessly reflect structural and functional changes in the brain. The current diagnostic criteria mostly use structural MRI reports of whole brain atrophy, medial temporal lobe atrophy or hippocampal atrophy, and other brain changes as the basis for diagnosis[12], however, functional MRI (fMRI) may reflect the state of brain function through the change of cerebral blood-oxygen signal, detect brain abnormalities earlier than detectable structural changes, to indicate cognitive decline.

Resting-state fMRI (rs-fMRI) is a noninvasive, harmless, and efficient detection method with high spatial resolution, which can reflect the functional status of the central nervous system. Since 1995, rs-fMRI has been increasingly used in scientific research[15]. At present, many indicators of rs-fMRI have been used to reflect functional activity, including functional connectivity[16], amplitude of low frequency fluctuation (ALFF)[17],

fractional ALFF (fALFF)[18], regional homogeneity (ReHo)[19], etc. Among the commonly used indicators, ALFF/fALFF and ReHo were reported to have relatively higher test–retest reliabilities[20], furthermore, the calculation process does not require a prior assumption of the specific brain regions to be studied. ALFF is considered to stand for spontaneous brain activities, and fALFF is derived from the improved algorithm of ALFF[21]. ReHo assesses the synchronization among one voxel and its neighboring voxels (e.g., 26 voxels), and is considered to stand for the homogeneity of the given cluster[21]. These indicators have also been proved to be correlated with disease progression in other diseases through general linear model or machine learning approaches, and considered as potential diagnostic markers[22-24]. Thus, we speculate that ALFF/fALFF and ReHo may be promising imaging markers for early diagnostic of AD.

Previous studies in the field of AD or amnestic MCI (aMCI) have reported many brain regions with increase or decrease ALFF/fALFF or ReHo[25 26]. However, seldom studies involved patients with SCD[27]. The diagnostic criteria of SCD have not been unified for decades, resulting in the heterogeneity of subjects in different studies[27]. This may be because the definitely concept of SCD was put forward in recent years[28], its definition and clinical significance have not been fully cleared, and researchers have not paid enough attention to this field. In addition, SCD is a naturally heterogenous state and greatly affected by the cultural background[29 30]. Thus, this systematic review and meta-analysis will not put SCD related studies into pooled estimations if there are not enough original studies with high quality and consistent diagnostic criteria. A previous meta-analysis summarized 12 original studies and reported that 8 brain regions showed changed ALFFs comparing aMCI patients with healthy controls[25]. Another meta-analysis included 10 original studies of aMCI patients and reported 11 brain regions with changed ReHos compared with healthy controls[26]. The above studies were published years before, and their findings may be changed with recent original studies.

Objective

We are conducting this systematic review and meta-analysis to summarize previous rs-fMRI studies on patients with AD or aMCI. SCD studies will also be included if possible. The primary outcomes will be ALFF/fALFF and ReHo. Furthermore, we will also identify the potential key brain regions that may associate with the severity of disease or cognitive decline by using meta-regression. Brain regions with increase or decrease ALFF/fALFF or ReHo will be determined through meta-analysis and reported for further clinical practice and establishment of diagnostic criteria.

METHODS AND ANALYSIS

Study guidelines and registration

This systematic review and meta-analysis will include studies reported ALFF/fALFF or ReHo of AD or aMCI patients comparing with cognitively healthy controls. Thus, the Quality of Reporting of Meta-Analyses (QUOROM) guidelines is not applicable

for this study[31]. The guidelines of Preferred reporting items for systematic review and meta-analysis (PRISMA) statement was updated from QUOROM and is applicable to all kinds of systematic reviews[32]. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) is also established for meta-analysis of observational studies[33]. Therefore, the systematic review and meta-analysis will be conducted and presented following the PRISMA statement[32], MOOSE guidelines[33] and Cochrane Handbook[34]. This protocol follows the PRISMA protocols (PRISMA-P)[35] statement, and is registered on PROSPERO (international prospective register of systematic reviews) (registration number CRD42021229009).

Search strategy

We will search the following electronic databases from inception to December 2021 for published literatures: Medline-Ovid, Medline-PubMed, EMBase-Ovid, and Cochrane Central. We will also search ClinicalTrials registration platform for missing, unpublished or ongoing studies. Two independent reviewers (DL and TL) will also check the reference lists of each literature that enters the full text screening step and each review article in this field. After data extraction, we will send e-mails to the corresponding authors of the included literatures for more information to avoid potential missing. The search strategy for Medline-PubMed is presented as Table 1.

Eligibility criteria

Studies will be included in the systematic review and meta-analysis following the below criteria:

i) Patients: The patients enrolled in the original studies should be diagnosed with AD or aMCI according to a clearly reported diagnostic criteria. Exact scores of cognitive assessments should be reported. We will not restrict the gender, age, race, etc. of participants in the original studies, however, above information should be reported in detail. In addition, studies focused on AD or aMCI with other complications such as post-dementia depression are applicable if there are clear descriptions in the literature.

ii) Control: The control group should include the cognitively healthy subjects matched with the demographic characteristics of the AD or aMCI group in the original studies.

iii) Outcomes: Only rs-fMRI studies will be included. The original studies should report results from the whole-brain analysis instead of analysis with any specific brain networks or regions. Brain regions with increased or decreased ALFF/fALFF or ReHo will be put into pooled estimation through activation likelihood estimation (ALE) analysis. The outcomes should be reported with the peak coordinate, cluster size, etc. of each brain region. In addition, only the results corrected by multiple comparison correction will be included in the study to avoid false positive results[15].

iv) Study design: Observational studies with or without follow-up repeated measurements will be included. Controlled clinical trials which reported the differences between patients and controls at baseline can also be included if meet the above criteria.

Study selection

Two independent reviewers (DL and TL) will screen the titles and abstracts of hit

literatures from each electronic database after removing duplications with EndNote X9 software. Literatures that are obviously inconsistent with our eligible criteria will be excluded by the two reviewers individually. Then, they will go through the full text of the rest literatures and further exclude those that do not meet our criteria. Reasons for each exclusion in this step will be record individually. An independent reviewer (XL) will solve the disagreement between the two reviewers. Any disagreement will be recorded with detailed reason. The complete process of study selection will be presented as a PRISMA flow diagram (Figure 1).

Data extraction

Two independent reviewers (TL and DL) will extract demographic information, study design and data analysis, and outcome data individually with Microsoft Excel. Demographic information includes the age, sex, nationality, race, Apolipoprotein E genotype (*APOE*), years since first symptom or first diagnosis, education level, cognitive assessment scores, etc. Information about study design and data analysis includes the field strength and exact machine model of the MRI scanner, the statistical method of multiple comparison correction, the software and packages used, the frequency range for ALFF/fALFF and the number of neighboring voxels for ReHo, the parameter of full width at half maximum (FWHM) smooth kernel, etc. of each original study. Result data includes anatomical label, peak coordinate, cluster size of each reported brain region from original studies, and cognitive assessment outcomes such as Mini-Mental State Examination scores. Continuous variables will be recorded as mean with standard deviation (SD) and discrete variables will be recorded as number with percentage. The recorded data will be verified through comparing between the two reviewers. For results without multiple comparison correction or missing data, we will also contact the corresponding authors when necessary.

Quality assessment

To our knowledge, there has been no standard checklist or tool for quality assessment of fMRI studies. Thus, we develop our quality assessment tool as Table 2 for this study based on some previous meta-analysis in this field[26 36] and the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) Statement[37]. Two independent reviewers (TL and DL) will go through the full text for any potential bias according to our quality assessment tool and grade each original study. The total quality assessment score will be reported in the main text and the detailed quality assessment table will be provided as well in our systematic review and meta-analysis.

Qualitative and quantitative synthesis

Qualitative synthesis

First, we will present a summary table to present the characteristics of the included studies, including the publication year, demographic characteristics, study design and analysis parameters, outcome indicators, etc. Then, we will summarize the studies by outcomes to give a general summary.

Quantitative synthesis

We will conduct a quantitative analysis for each outcome. First, all coordinates will be converted to Montreal Neurological Institute (MNI) space. After that, we will perform an ALE meta-analysis for ALFF/fALFF and ReHo, respectively. The pooled estimations will reveal the consistent brain regions with significant functional alteration reported from different studies by using the Seed-based *d* Mapping- Permutation of subject images (SDM-PSI) 6.21 software (www.sdmproject.com)[38]. The algorithm of SDM-PSI is updated from ALE algorithm. Comparing with software using ALE algorithm, SDM-PSI can retain positive and negative activation results and avoid bias due to overlapping activation positions in the brain. The peak coordinates in MNI space and their effect sizes (e.g., t-values) extracted from original studies will be registered as centers in the 3D Gaussian probability distribution to recreate standard MNI brain maps for each study respectively[39 40]. Later the brain maps will be used to generate pooled estimation for each outcome separately. The sample size, sex, age, and other demographic information of each group will also be used to achieve a linear model analysis to control potential confounding factors. We will also employ family-wise error correction to control false positive rate.

Subgroup analysis

We will further analyze the results with subgroup analysis to detect potential heterogeneity and explain possible reasons. Data from different disease stage (AD or aMCI), from MRI scanner with different field strength (1.5T or 3.0T), and with or without complications will be analyzed separately.

Sensitivity analysis

After subgroup analysis, we will perform a sensitivity analysis by excluding studies one by one to observe whether the pooled estimations are stable or not. In this step, significant changes may imply significant heterogeneity among studies. Any significant heterogeneity will be reported when occurs.

Assessment of publication bias

We will apply funnel plot to detect potential reporting bias while no less than 10 original studies are pooled in a meta-analysis[41]. For continuous variables, we will also apply Egger's test for funnel plot asymmetry. We will try to figure out possible reasons and give interpretation for existing publication bias.

Meta-regression

To identify potential factors that may affect the changes of brain regions in different stage of disease, we will perform a meta-regression. In this step, studies of patients with aMCI and AD will be combined to regard cognitive decline as a continuous progression. Following variables will be analyzed: age, cognitive assessment score, *APOE* genotype, etc. We will perform meta-regression using the SDM-PSI software 6.21. For missing values, we will contact the correspondence authors by E-mail. If missing values are unavailable, we will remove the related regression factors or original studies with too

many missing values. For those regression factors or original studies with less than 10% missing values, we will use "mice" package in R v4.0.3 for multiple imputation to deal with them.

Ethics and dissemination

This study does not require formal ethical approval. The findings will be submitted for publication in a peer-review journal.

Patient and public involvement

As this is a protocol for our systematic review and meta-analysis, we will obtain public data from published literatures or from corresponding authors. Thus, patients or the public were not involved.

DISCUSSION

This systematic review will comprehensively summarize and analyze the results of previous fMRI studies investigated AD or aMCI by ALFF/fALFF or ReHo. We will present whole-brain ALFF/fALFF and ReHo analyses and report significant differences between AD or aMCI patients and cognitively healthy controls.

A previous meta-analysis summarized 12 original studies and reported that 4 brain regions showed decreased ALFFs and another 4 showed increased ALFFs comparing aMCI patients with healthy controls[25]. They also reported that a greater decrease in ALFFs in the cuneus/precuneus cortices may associate with the increased severity of cognitive impairment[25]. Another meta-analysis included 10 original studies of aMCI patients and focused on ReHo[26]. They found that ReHo of 11 brain regions from 4 brain networks differs between aMCI patients and healthy controls. The above meta-analyses were done several years before, and their results varies. However, these studies included AD and aMCI only, and we will include studies on SCD if possible. In addition, our meta-analysis may provide different estimations by including recent studies in this field.

To our knowledge, only 1 previous meta-analysis investigated the functional characteristics of AD and aMCI patients compared with healthy controls through both ALFF/fALFF and ReHo[42]. Their findings reported that patients with aMCI and AD displayed consistently decreased functional characteristics, and the changed brain regions were relatively consistent. Although this study was published 6 years before, their finding supports our idea of combining studies of AD and aMCI patients together, even including SCD studies if possible, and trying to find brain regions that have changed ALFF/fALFF or ReHo with strong correlation with cognitive decline measured by neuropsychological scales through meta regression. Our findings may provide reliable biomarkers for further early diagnosis and prediction of progressing cognitive impairment.

Author Contributions

DL and XL designed this study. DL developed the search strategy, TL and DL established the data extraction list. DL drafted the manuscript. TL and XL revised the manuscript and provided methodological perspectives. DL and TL will search and screen literatures, perform data extraction. DL will assess the quality of included studies and conduct data analyses. All authors read and approved the final manuscript.

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Competing interests
The authors declare no competing interests.

Patient consent for publication
Not applicable.

Data sharing statement
Results of the current review will be disseminated through peer-reviewed publications.

Provenance and peer review

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Table 1. Search strategy for Medline-PubMed.

Search	Query
#1	(alzheimer*[Title/Abstract]) OR (alzheimer disease[MeSH Terms])
#2	(dementia[MeSH Terms]) OR (dement*[Title/Abstract])
#3	(((((MCI[Title/Abstract]) OR ("mild cognitive impairment"[Title/Abstract])) OR ("amnestic mild cognitive impairment"[Title/Abstract])) OR (aMCI[Title/Abstract])) OR (SCD[Title/Abstract])) OR ("subjective cognitive decline"[Title/Abstract])
#4	(cognit*[Title/Abstract]) OR (memor*[Title/Abstract])
#5	((impair*[Title/Abstract]) OR (decline[Title/Abstract])) OR (reduc*[Title/Abstract])
#6	#4 and #5
#7	(((((("functional magnetic resonance imaging"[Title/Abstract]) OR ("magnetic resonance imaging"[Title/Abstract])) OR ("resting-state functional magnetic resonance imaging"[Title/Abstract])) OR ("resting state functional magnetic resonance imaging"[Title/Abstract])) OR (fMRI[Title/Abstract])) OR ("functional MRI"[Title/Abstract])) OR (MRI[Title/Abstract])) OR ("rs-fMRI"[Title/Abstract])
#8	(((((("amplitude of low frequency fluctuation*"[Title/Abstract]) OR ("fractional amplitude of low frequency fluctuation*"[Title/Abstract])) OR ("regional homogeneit*"[Title/Abstract])) OR (ReHo*[Title/Abstract])) OR (ALFF*[Title/Abstract])) OR (fALFF*[Title/Abstract])
#9	#1 or #2 or #3 or #6
#10	#7 and #8 and #9

Table 2. Quality assessment tool of original fMRI studies.

	Category 1: sample characteristics (10)
1	Participants were enrolled with clearly described standardized diagnostic criteria (2).
2	Comprehensive demographic data with comparable baseline between groups (1).
3	Inclusion and exclusion criteria are reasonable, taking into account the possible affecting factors (2).
4	Cognitive outcomes were reported in detail as mean±SD (3).
5	Sample size >10 in each group (2).
	Category 2: methodology and reporting (10)
6	The machine model and field strength of MRI scanner is reported (1).
7	Clear description of study procedure and quality control, includes the methods to ensure that the subjects are in "resting state" (2).
8	At least 5 min of resting state acquisition (2).
9	The report of scanning parameters is comprehensive and reasonable, and the quality control method of image scanning is reported (1).
10	Detailed description of software and toolkits used (1).
11	Results were applied and reported in original literature, including peak coordinate, cluster size of each brain region (1).
12	Multiple comparison correction was applied and reported in original literature (1).
13	Conclusions were consistent with the results obtained and the limitations were discussed (1).

MRI, magnetic resonance imaging; SD, standard deviation.

Figure 1. The PRISMA flow diagram of this systematic review and meta-analysis.

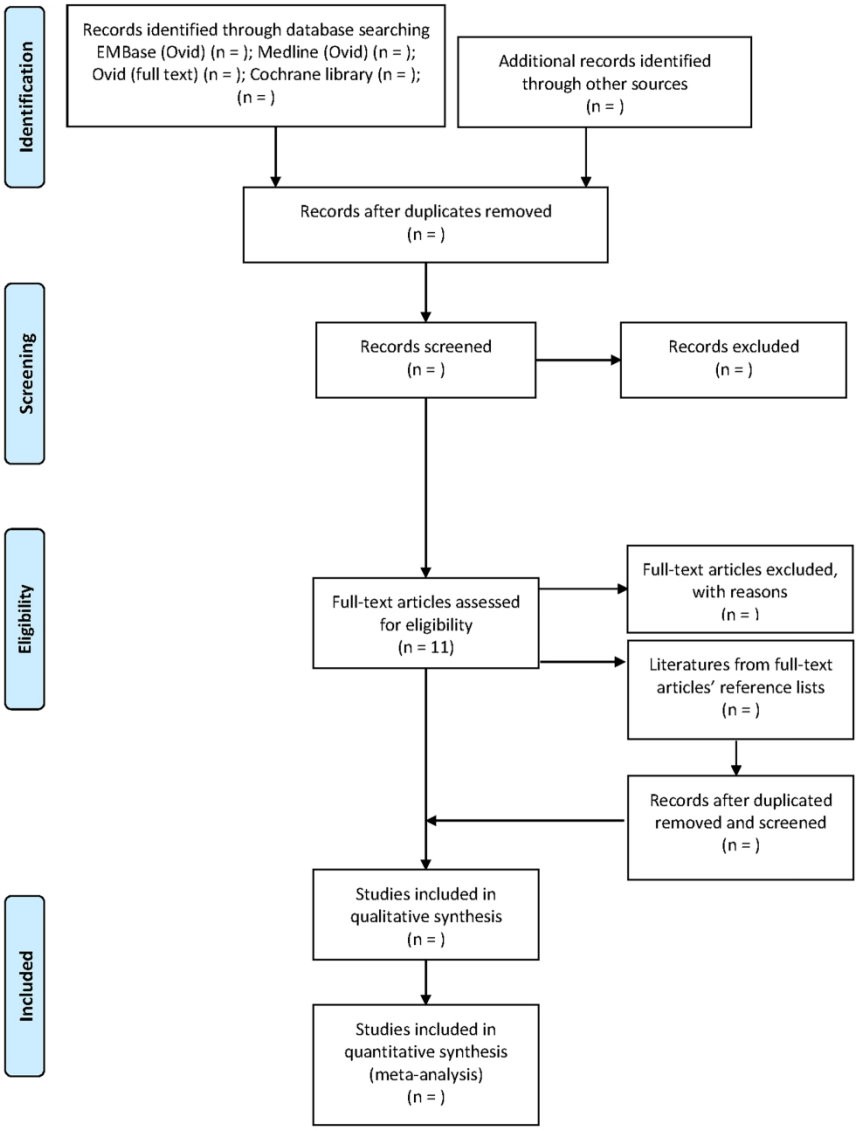

Figure 1. The PRISMA flow diagram of this systematic review and meta-analysis.

PRISMA-P 2015 Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in: Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☑	☐	Page 1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☑	NA
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☑	☐	Page2
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☑	☐	Page1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☑	☐	Page 10
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☐	☑	NA
Support					
Sources	5a	Indicate sources of financial or other support for the review	☑	☐	Page 10
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☑	NA
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☑	NA
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☑	☐	Page 4-5

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☑	☐	Page 5
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☑	☐	Page 6
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☑	☐	Page 6-7
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☑	☐	Page 15
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☑	☐	Page 6-7
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☑	☐	Page6, Figure 1
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☑	☐	Page 6-7
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☑	☐	Page 7 and 16
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☑	☐	Page 6-7
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☑	☐	Page 7 and 16
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☑	☐	Page 7-8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☑	☐	Page 8-9

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☑	☐	Page 8-9
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☑	☐	Page 7
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☑	☐	Page 8-9
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☐	☑	N/A

BMJ Open

Resting-state functional reorganization in Alzheimer's disease and amnestic mild cognitive impairment: protocol for a systematic review and meta-analysis

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Resting-state functional reorganization in Alzheimer's disease and amnestic mild cognitive impairment: protocol for a systematic review and meta-analysis

Diyang Lyu[1#], Taoran Li[1#], Xuanxin Lyu[2*]

1 Capital Medical University, Beijing, 100069, China
2 Neurological Rehabilitation Center, Beijing Rehabilitation Hospital, Capital Medical University, Beijing, 100144, China

#These authors contributed equally to this work.
*Correspondence to: Dr. Xuanxin Lyu, lvxvanxin@163.com

ABSTRACT

Introduction: The incidence of Alzheimer's disease (AD) is increasing rapidly, causing a growing health and economic burden worldwide. Previous clinical trials have failed in the past decade, and there is still a lack of satisfactory treatment. Scientists have pointed out that early intervention for dementia is a possible factor. Cognitive decline in AD occurs continuously over a long period; however, there is still a lack of simple, rapid, and accurate diagnostic approaches for amnestic mild cognitive impairment (aMCI) or subjective cognitive decline (SCD) to help doctors, especially non-experts, identify patients with the disease. Resting-state functional magnetic resonance imaging (rs-fMRI) can determine the functional activities of the human brain noninvasively. The amplitude of low-frequency fluctuation (ALFF), fractional ALFF (fALFF), and regional homogeneity (ReHo) are rs-fMRI indicators with good repeatability. They have been studied in the early diagnosis of other diseases and may be promising early diagnostic imaging markers of AD.

Methods and analysis: The following electronic literature databases will be searched from inception to December 2021: Medline-Ovid, Medline-PubMed, EMBase-Ovid, Cochrane Central, and ClinicalTrials.gov. Two independent reviewers will select studies with eligible criteria, extract data, and assess the quality of the original studies with our quality assessment tool individually. Missing data will be obtained by sending e-mails to the corresponding authors. Brain regions will be presented for ALFF/fALFF and ReHo by performing activation likelihood estimation (ALE) with the Seed-based *d* Mapping-Permutation of subject images (SDM-PSI) 6.21 software. Meta-regression will be performed to determine the potential brain regions that may have a strong correlation with cognitive decline progression. Subgroup analysis, funnel plot, Egger's test, and sensitivity analysis will be conducted to detect and explain potential heterogeneity.

Ethics and dissemination: This study does not require formal ethical approval. The findings will be submitted to a peer-review journal.

PROSPERO registration number: CRD42021229009

Key words: Alzheimer's disease, amnestic mild cognitive impairment, systematic review, meta-analysis, rs-fMRI, ALFF, ReHo

Strengths and limitations of this study

- This systematic review and meta-analysis will summarize brain regions with ALFF/fALFF or ReHo changes in AD and aMCI patients using qualitative and quantitative analyses.
- This study will consider AD and aMCI as different stages of cognitive decline

and conduct meta-regression with the pooled population to explore the brain regions closely related to the whole process of cognitive decline.

- We established a modified quality assessment tool to assess the quality of the original studies for rs-fMRI systematic review and meta-analysis.
- We will only retrieve data from English databases and may overlook a few valuable original studies in other languages.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease that is causing a growing health and economic burden[1]. AD will reportedly affect 131 million people worldwide by 2050 and will cause over $2 trillion in economic losses by 2030[2]. However, there has yet to be a satisfactory breakthrough in the field of AD treatment. Since the approval of memantine in 2003[3], all clinical trials of new drugs developed for the different pathogeneses of AD have failed[4]. Newly developed drugs can reduce the pathological products of AD in the human body but are not effective in improving the patient's ability to perform daily living and cognitive function, especially memory[5]. Some scientists have suggested that this current situation may be due to the fact that most of the study participants were patients with moderate or severe AD. These patients have pathological changes in the brain that may be very severe to reverse or stop progression and for the optimal time for treatment may have already elapsed[6 7]. Thus, early diagnosis and treatment of patients with AD may be the key. In the Alzheimer's continuum, for patients in the predementia stages, their clinical symptoms, such as mild cognitive impairment (MCI) or subjective cognitive decline (SCD), are not obvious, and it is difficult to efficiently and accurately differentiate them from cognitively healthy individuals through clinical information and neuropsychological scales, especially for clinicians not in the AD field[8]. Therefore, there is currently a high demand for convenient and reliable markers for diagnosis[9].

The accurate diagnosis of AD was confirmed on autopsy; however, in the recent decade, with the development of laboratory tests and neuroimaging, scientists can directly detect biomarkers in vivo to identify patients with AD[9-11]. More specifically, these biomarkers include amyloid-β (Aβ) and hyperphosphorylated tau in plasma and cerebrospinal fluid[6] and positron emission tomography (PET) imaging[12]. However, interlaboratory variations lead to a lack of robustness of these biomarkers for early diagnosis. Although many other biomarkers have been proposed, repeated validation is still needed to prove their reliability[13 14]. Moreover, most hospital laboratories are unable to detect these biomarkers, especially in non-tertiary hospital settings. Similarly, PET is difficult to use generally. In addition, these examinations can cause harm to the body through vascular puncture, lumbar puncture, or the administration of radioactive substances. In contrast, magnetic resonance imaging (MRI) can noninvasively reflect structural and functional changes in the brain. The current diagnostic criteria mostly use structural MRI reports of whole brain atrophy, medial temporal lobe atrophy, hippocampal atrophy, and other brain changes as the basis for diagnosis[12]; however, functional MRI (fMRI) may reflect the state of brain function through the change of cerebral blood-oxygen signal, detect brain abnormalities earlier than detectable structural changes, and indicate cognitive decline.

Resting-state fMRI (rs-fMRI) is a noninvasive, harmless, and efficient detection method with high spatial resolution, which can reflect the functional status of the central nervous system. Since 1995, rs-fMRI has been increasingly used in scientific research[15]. At present, many indicators of rs-fMRI have been used to reflect functional activity,

including functional connectivity[16], amplitude of low-frequency fluctuation (ALFF)[17], fractional ALFF (fALFF)[18], regional homogeneity (ReHo)[19], among others. Among the commonly used indicators, ALFF/fALFF and ReHo were reported to have relatively higher test–retest reliabilities[20], and the calculation process does not require a prior assumption of the specific brain regions to be studied. ALFF is considered to represent spontaneous brain activity, and fALFF is derived from the improved algorithm of ALFF[21]. ReHo assesses the synchronization among one voxel and its neighboring voxels (e.g., 26 voxels) and is considered to represent the homogeneity of the given cluster[21]. These indicators have also been proven to be correlated with disease progression in other diseases through general linear models or machine learning approaches and are considered as potential diagnostic markers[22-24]. However, fMRI is still used as an additional resource in conjunction with other tests. Thus, we speculate that ALFF/fALFF and ReHo may be promising imaging markers for the early diagnosis of AD.

Previous studies in the field of AD or amnestic MCI (aMCI) have reported many brain regions with increased or decreased ALFF/fALFF or ReHo[25 26]. However, few studies have examined patients with SCD[27]. Uniform diagnostic criteria for SCD have not been established for decades, resulting in the heterogeneity of subjects in different studies[27]. This may be because the definite concept of SCD was only determined in recent years[28], its definition and clinical significance have not been fully clarified, and researchers have not paid enough attention to this field. In addition, SCD is a naturally heterogeneous state and is greatly affected by cultural background[29 30]. Thus, this systematic review and meta-analysis will not put SCD-related studies into pooled estimations if there are not enough original studies with high quality and consistent diagnostic criteria. A previous meta-analysis summarized 12 original studies and reported that 8 brain regions showed altered ALFFs compared to aMCI patients with healthy controls[25]. Another meta-analysis included 10 original studies of aMCI patients and reported 11 brain regions with changed ReHos compared with healthy controls[26]. The above studies were published years before, and their findings may have changed with recent original studies.

Objective

We are conducting this systematic review and meta-analysis to summarize previous rs-fMRI studies comparing patients with AD or aMCI with adults with normal cognition to determine their differences in ALFF/fALFF or ReHo. SCD studies will also be included, if available. Furthermore, we will also identify the potential key brain regions that may be associated with the severity of disease or cognitive decline by using meta-regression. Brain regions with increased or decreased ALFF/fALFF or ReHo will be determined through meta-analysis and reported for further clinical practice and establishment of diagnostic criteria.

METHODS AND ANALYSIS

Study guidelines and registration

This systematic review and meta-analysis will include studies reporting ALFF/fALFF or ReHo in AD or aMCI patients compared with cognitively healthy controls. Thus, the Quality of Reporting of Meta-Analyses guidelines is not applicable for this study[31]. The guidelines of the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement were updated from QUOROM and are applicable to all kinds of systematic reviews[32]. Meta-analysis of Observational Studies in Epidemiology (MOOSE) has also been established for meta-analysis of observational studies[33]. Therefore, a systematic review and meta-analysis will be conducted and presented following the PRISMA statement[32], MOOSE guidelines[33] and Cochrane Handbook[34]. This protocol follows the PRISMA protocols (PRISMA-P)[35] statement and is registered on the International Prospective Register of Systematic Reviews (PROSPERO) (registration number CRD42021229009).

Search strategy

We will search the following electronic databases from inception to December 2021 for published literature: Medline-Ovid, Medline-PubMed, EMBase-Ovid, and Cochrane Central. We will also search the ClinicalTrials registration platform for missing, unpublished, or ongoing studies. Two independent reviewers (DL and TL) will also check the reference lists of each literature that enters the full-text screening step and each review article in this field. After data extraction, we will send e-mails to the corresponding authors of the included literature for more information to avoid potential missing data. The search strategy for Medline-PubMed is presented in Table 1, and the full search strategy is presented in Supplementary File 1.

Eligibility criteria

Studies will be included in the systematic review and meta-analysis according to the following criteria:

i) Patients: The patients enrolled in the original studies should be diagnosed with AD or aMCI according to clearly reported diagnostic criteria. Exact scores for cognitive assessments should be reported; however, a specific format is not required. We will not restrict the age, sex, or race of participants in the original studies; however, the above information should be reported in detail. In addition, studies focused on AD or aMCI with other complications, such as post-dementia depression, are applicable if there are clear descriptions in the literature.

ii) Control: The control group should include cognitively healthy subjects with comparable demographic characteristics to the AD or aMCI group in the original studies.

iii) Outcomes: Only rs-fMRI studies will be included. The original studies should report results from whole-brain analysis instead of analysis with specific brain networks or regions. Brain regions with increased or decreased ALFF/fALFF or ReHo will be put into pooled estimation through activation likelihood estimation (ALE) analysis. The outcomes should be reported with the peak coordinate, cluster size, and statistics of each brain region. In addition, only the results corrected by multiple comparison

correction will be included in the study to avoid false positive results[15].

iv) Study design: Observational studies with or without repeated follow-up measurements will be included. Controlled clinical trials that reported the differences between patients and controls at baseline can also be included if they meet the above criteria.

Study selection

Two independent reviewers (DL and TL) will screen the titles and abstracts of the hit literature from each electronic database after removing duplications with EndNote X9 software. The literature that are obviously inconsistent with our eligibility criteria will be excluded by the two reviewers individually. Then, they will go through the full text of the literature and further exclude those that do not meet our criteria. The reasons for each exclusion in this step will be record individually. An independent reviewer (XL) will solve the disagreement between the two reviewers. Any disagreement will be recorded with detailed explanation. The complete process of study selection will be presented in a PRISMA flow diagram (Figure 1).

Data extraction

Two independent reviewers (DL and TL) will extract demographic information, study design, data analysis, and outcome data individually using Microsoft Excel. The complete information and data extraction lists are presented in Table 2. Demographic information includes the age, sex, nationality, race, apolipoprotein E genotype (*APOE*), years since first symptom or first diagnosis, educational level, and cognitive assessment scores. Information about the study design and data analysis includes the field strength and exact machine model of the MRI scanner, the statistical method of multiple comparison correction, the software and packages used, the frequency range for ALFF/fALFF, the number of neighboring voxels for ReHo, and the parameter of full width at half maximum (FWHM) smooth kernel of each original study. The results include the anatomical labels, peak coordinates, cluster size of each reported brain region from original studies, and the cognitive assessment outcomes, such as Mini-Mental State Examination scores. Continuous variables will be recorded as mean ± standard deviation (SD), and discrete variables will be recorded in percentage. For those reported as median with range or interquartile range, we will convert them into mean with SD according to validated algorithms[36 37]. The recorded data will be verified by comparing the two reviewers. For results without multiple comparison correction or missing data, we will also contact the corresponding authors when necessary.

Quality assessment

To our knowledge, there has been no standard checklist or tool for the quality assessment of fMRI studies. Thus, we developed our quality assessment tool as shown in Table 3 for this study based on a previous meta-analysis in this field[26 38]. Two independent reviewers (DL and TL) will go through the full text for any potential bias according to our quality assessment tool and grade each original study. The total quality assessment score will be reported in the main text, and a detailed quality assessment

table will be provided in our systematic review and meta-analysis.

Qualitative and quantitative synthesis

Qualitative data synthesis

First, we will create a summary table to present the characteristics of the included studies, including the information to be extracted, such as publication year, demographic characteristics, study design, analysis parameters, and outcome indicators, as shown in Table 2. Then, we will summarize the studies by outcomes to provide a general summary.

Quantitative data synthesis

We will conduct a quantitative analysis of each outcome. First, all coordinates will be converted to the Montreal Neurological Institute (MNI) space. Subsequently, we will perform an ALE meta-analysis for ALFF/fALFF and ReHo. The pooled estimations will reveal consistent brain regions with significant functional alterations reported from different studies using the Seed-based *d* Mapping-Permutation of subject images (SDM-PSI) 6.21 software (www.sdmproject.com)[39]. The SDM-PSI algorithm is updated using the ALE algorithm. Compared with software using the ALE algorithm, SDM-PSI can retain positive and negative activation results and avoid bias due to overlapping activation positions in the brain. The peak coordinates in the MNI space and their effect sizes (e.g., t-values) extracted from original studies will be registered as centers in the 3D Gaussian probability distribution to recreate standard MNI brain maps for each study[40 41]. Subsequently, the brain maps will be used to generate pooled estimations for each outcome separately. The sample size, sex, age, and other demographic information of each group will also be used to achieve a linear mixed model analysis to control for potential confounding factors. To avoid potential collinearity, we will perform correlation analysis or partial correlation analysis to exclude variables with strong collinearity. Subsequently, principal component analysis will be performed to extract several principal components, if necessary, and the principal components will be used as variables. The above analyses will be performed with R v4.0.3. We will also employ family-wise error correction to control the false positive rate.

We will further analyze the results with subgroup analysis to detect potential heterogeneity and explain the possible reasons. Data from different disease stages (AD or aMCI), MRI scanners with different field strengths (1.5T or 3.0T), and with or without complications will be analyzed separately. After subgroup analysis, we will perform a sensitivity analysis by excluding studies one by one to observe whether the pooled estimations are stable or not. In this step, significant changes may imply significant heterogeneity among the studies. Significant heterogeneity will be reported upon occurrence. We will apply funnel plots to detect potential reporting bias, while no less than 10 original studies are pooled in a meta-analysis[42]. We will attempt to identify possible reasons and interpret existing publication bias. To identify potential factors that may contribute to the changes in brain regions in different stages of the disease, we

will perform a meta-regression. In this step, studies of patients with aMCI and AD will be combined to regard cognitive decline as a continuous progression. The following variables will be analyzed: age, sex, cognitive assessment score, and *APOE* genotype. We will perform meta-regression using the SDM-PSI software 6.21. For missing values, we will contact the corresponding authors through e-mail. If missing values are unavailable, we will remove the related regression factors or original studies.

Patient and public involvement

As this is a protocol for our systematic review and meta-analysis, we will obtain public data from published literature or from the corresponding authors. Thus, patients or the public will not be involved.

ETHICS AND DISSEMINATION

This study does not require any formal ethical approval. The findings will be submitted for publication in a peer-review journal.

DISCUSSION

This systematic review will comprehensively summarize and analyze the results of previous fMRI studies investigating AD or aMCI using ALFF/fALFF or ReHo. We will present whole-brain ALFF/fALFF and ReHo analyses and report significant differences between patients with AD or aMCI and cognitively healthy controls.

A previous meta-analysis summarized 12 original studies and reported that 4 brain regions showed decreased ALFFs and another 4 showed increased ALFFs compared to aMCI patients with healthy controls[25]. They also reported that a greater decrease in ALFFs in the cuneus/precuneus cortices may be associated with an increased severity of cognitive impairment[25]. Another meta-analysis included 10 original studies of patients with aMCI and focused on ReHo[26]. They found that the ReHo of 11 brain regions from four brain networks differed between aMCI patients and healthy controls. The above meta-analyses were performed several years before, and their results varied. However, these studies included AD and aMCI only, and we will include studies on SCD, if possible. In addition, our meta-analysis may provide different estimations by including recent studies in this field.

To our knowledge, only one previous meta-analysis investigated the functional characteristics of AD and aMCI patients compared with healthy controls through both ALFF/fALFF and ReHo[43]. Their findings reported that patients with aMCI and AD displayed consistently decreased functional characteristics, and the changes in brain regions were relatively consistent. Although this study was published six years ago, their findings support our idea of combining studies of AD and aMCI patients, including SCD studies, if possible and attempting to determine the brain regions that have altered ALFF/fALFF or ReHo with strong correlation with cognitive decline

measured by neuropsychological scales through meta-regression.

It is generally known that fMRI is expensive and inconvenient as it is difficult for clinicians to understand and interpret. However, previous studies have reported a large amount of data in this field, making it feasible for scientists to establish diagnostic tools for clinicians through data mining or machine learning. Similarly, our findings may provide reliable biomarkers for the early diagnosis and prediction of progressive cognitive impairment.

Author Contributions

DL and XL designed this study. DL and TL developed the search strategy, established the data extraction list. DL drafted the manuscript. TL and XL revised the manuscript and provided methodological perspectives. DL and TL will search and screen literatures and perform data extraction. DL will assess the quality of included studies and conduct data analyses. All authors read and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

Patient consent for publication

Not applicable.

Data sharing statement

Results of the current review will be disseminated through peer-reviewed publications.

Provenance and peer review

Word Count

Main text: 2780 words Abstract: 273 words Figure: 1 Tables: 3

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Table 1. Search strategy for Medline-PubMed.

Search	Query
#1	(alzheimer*[Title/Abstract]) OR (alzheimer disease[MeSH Terms])
#2	(dementia[MeSH Terms]) OR (dement*[Title/Abstract])
#3	(((((MCI[Title/Abstract]) OR ("mild cognitive impairment"[Title/Abstract])) OR ("amnestic mild cognitive impairment"[Title/Abstract])) OR (aMCI[Title/Abstract])) OR (SCD[Title/Abstract])) OR ("subjective cognitive decline"[Title/Abstract])
#4	(cognit*[Title/Abstract]) OR (memor*[Title/Abstract])
#5	((impair*[Title/Abstract]) OR (decline[Title/Abstract])) OR (reduc*[Title/Abstract])
#6	#4 and #5
#7	(((((("functional magnetic resonance imaging"[Title/Abstract]) OR ("magnetic resonance imaging"[Title/Abstract])) OR ("resting-state functional magnetic resonance imaging"[Title/Abstract])) OR ("resting state functional magnetic resonance imaging"[Title/Abstract])) OR (fMRI[Title/Abstract])) OR ("functional MRI"[Title/Abstract])) OR (MRI[Title/Abstract])) OR ("rs-fMRI"[Title/Abstract])
#8	(((((("amplitude of low frequency fluctuation*"[Title/Abstract]) OR ("fractional amplitude of low frequency fluctuation*"[Title/Abstract])) OR ("regional homogeneit*"[Title/Abstract])) OR (ReHo*[Title/Abstract])) OR (ALFF*[Title/Abstract])) OR (fALFF*[Title/Abstract])
#9	#1 or #2 or #3 or #6
#10	#7 and #8 and #9

Table 2. Data and information extraction list.

Item	Content
Publication information	Authors, publish year, e-mail of the corresponding author, country.
Demographic data	Age, sex, human race, right-handed or not, *APOE* genotype, sample size, education level, diagnostic criteria, years since first symptom or first diagnosis, disease stage, cognitive assessment scores.
Data acquisition	The field strength and the exact machine model of the MRI scanner, slice numbers and thickness of each sequence, time points and total scanning time of functional MRI.
Outcome data	Coordinate space, anatomical label, peak coordinate, cluster size, t-value or other statistics.
Data processing	Software used, number of time points to be discarded, resampling parameters, full-width at half-maximum smooth kernel size, variables to be regressed out, the frequency range for ALFF/fALFF and the number of neighboring voxels for ReHo, method to control false positive rate.

ALFF, amplitude of low frequency fluctuation ; fALFF, fractional ALFF;*APOE*, apolipoprotein E; MRI, magnetic resonance imaging; ReHo, regional homogeneity.

Table 3. Quality assessment tool of original fMRI studies.

	Category 1: sample characteristics (10)
1	Participants were enrolled with clearly described standardized diagnostic criteria (2).
2	Comprehensive demographic data with comparable baseline between groups (1).
3	Inclusion and exclusion criteria are reasonable, taking into account the possible affecting factors (2).
4	Cognitive outcomes were reported in detail (3).
5	Sample size >10 in each group (2).
	Category 2: methodology and reporting (10)
6	The machine model and field strength of the MRI scanner is reported (1).
7	A clear description of the study procedure and quality control, includes the methods to ensure that the subjects are in a "resting state" (2).
8	At least 5 min of resting state acquisition (2).
9	The report of scanning parameters is comprehensive and reasonable, and the quality control method of image scanning is reported (1).
10	Detailed description of software and toolkits used (1).
11	Results were applied and reported in original literature, including peak coordinate and cluster size of each brain region (1).
12	Multiple comparison corrections were applied and reported in the original literature (1).
13	Conclusions were consistent with the results obtained and the limitations were discussed (1).

Figure 1. The PRISMA flow diagram of this systematic review and meta-analysis.

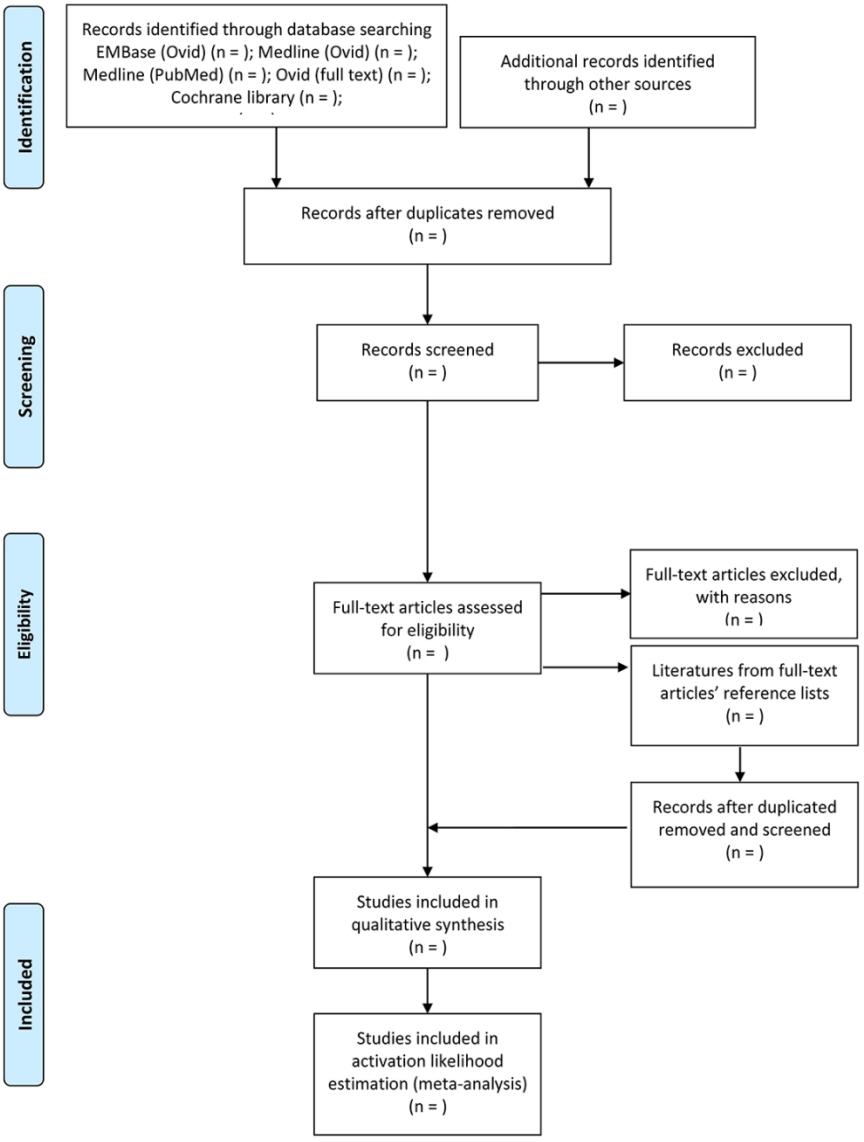

Figure 1. The PRISMA flow diagram of this systematic review and meta-analysis.

99x129mm (300 x 300 DPI)

Supplementary File 1. Search strategies.

Search strategy for Medline-PubMed:

#1 (alzheimer*[Title/Abstract]) OR (alzheimer disease[MeSH Terms])

#2 (dementia[MeSH Terms]) OR (dement*[Title/Abstract])

#3 (((((MCI[Title/Abstract]) OR ("mild cognitive impairment"[Title/Abstract])) OR ("amnestic mild cognitive impairment"[Title/Abstract])) OR (aMCI[Title/Abstract])) OR (SCD[Title/Abstract])) OR ("subjective cognitive decline"[Title/Abstract])

#4 (cognit*[Title/Abstract]) OR (memor*[Title/Abstract])

#5 ((impair*[Title/Abstract]) OR (decline[Title/Abstract])) OR (reduc*[Title/Abstract])

#6 #4 and #5

#7 (((((("functional magnetic resonance imaging"[Title/Abstract]) OR ("magnetic resonance imaging"[Title/Abstract])) OR ("resting-state functional magnetic resonance imaging"[Title/Abstract])) OR ("resting state functional magnetic resonance imaging"[Title/Abstract])) OR (fMRI[Title/Abstract])) OR ("functional MRI"[Title/Abstract])) OR (MRI[Title/Abstract])) OR ("rs-fMRI"[Title/Abstract])

#8 (((((("amplitude of low frequency fluctuation*"[Title/Abstract]) OR ("fractional amplitude of low frequency fluctuation*"[Title/Abstract])) OR ("regional homogeneit*"[Title/Abstract])) OR (ReHo*[Title/Abstract])) OR (ALFF*[Title/Abstract])) OR (fALFF*[Title/Abstract])

#9 #1 or #2 or #3 or #6

#10 #7 and #8 and #9

Search strategy for Medline-Ovid and EMBase-Ovid:

#1 exp alzheimer disease/ or dementia/

#2 (alzheimer* or dement*).tw.

#3 (MCI or “mild cognitive impairment” or “amnestic mild cognitive impairment” or aMCI or SCD or “subjective cognitive decline”).tw.

#4 (cognit* or memor*).tw.

#5 (impair* or decline or reduc*).tw.

#6 #4 and #5

#7 (“functional magnetic resonance imaging” or “magnetic resonance imaging” or “resting-state functional magnetic resonance imaging” or “resting state functional magnetic resonance imaging” or fMRI or “functional MRI” or MRI or “rs-fMRI”).tw.

#8 (“amplitude of low frequency fluctuation” or “fractional amplitude of low frequency fluctuation” or “regional homogeneity” or REHO* or ALFF* or fALFF*).tw.

#9 #1 or #3 or #6

#10 #7 and #8 and #9

Search strategy for Cochrane Central:

#1 'Alzheimer disease':ti,ab,kw OR 'dementia':ti,ab,kw OR 'alzheimer*':ti,ab,kw OR 'dement*':ti,ab,kw OR 'MCI':ti,ab,kw OR 'mild cognitive impairment':ti,ab,kw OR 'amnestic mild cognitive impairment':ti,ab,kw OR 'aMCI':ti,ab,kw OR 'SCD':ti,ab,kw OR 'subjective cognitive decline'

#2 ('cognit*':ti,ab,kw OR 'memor*':ti,ab,kw) AND ('impair*':ti,ab,kw OR 'decline':ti,ab,kw OR 'reduc*':ti,ab,kw)

#3 'functional magnetic resonance imaging':ti,ab,kw OR 'magnetic resonance imaging':ti,ab,kw OR 'resting-state functional magnetic resonance imaging':ti,ab,kw OR 'resting state functional magnetic resonance imaging':ti,ab,kw OR 'fMRI':ti,ab,kw OR 'functional MRI':ti,ab,kw OR 'MRI':ti,ab,kw OR 'rs-fMRI':ti,ab,kw

#4 'amplitude of low frequency fluctuation':ti,ab,kw OR 'fractional amplitude of low frequency fluctuation':ti,ab,kw OR 'regional homogeneity':ti,ab,kw OR 'REHO*':ti,ab,kw OR 'ALFF*':ti,ab,kw OR 'fALFF*':ti,ab,kw

#5 #1 OR #2

#6 #3 AND #4 AND #5

Search strategy for clinicaltrials.gov:
Condition or disease: Alzheimer Disease OR MCI
Study type: All Studies
Study Results: All Studies
Outcome Measure: MRI or magnetic resonance imaging

PRISMA-P 2015 Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in: Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☑	☐	Page 1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☑	NA
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☑	☐	Page2
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☑	☐	Page1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☑	☐	Page 10
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☐	☑	NA
Support					
Sources	5a	Indicate sources of financial or other support for the review	☑	☐	Page 10
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☑	NA
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☑	NA
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☑	☐	Page 4-5

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☑	☐	Page 5
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☑	☐	Page 6
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☑	☐	Page 6-7
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☑	☐	Page 15
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☑	☐	Page 6-7
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☑	☐	Page6, Figure 1
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☑	☐	Page 6-7
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☑	☐	Page 7 and 16
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☑	☐	Page 6-7
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☑	☐	Page 7 and 16
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☑	☐	Page 7-8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☑	☐	Page 8-9

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☑	☐	Page 8-9
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☑	☐	Page 7
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☑	☐	Page 8-9
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☐	☑	N/A

BMJ Open

Resting-state functional reorganization in Alzheimer's disease and amnestic mild cognitive impairment: protocol for a systematic review and meta-analysis

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Resting-state functional reorganization in Alzheimer's disease and amnestic mild cognitive impairment: protocol for a systematic review and meta-analysis

Diyang Lyu[1#], Taoran Li[1#], Xuanxin Lyu[2*]

1 Capital Medical University, Beijing, 100069, China
2 Neurological Rehabilitation Center, Beijing Rehabilitation Hospital, The Affiliated Hospital of Capital Medical University, Beijing, 100144, China

#These authors contributed equally to this work.
*Correspondence to: Dr. Xuanxin Lyu, lvxvanxin@163.com

ABSTRACT

Introduction: The incidence of Alzheimer's disease (AD) is increasing rapidly, causing a growing burden to health and economic worldwide. Several clinical trials in the past decade failed to find solutions, and there remains a lack of an effective treatment. The evidence suggests that early intervention for neurodegeneration would likely be effective in preventing cognitive decline. Cognitive decline in AD occurs continuously over a long period; however, there remains a lack of simple, rapid, and accurate approach for diagnosis of amnestic mild cognitive impairment (aMCI) or subjective cognitive decline (SCD) due to underlying Alzheimer's pathology. Resting-state functional magnetic resonance imaging (rs-fMRI) determines the functional activities of the human brain non-invasively. The amplitude of low-frequency fluctuation (ALFF), fractional ALFF (fALFF), and regional homogeneity (ReHo) are rs-fMRI indicators with high repeatability. They have been studied as early diagnostic imaging markers for other diseases and may be promising markers also for AD.

Methods and analysis: The following electronic literature databases will be searched from inception to December 2021: Medline-Ovid, Medline-PubMed, EMBase-Ovid, Cochrane Central, and ClinicalTrials.gov. Two independent reviewers will select studies with eligible criteria, extract data, and assess the quality of the original studies with our quality assessment tool individually. Missing data will be requested by sending e-mails to the corresponding authors. Brain regions will be presented for ALFF/fALFF and ReHo by performing activation likelihood estimation (ALE) with the Seed-based *d* Mapping-Permutation of subject images (SDM-PSI) 6.21 software. Meta-regression will be performed to determine the potential brain regions that may strongly correlate with cognitive decline progression. Subgroup analysis, funnel plot, Egger's test, and sensitivity analysis will be conducted to detect and explain potential heterogeneity.

Ethics and dissemination: This study does not require formal ethical approval. The findings will be submitted to a peer-review journal.

PROSPERO registration number: CRD42021229009

Key words: Alzheimer's disease, amnestic mild cognitive impairment, systematic review, meta-analysis, rs-fMRI, ALFF, ReHo

Strengths and limitations of this study

- This systematic review and meta-analysis will summarize brain regions with ALFF/fALFF or ReHo alterations in AD and aMCI patients using qualitative and quantitative analyses.

- This study will consider AD and aMCI as different stages of cognitive decline and conduct a meta-regression analysiswith the pooled population to explore the brain regions closely related to the stages of cognitive decline.
- We established a modified quality assessment tool to assess the quality of the original studies for rs-fMRI systematic review and meta-analysis.
- We will only retrieve data from English-language databases and may overlook a few valuable original studies in other languages.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease that is causing a growing burden on health and economic[1]. AD will reportedly affect 131 million people worldwide by 2050 and will cause over $2 trillion in economic losses by 2030[2]. However, a satisfactory therapeutic breakthrough in the field of AD is still lacking. Since the approval of memantine in 2003[3], all clinical trials of new drugs developed for the different pathogeneses of AD have failed to improve clinical outcome[4]. The molecular targeted therapies can reduce the pathological products of AD in the human brain but it is yet uncertain if they are effective in AD[5]. The failure of such therapies has been attributed to the fact that most of the study participants were patients with moderate or severe AD. These patients exhibit pathological changes in the brain, which may be irreversible or challenging to stop the progression, and the optimal time for treatment may have already elapsed[6 7]. Thus, the early diagnosis and treatment of patients with AD may be the key to halt the disease progression. In the Alzheimer's continuum, patients in the predementia stages may exhibit clinical symptoms difficult to identify, such as mild cognitive impairment (MCI) or subjective cognitive decline (SCD). Efficiently and accurately differentiating them from cognitively healthy individuals through clinical information and neuropsychological scales is complicated, especially for clinicians who are not experts in the AD field[8]. Therefore, there is currently a high demand for convenient and reliable markers for the diagnosis of AD[9].

The accurate diagnosis of AD is usually confirmed by autopsy; however, in the recent decade, with the development of laboratory tests and neuroimaging, scientists can directly detect biomarkers in vivo to identify patients with AD[9-11]. These biomarkers include detection of amyloid-β (Aβ) and hyperphosphorylated tau, found in the plasma, cerebrospinal fluid[6], and with positron emission tomography (PET) imaging[12]. However, interlaboratory variations lead to a lack of robustness of these biomarkers for early diagnosis. Although several other biomarkers have been proposed, repeated validation is still required to prove their reliability[13 14]. In addition, these examinations can be harmful through vascular punctures, lumbar punctures, or the administration of radioactive substances. Conversely, magnetic resonance imaging (MRI) can non-invasively visualize structural and functional changes in the brain. The current diagnostic criteria are mainly based on the structural MRI reports of atrophy of the whole brain, medial temporal lobe, or hippocampus, and other brain alterations[12]. However, functional MRI (fMRI) may reflect the brain's functional state through the changes in cerebral blood-oxygen signal, detect brain abnormalities before detectable structural changes, and indicate risk of cognitive decline.

Resting-state fMRI (rs-fMRI) is a non-invasive, harmless, and efficient imaging detection method with high spatial resolution, showing the functional status of the central nervous system. Since 1995, rs-fMRI has been increasingly used in scientific research[15]. Several indicators of rs-fMRI have been used to reflect functional activity, including functional connectivity[16], the amplitude of low-frequency fluctuation (ALFF)[17], fractional ALFF (fALFF)[18], and regional homogeneity (ReHo)[19]. Among the

commonly used indicators, ALFF/fALFF and ReHo are reported to have relatively higher test-retest reliability than other biomarkers[20], and the calculation process does not require prior assumptions of the specific brain regions to be studied. ALFF is considered to represent spontaneous brain activity, and fALFF is derived from an improved algorithm of ALFF[21]. ReHo assesses the synchronization among one voxel and its neighbors (e.g., 26 voxels) and is considered to represent the homogeneity of a given cluster[21]. These indicators were also proven to correlatewith disease progression in other diseases through general linear models or machine learning approaches and are considered potential diagnostic markers[22-24]. However, fMRI is still used as an additional resource in conjunction with other tests. We speculate that ALFF/fALFF and ReHo may be promising imaging markers for the early diagnosis of AD.

Previous studies in the field of AD or amnestic MCI (aMCI) have reported several brain regions with increased or decreased ALFF/fALFF or ReHo[25 26]. However, few studies have examined patients with SCD[27]. Uniform diagnostic criteria for SCD was not established for decades, resulting in the heterogeneity of subjects in different studies[27]. This lack of clarity may be due to the concept of SCD was introduced in recent years[28], its definition and clinical significance has not been fully clarified. Additionally, SCD is a naturally heterogeneous state and a construct that is greatly affected by psychosocial factors including cultural background[29 30]. Thus, this systematic review and meta-analysis will exclude SCD-related studies from pooled estimations if there are not enough original studies with high quality and consistent diagnostic criteria. A previous meta-analysis summarized 12 original studies and reported that eight brain regions showed altered ALFFs in aMCI patients compared to healthy controls[25]. Another meta-analysis included ten original studies of aMCI patients and reported 11 brain regions with altered ReHos in these patients compared to healthy controls[26]. The above reviews were published severalyears ago, and their findings might change with recent original studies.

Objective

We will conduct this systematic review and meta-analysis to summarize previous rs-fMRI studies comparing patients with AD or aMCI to adults with normal cognition and determine the group differences in ALFF/fALFF or ReHo. SCD studies will also be included, if available. Furthermore, we will also identify the brain regions associated with the severity of the disease or cognitive decline by using meta-regression. Brain regions with increased or decreased ALFF/fALFF or ReHo will be determined through a meta-analysis, and reported for potential incorporation in clinical practice and the establishment of diagnostic criteria.

METHODS AND ANALYSIS

Study guidelines and registration

This systematic review and meta-analysis will include studies reporting ALFF/fALFF or ReHo in AD or aMCI patients compared with cognitively healthy controls. Thus, the

Quality of Reporting of Meta-Analyses guidelines is not applicable for this study[31]. The guidelines of the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement were updated from QUOROM and apply to all types of systematic reviews[32]. Meta-analysis of Observational Studies in Epidemiology (MOOSE) has also been established for meta-analysis of observational studies[33]. Therefore, a systematic review and meta-analysis will be conducted and presented following the PRISMA statement[32], MOOSE guidelines[33] and Cochrane Handbook[34]. This protocol follows the PRISMA protocols (PRISMA-P)[35] statement and is registered on the International Prospective Register of Systematic Reviews (PROSPERO) (registration number CRD42021229009).

Search strategy

We will search the following electronic databases from inception to December 2021 for published literature: Medline-Ovid, Medline-PubMed, EMBase-Ovid, and Cochrane Central. We will also search the ClinicalTrials registration platform for missing, unpublished, or ongoing studies. Two independent reviewers (DL and TL) will also examine the reference lists of each article that qualifies for the full-text screening step and of each review article in this field. After data extraction, we will send e-mails to the corresponding authors of the included studies for additional information to avoid potentially missing data. The search strategy for Medline-PubMed is presented in Table 1, and the full search strategy is presented in Supplementary File 1.

Eligibility criteria

Studies will be included in the systematic review and meta-analysis according to the following criteria:

i) Patients: The patients enrolled in the original studies have been diagnosed with mild, moderate or severe AD or aMCI according to clearly reported diagnostic criteria. We will not limit the exact diagnostic criteria in the original studies, although there are some of the most commonly used criteria for AD[9 12] or aMCI[36 37]. The exact scores for cognitive assessments are preferably reported; however, a specific format is not required. We will not restrict data collection on the age, sex, or race of participants in the original studies; however, it will be reported in detail. Moreover, studies focused on AD or aMCI with other complications, such as post-dementia depression, are eligible if there are clear descriptions in the literature.

ii) Controls: The control group will include cognitively healthy subjects with comparable demographic characteristics to the AD or aMCI group in the original studies.

iii) Outcomes: Only rs-fMRI studies will be included. The original studies should report the results from whole-brain analysis, rather than analyses of specific brain networks or regions. Brain regions with increased or decreased ALFF/fALFF or ReHo will be included in the pooled estimation through activation likelihood estimation (ALE) analysis. The outcomes will preferably be reported with the peak coordinates, cluster size, and statistics of each brain region. Additionally, only the studies which mentioned the use of multiple comparison correction will be included in the meta-analysis to avoid

significant false-positive results induced by the authors[15], while all results reported in these studies will be put into pooled estimation to avoid missing important data.
iv) Study design: Observational studies with or without further follow-up measurements will be included. Controlled clinical trials that reported the differences between patients and controls at baseline will also be included if they meet the above criteria.

Study selection

Two independent reviewers (DL and TL) will screen the titles and abstracts of the articles found in each electronic database after removing duplications with EndNote X9 software. The studies that are inconsistent with our eligibility criteria will be excluded by the each reviewer individually. Then, the reviewers will examine the full text of the articles and further exclude those that do not meet our criteria. The reasons for each exclusion in this step will be recorded individually. An independent reviewer (XL) will solve any disagreement between the two reviewers, which will be recorded with detailed explanation. The complete process of the study selection will be presented in a PRISMA flow diagram (Figure 1).

Data extraction

The same reviewers (DL and TL) will extract demographic information, the study design, data analysis, and outcomes individually using Microsoft Excel. The complete information and data extraction lists are presented in Table 2. Demographic information includes age, sex, nationality, race, apolipoprotein E genotype (*APOE*), years since first symptom or initial diagnosis, education level, and cognitive assessment scores. Information about the disease stage will be extracted directly from the original articles and determined through their clinical dementia rating (CDR) scores (0.5 for aMCI, 1 for mild AD, 2 for moderate AD, and 3 for severe AD)[38]. Information about the study design and data analysis will include the field strength and exact machine model of the MRI scanner, the statistical method of multiple comparison correction, the software and packages used, the frequency range for ALFF/fALFF, the number of neighboring voxels for ReHo, and the parameter of full width at half maximum (FWHM) smooth kernel of each original study. The results include the anatomical labels, peak coordinates, and cluster size of each reported brain region from the original studies, and the cognitive assessment outcomes, such as the Mini-Mental State Examination scores. Continuous variables will be recorded as means ± standard deviations (SD), and discrete variables will be recorded in percentages. Values reported as median with range or interquartile range will be converted into mean ±SD with validated algorithms[39 40]. The recorded data will be verified by comparing the reports of the two reviewers. In case of studies without multiple comparison correction or with missing data, we will also contact the corresponding authors as necessary.

Quality assessment

To our knowledge, there is no standard checklist or tool for the quality assessment of fMRI studies. Thus, we have developed a quality assessment tool for this study, as

shown in Table 3, based on a previous meta-analysis[26 41]. The two independent reviewers (DL and TL) will examine the full texts for any potential bias according to our quality assessment tool and grade each original study. The total quality assessment score will be reported in the main text, and a detailed quality assessment table will be provided in our systematic review and meta-analysis.

Qualitative and quantitative synthesis

Qualitative data synthesis

First, we will create a summary table to present the characteristics of the selected studies, including the information to be extracted, such as the publication year, demographic characteristics of participants, study design, analysis parameters, and outcome indicators, as shown in Table 2. Then, we will provide a general summary of the study outcomes.

Quantitative data synthesis

We will conduct a quantitative analysis of each outcome. First, all brain coordinates will be converted to their corresponding Montreal Neurological Institute (MNI) space. Then, we will perform an ALE meta-analysis for ALFF/fALFF and ReHo. The pooled estimations will reveal consistent brain regions with significant functional alterations reported from different studies using the Seed-based *d* Mapping-Permutation of subject images (SDM-PSI) 6.21 software (www.sdmproject.com)[42]. The SDM-PSI algorithm is updated using the ALE algorithm. Compared to the software using the ALE algorithm, SDM-PSI can retain positive and negative activation results and avoid bias due to overlapping activation positions in the brain. The peak coordinates in the MNI space and their effect sizes (e.g., t-values) extracted from original studies will be registered as centers in the 3D Gaussian probability distribution to recreate standard MNI brain maps for each study[43 44]. Subsequently, the brain maps will be used to generate pooled estimations for each outcome separately. The sample size, sex, age, and other demographic information of each group will also be used to achieve a linear mixed model analysis to control the effect of potential confounding factors. We will perform correlation analyses or partial correlation analyses to exclude variables with strong collinearity. Finally, a principal component analysis will be performed to extract several principal components as necessary; the principal components will be used as variables. The above analyses will be performed with R v4.0.3. We will also employ a family-wise error correction to control the false-positive rate.

We will further analyze the results with subgroup analyses to detect potential heterogeneity and explain its possible reasons. The data from different disease stages (AD or aMCI), with or without complications, and MRI scanners with different field strengths (1.5T or 3.0T) will be analyzed separately. After the subgroup analyses, we will perform a sensitivity analysis by excluding studies one by one to determine whether the pooled estimations are stable or not. In this step, significant changes may indicate significant heterogeneity among the studies. Significant heterogeneity will be reported upon occurrence. We will apply funnel plots to detect potential reporting biases, when

no less than ten original studies are pooled in a meta-analysis[45]. We will attempt to identify possible reasons and interpret existing publication biases. We will perform a meta-regression to identify potential factors that may contribute to the stage-specific changes in different brain regions, we will perform a meta-regression. In this step, we will put all included studies into pooled estimation. The following variables will be analyzed: age, sex, cognitive assessment score, and *APOE* genotype. We will perform a meta-regression using the SDM-PSI software 6.21. We will contact the corresponding authors via e-mail To retrieve missing data; if these values are unavailable, we will remove the related regression factors or the original study.

Patient and public involvement

As this is a protocol for a systematic review and meta-analysis, we will obtain data from published literature or the corresponding authors. Thus, patients or the public will not be involved.

ETHICS AND DISSEMINATION

This study does not require any formal ethical approval. The findings will be submitted for publication in a peer-reviewed journal.

DISCUSSION

This systematic review will comprehensively summarize and analyze the results of previous fMRI studies investigating AD or aMCI using ALFF/fALFF or ReHo. We will present whole-brain ALFF/fALFF and ReHo analyses and report significant differences between patients with AD or aMCI and cognitively healthy controls.

A previous meta-analysis summarized 12 original studies and reported that 4 brain regions exhibited decreased ALFFs, while another four showed increased ALFFs in aMCI patients compared with healthy controls[25]. The authors also reported that a greater decrease in ALFFs in the cuneus/precuneus cortex may be associated with the severity of cognitive impairment[25]. Another meta-analysis included 10 original studies of patients with aMCI and focused on ReHo[26]. The authors found that the ReHo of 11 brain regions from four brain networks differed between aMCI patients and healthy controls. The above meta-analyses were conducted several years ago, and their results varied. However, these studies included AD and aMCI only, and we will also include studies on SCD, if possible. Furthermore, our meta-analysis may provide different estimations by including recent studies in this field.

To our knowledge, only one previous meta-analysis investigated the functional characteristics of AD and aMCI patients compared with healthy controls through both ALFF/fALFF and ReHo[46]. Their findings revealed that patients with aMCI and AD exhibited consistently impaired functional characteristics, and the alterations in the brain regions were relatively consistent. Although this study was published six years

ago, the findings support our idea of combining studies of AD and aMCI patients, including SCD studies if possible, and attempting to determine the brain regions that have altered ALFF/fALFF or ReHo with strong correlations with cognitive decline measured by neuropsychological scales through a meta-regression.

It is generally known that there are various limitations of fMRI including it being expensive and need of specific expert interpretation so that it can used by non-experts to applying in various clinical scenarios. However, previous studies have reported a large amount of data in this field, making it feasible for scientists to establish diagnostic tools for clinician use through data mining or machine learning. Similarly, our findings may highlight utility of fMRI as an adjunctive tool in early diagnosis of AD and predicting cognitive decline in addition to other diagnostic tools.

Author Contributions

DL and XL designed this study. DL and TL developed the search strategy, established the data extraction list. DL drafted the manuscript. TL and XL revised the manuscript and provided methodological perspectives. DL and TL will search and screen the literature and perform data extraction. DL will assess the quality of included studies and conduct data analyses. All authors read and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

Patient consent for publication

Not applicable.

Data sharing statement

Results of the current review will be disseminated through peer-reviewed publications.

Provenance and peer review

Word Count

Main text: 2873 words Abstract: 290 words Figure: 1 Tables: 3

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Table 1. Search strategy for Medline-PubMed.

Search	Query
#1	(alzheimer*[Title/Abstract]) OR (alzheimer disease[MeSH Terms])
#2	(dementia[MeSH Terms]) OR (dement*[Title/Abstract])
#3	(((((MCI[Title/Abstract]) OR ("mild cognitive impairment"[Title/Abstract])) OR ("amnestic mild cognitive impairment"[Title/Abstract])) OR (aMCI[Title/Abstract])) OR (SCD[Title/Abstract])) OR ("subjective cognitive decline"[Title/Abstract])
#4	(cognit*[Title/Abstract]) OR (memor*[Title/Abstract])
#5	((impair*[Title/Abstract]) OR (decline[Title/Abstract])) OR (reduc*[Title/Abstract])
#6	#4 and #5
#7	(((((("functional magnetic resonance imaging"[Title/Abstract]) OR ("magnetic resonance imaging"[Title/Abstract])) OR ("resting-state functional magnetic resonance imaging"[Title/Abstract])) OR ("resting state functional magnetic resonance imaging"[Title/Abstract])) OR (fMRI[Title/Abstract])) OR ("functional MRI"[Title/Abstract])) OR (MRI[Title/Abstract])) OR ("rs-fMRI"[Title/Abstract])
#8	(((((("amplitude of low frequency fluctuation*"[Title/Abstract]) OR ("fractional amplitude of low frequency fluctuation*"[Title/Abstract])) OR ("regional homogeneit*"[Title/Abstract])) OR (ReHo*[Title/Abstract])) OR (ALFF*[Title/Abstract])) OR (fALFF*[Title/Abstract])
#9	#1 or #2 or #3 or #6
#10	#7 and #8 and #9

Table 2. Data and information extraction list.

Item	Content
Publication information	Authors, publish year, e-mail of the corresponding author, country.
Demographic data	Age, sex, human race, right-handed or not, *APOE* genotype, sample size, education level, diagnostic criteria, years since first symptom or first diagnosis, disease stage, cognitive assessment scores.
Data acquisition	The field strength and the exact machine model of the MRI scanner, slice numbers and thickness of each sequence, time points and total scanning time of functional MRI.
Outcome data	Coordinate space, anatomical label, peak coordinate, cluster size, t-value or other statistics.
Data processing	Software used, number of time points to be discarded, resampling parameters, full-width at half-maximum smooth kernel size, variables to be regressed out, the frequency range for ALFF/fALFF and the number of neighboring voxels for ReHo, method to control false positive rate.

ALFF, amplitude of low frequency fluctuation ; fALFF, fractional ALFF;*APOE*, apolipoprotein E; MRI, magnetic resonance imaging; ReHo, regional homogeneity.

Table 3. Quality assessment tool of original fMRI studies.

	Category 1: sample characteristics (10)
1	Participants were enrolled with clearly described standardized diagnostic criteria (2).
2	Comprehensive demographic data with comparable baseline between groups (1).
3	Inclusion and exclusion criteria are reasonable, taking into account the possible affecting factors (2).
4	Cognitive outcomes were reported in detail (3).
5	Sample size >10 in each group (2).
	Category 2: methodology and reporting (10)
6	The machine model and field strength of the MRI scanner is reported (1).
7	A clear description of the study procedure and quality control, includes the methods to ensure that the subjects are in a "resting state" (2).
8	At least 5 min of resting state acquisition (2).
9	The report of scanning parameters is comprehensive and reasonable, and the quality control method of image scanning is reported (1).
10	Detailed description of software and toolkits used (1).
11	Results were applied and reported in original literature, including peak coordinate and cluster size of each brain region (1).
12	Multiple comparison corrections were applied and reported in the original literature (1).
13	Conclusions were consistent with the results obtained and the limitations were discussed (1).

Figure 1. The PRISMA flow diagram of this systematic review and meta-analysis.

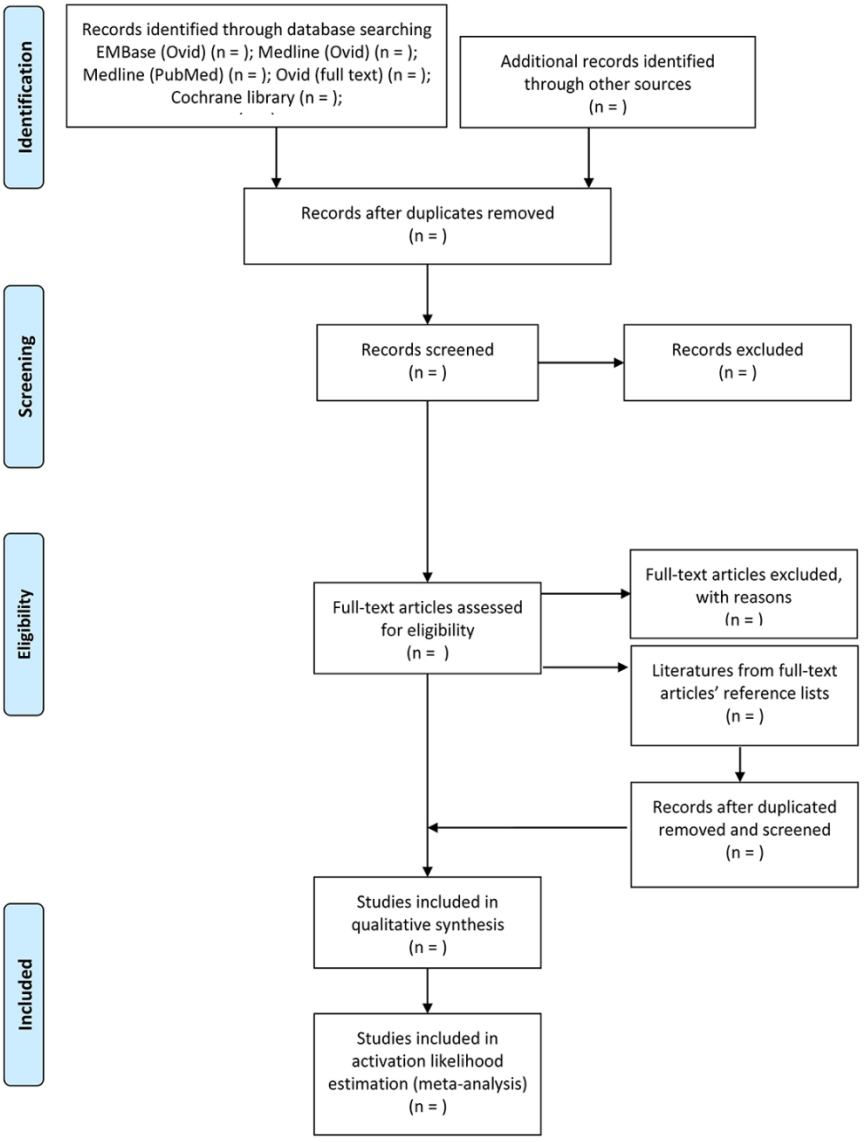

Figure 1. The PRISMA flow diagram of this systematic review and meta-analysis.

99x129mm (300 x 300 DPI)

Supplementary File 1. Search strategies.

Search strategy for Medline-PubMed:

#1 (alzheimer*[Title/Abstract]) OR (alzheimer disease[MeSH Terms])
#2 (dementia[MeSH Terms]) OR (dement*[Title/Abstract])
#3 (((((MCI[Title/Abstract]) OR ("mild cognitive impairment"[Title/Abstract])) OR ("amnestic mild cognitive impairment"[Title/Abstract])) OR (aMCI[Title/Abstract])) OR (SCD[Title/Abstract])) OR ("subjective cognitive decline"[Title/Abstract])
#4 (cognit*[Title/Abstract]) OR (memor*[Title/Abstract])
#5 ((impair*[Title/Abstract]) OR (decline[Title/Abstract])) OR (reduc*[Title/Abstract])
#6 #4 and #5
#7 (((((("functional magnetic resonance imaging"[Title/Abstract]) OR ("magnetic resonance imaging"[Title/Abstract])) OR ("resting-state functional magnetic resonance imaging"[Title/Abstract])) OR ("resting state functional magnetic resonance imaging"[Title/Abstract])) OR (fMRI[Title/Abstract])) OR ("functional MRI"[Title/Abstract])) OR (MRI[Title/Abstract])) OR ("rs-fMRI"[Title/Abstract])
#8 (((((("amplitude of low frequency fluctuation*"[Title/Abstract]) OR ("fractional amplitude of low frequency fluctuation*"[Title/Abstract])) OR ("regional homogeneit*"[Title/Abstract])) OR (ReHo*[Title/Abstract])) OR (ALFF*[Title/Abstract])) OR (fALFF*[Title/Abstract])
#9 #1 or #2 or #3 or #6
#10 #7 and #8 and #9

Search strategy for Medline-Ovid and EMBase-Ovid:

#1 exp alzheimer disease/ or dementia/
#2 (alzheimer* or dement*).tw.
#3 (MCI or “mild cognitive impairment” or “amnestic mild cognitive impairment” or aMCI or SCD or “subjective cognitive decline”).tw.
#4 (cognit* or memor*).tw.
#5 (impair* or decline or reduc*).tw.
#6 #4 and #5
#7 (“functional magnetic resonance imaging” or “magnetic resonance imaging” or “resting-state functional magnetic resonance imaging” or “resting state functional magnetic resonance imaging” or fMRI or “functional MRI” or MRI or “rs-fMRI”).tw.
#8 (“amplitude of low frequency fluctuation” or “fractional amplitude of low frequency fluctuation” or “regional homogeneity” or REHO* or ALFF* or fALFF*).tw.
#9 #1 or #3 or #6
#10 #7 and #8 and #9

Search strategy for Cochrane Central:

#1 'Alzheimer disease':ti,ab,kw OR 'dementia':ti,ab,kw OR 'alzheimer*':ti,ab,kw OR 'dement*':ti,ab,kw OR 'MCI':ti,ab,kw OR 'mild cognitive impairment':ti,ab,kw OR 'amnestic mild cognitive impairment':ti,ab,kw OR 'aMCI':ti,ab,kw OR 'SCD':ti,ab,kw OR 'subjective cognitive decline'

#2 ('cognit*':ti,ab,kw OR 'memor*':ti,ab,kw) AND ('impair*':ti,ab,kw OR 'decline':ti,ab,kw OR 'reduc*':ti,ab,kw)

#3 'functional magnetic resonance imaging':ti,ab,kw OR 'magnetic resonance imaging':ti,ab,kw OR 'resting-state functional magnetic resonance imaging':ti,ab,kw OR 'resting state functional magnetic resonance imaging':ti,ab,kw OR 'fMRI':ti,ab,kw OR 'functional MRI':ti,ab,kw OR 'MRI':ti,ab,kw OR 'rs-fMRI':ti,ab,kw

#4 'amplitude of low frequency fluctuation':ti,ab,kw OR 'fractional amplitude of low frequency fluctuation':ti,ab,kw OR 'regional homogeneity':ti,ab,kw OR 'REHO*':ti,ab,kw OR 'ALFF*':ti,ab,kw OR 'fALFF*':ti,ab,kw

#5 #1 OR #2

#6 #3 AND #4 AND #5

Search strategy for clinicaltrials.gov:

Condition or disease: Alzheimer Disease OR MCI
Study type: All Studies
Study Results: All Studies
Outcome Measure: MRI or magnetic resonance imaging

PRISMA-P 2015 Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in: Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☑	☐	Page 1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☑	NA
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☑	☐	Page2
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☑	☐	Page1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☑	☐	Page 10
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☐	☑	NA
Support					
Sources	5a	Indicate sources of financial or other support for the review	☑	☐	Page 10
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☑	NA
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☑	NA
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☑	☐	Page 4-5

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☑	☐	Page 5
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☑	☐	Page 6
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☑	☐	Page 6-7
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☑	☐	Page 15
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☑	☐	Page 6-7
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☑	☐	Page6, Figure 1
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☑	☐	Page 6-7
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☑	☐	Page 7 and 16
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☑	☐	Page 6-7
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☑	☐	Page 7 and 16
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☑	☐	Page 7-8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☑	☐	Page 8-9

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☑	☐	Page 8-9
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☑	☐	Page 7
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☑	☐	Page 8-9
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☐	☑	N/A