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Magnetic Resonance Imaging-based Comparative Research in Different Mild Cognitive Impairment Subtype: Study Protocol of an Observational Case-Control Study

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Abstract

Introduction:

Amnestic mild cognitive impairment (aMCI) and vascular mild cognitive impairment (VaMCI) are the two main types of mild cognitive

impairment (MCI), one would probably progress to Alzheimer's disease (AD) and the other is likely to be vascular dementia. The brain structure and function are different from normal elders. However, whether this two MCI are different in brain structure hasn't been detected. This study is designed to analysis the brain neuroimage in VaMCI and Amci with multi-modality magnetic resonance imaging (structural MRI, function MRI and diffusion tensor imaging).

Methods and analysis:

In this study, 80 subjects who are diagnosed as aMCI and 80 VaMCI will be recruited at the Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. All subjects will undergo the neuroimaging and neuropsychological evaluation. The primary outcome measures are 1) Microstructural alterations revealed with multimodal MRI scans including structure MRI (sMRI), resting state functional MRI (rs-fMRI), diffusion tensor imaging (DTI); 2) neuropsychological evaluation, including Auditory Verbal Learning Test (AVLT), mini-mental state examination (MMSE), Montreal Cognitive Assessment (MoCA), Clinical Dementia Rating scale (CDR).

Ethics and Dissemination:

Ethical approval of this has been obtained from the medical research ethics committee and institutional review board of Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. Study findings will

be disseminated widely through conference presentations and peer-reviewed publications.

Trial Registration:

Protocol Registered on ClinicalTrials.gov (NCT02706210)

Keywords: Amnestic mild cognitive impairment, Vascular mild cognitive impairment, Neuropsychological, Neuroimaging techniques,

Introduction

As people live longer, so chronic diseases become more prevalent. Dementia is a progressive brain disease, which is one of the main chronic non-communicable diseases associated with disability and mortality among elderly individuals. Dementia resulting from Alzheimer's disease (AD) has catapulted into the public's consciousness while dementia has many causes—not just Alzheimer's, vascular dementia (VD) is also considered to be the main types of dementia. Mild cognitive impairment (MCI) constitutes an intermediate stage between normal aging and dementia[1]. A widely shared view is that future treatment strategies need to focus on treatment of the MCI.

Amnestic MCI (aMCI) has been reported to be the clinical transition stage of AD[2] and vascular MCI (VaMCI) is considered to be the early stage of VD[3 4]. The pathogenesis of the two diseases are different, the aMCI is considered to be neurodegenerative disease caused by the

endogenous neuronal factors such as tau and amyloid pathology[5] while VaMCI is caused by vascular diseases such as infarcts or profuse white matter disease [3 6]. Both VaMCI and aMCI are associated with deficits in multiple cognitive domains, with the same chief complaints in memory deficits[7 8]. A considerable body of literature has concluded that patients with VaMCI generally shows more impairment in semantic memory, executive/attentional functioning, and visual-spatial and perceptual skills, whereas the clinical picture of AD is characterized by deficits in episodic memory[3 9 10]. Under some specific neuropsychological tests, they may have different performances. However, few studies have examined the relationship between these two kinds of MCI in their brain structural and cognitive behavior. If it can be clear which will turn into what type of dementia in patients with cognitive impairment stage, it may be helpful to the understanding of the mechanism of the two MCI.

According to previous publications, aMCI and VaMCI are considered to be a kind of "disconnection syndrome"[11 12]. By applying the structural magnetic resonance imaging (sMRI), resting state functional magnetic resonance (fMRI) and diffusion tensor magnetic resonance imaging (DTI) technology, the project comprehensive analysis comparison of brain structure and function in patients with aMCI and VaMCI. Previous studies have detected the atrophy of brain volume, reduced white matter integrity and abnormal functional connectivity in those MCI patients[13-17].

Previous studies also have detected that the brain structures impaired in VD seem not consistent with AD[18 19], but whether they are different in MCI hasn't been detected. Moreover, the changes in brain structural and function are not completely similar[20-22]. Unlike using a single technique, by using multimodal magnetic resonance, the brain structure and function can be analyzed omni-directionally and multi-angularly. By using multimodal magnetic resonance could also consolidate our understanding of how functional networks interact with their structural substrates.

This project in order to reveal the cognitive impairment disease neural circuits in the development of the network connection and its change rule. People can further understand the pathogenesis of cognitive impairment, discover the relationship between brain structure, function and cognitive behavior.

Methods

Subject

The recruited patients were patients (inpatients and/or outpatients) who were registered at the Neurology Department of Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. All participants received baseline evaluation, including complete sociodemographic and clinical data collection. Patient histories were collected from informants, usually from their spouses or children. The diagnosis for the two groups was performed by two experienced neurologists, respectively. This study

was approved by the medical research ethics committee and the institutional review board of Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. The study was conducted in accordance with the approved guidelines. Written informed consent was obtained from all participants.

Inclusion Criteria

Criteria for aMCI

Diagnosis of aMCI was made based on the recent international consensus criteria, which were adapted as follows[1 3 23-25]: 1) subjective cognitive complaints reported by the informant; 2) objective cognitive impairments that do not meet the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-R, fourth-revised edition) criteria for dementia; 3) normal or near-normal performance of general cognitive functioning and no or minimum impairments in daily life activities; 4) abnormal memory function, documented by extensive neuropsychological evaluation; normal general cognitive function, Clinical Dementia Rating Scale (CDR) score = 0.5; 5) Hippocampal atrophy confirmed by structural MRI was met simultaneously in the aMCI group[1]. 6) Neuropsychological testing included a Hanchinski ischemic (HIS) score (HIS score ≤ to 4) and Montreal cognitive assessment score (MoCA, Beijing Version)[26].

Criteria for VaMCI

Diagnosis of VaMCI depended on the following criteria[1 3 17 26]: 1) subjective cognitive complaints reported by the participant or his/her caregiver; 2) insufficient cognitive impairment to meet the DSM-IV-R criteria for dementia; vascular etiology as follows: cognitive impairment due to subcortical small vessel disease (SIVD) was defined as moderate white matter changes (at least 1 region score <2 according to the Wahlund rating scale), and/or multiple lacunar infarcts (<2) on brain imaging. And may or not be suggested by minor neurological signs (such as hemiparesis, lower facial weakness, Babinski sign, sensory deficit, dysarthria, gait disorder, urine urgency, or motor slowness). Neuropsychological testings for aMCI, the MoCA scoring, and HIS determination (HIS ≥ 7) were also performed.

Exclusion Criteria

Participants were excluded if they had the following: 1) psychiatric disease (eg, depression, Hamilton depression rating scale >20, Center for Epidemiologic Studies Depression Scale >21), systemic disease (eg, brain tumor, Parkinson disease, encephalitis or epilepsy) or other neurological disorder, 2) visual or auditory abnormalities, severe aphasia or palsy that made clinical assessments infeasible; 3) any medical or psychological conditions that might interfere with clinical and neuropsychological assessment, 4) insufficient Mandarin language abilities to complete the assessment.

The exclusive criteria for VaMCI also included the following: 1) signs of large vascular disease, such as cortical, and/or cortico-subcortical, or non-lacunar territorial infarcts and watershed infarcts or hemorrhages, 2) patients who may also be considered as aMCI. For aMCI, participants, those who were considered as VaMCI were excluded. This selection procedure was applied to ensure the purity of each group.

Neuropsychological Evaluations

All participants received a battery of neuropsychological tests to assess general mental status and other cognitive domains. These tests included the CDR scale[1 25], the Mini-Mental State Examination (MMSE)[27], the Montreal Cognitive Assessment (MoCA)[26], and the Auditory Verbal Learning Test (AVLT)[28], and also Hamilton Depression Scale (HAMD)[29].

Magnetic resonance brain imaging Imaging protocol

For each participant, conventional brain T1-weighted (T1WI), T2-weighted (T2WI) will be obtained to exclude serious brain diseases. For inpatients, the data will be collected at Hongqi Hospital of Mudanjiang Medical University while for outpatients, the T1WI and T2WI may be acquired from other hospitals. The imaging data for analyzing will be acquired using a 3.0 T Trio Siemens scanner at Hongqi Hospital of Mudanjiang Medical University.

sMRI

Using a sagittal MP-RAGE sequence with the following imaging parameters: TR = 1900 ms; TE = 2.2 ms; inversion time = 900 ms; flip angle = 9°; FOV = 256 mm × 256 mm; matrix = 256 × 256;176 slices, thickness = 1.0 mm.

MRI data acquisition was performed on a 3.0 T Siemens scanner by employing a sagittal magnetization-prepared rapid gradient echo (MP-RAGE) sequence with the following imaging parameters: repetition time (*T*R) = 1900 ms; echo time (*T*E) = 2.2 ms; inversion time = 900 ms; flip angle = 90∘ ; field of view (FOV) = 250 mm × 250 mm; matrix = 256×256; 176 slices, thickness = 1.0 mm. Brain MR images were inspected by an experienced neuroradiologist, and no gross abnormalities were observed for any subject.

fMRI

Using an echo-planar imaging sequence with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 40 ms, flip angle (FA) = 90°, number of slices = 28, slice thickness = 4 mm, gap = 1 mm, voxel size = 4 × 4 × 4 mm^3, and matrix = 64 × 64. Participants will be asked to lie quietly in the scanner with their eyes closed during data acquisition. Each scan lasted for 478 s.

DTI

Using an echo planar imaging (EPI) sequence in 32 independent, non-collinear directions of a b-value = 1000 s/mm^2 , and one additional

image with no diffusion weighting (b = 0). TR = 11000 ms, TE = 98 ms, flip angle = 90°, field of view (FOV) = 256 mm × 256 mm, imaging matrix = 128 × 128, number of slices = 60, and slice thickness = 2 mm. Three acquisitions will be averaged to increase the signal-to-noise ratio.

MRI image analysis

sMRI data analysis

Both the cortical reconstruction and morphological features extraction will be obtained by using the FreeSurfer software (http://surfer.nmr.mgh.harvard.edu/) with a standard cortical automatic handling protocol. First, the data will be normalized to a standard anatomical template[30] and corrected for bias-field inhomogeneity. Subsequently, the images will be skull-stripped using a watershed algorithm[31] and subsequently segmented into subcortical white matter and deep gray matter volumetric structures[32 33]. The initial tessellation will be formed by reconstructing the boundary of gray matter/white matter (white surface) and the outer cortical surface (pial surface)[34 35]. After that, a series of deformable procedures will be performed. All reconstructed surfaces will be visually inspected for gross-anatomical topological defects. Finally, a variety of morphological features at each vertex on the pial surface will be computed.

fMRI data analysis

Image preprocessing will be performed by using SPM8

(http://www.fil.ion.ucl.ac.uk/spm/) and Data Processing Assistant for Resting-State fMRI. The preprocessing procedures will be performed including removal of the first 10 volumes, slice timing, and head motion correction. All fMRI data will be satisfied the criteria of spatial movement in any direction < 3 mm or 3° and the subjects will be demonstrated no significant group differences in the head motion parameters (i.e, three translation and three rotation parameters). To normalize the fMRI data spatially, the T1-weighted images will be firstly registered to the mean functional data, and the resulting aligned T1 data set will be segmented and transformed into MNI space using the DARTEL toolbox, and a group template will be generated. Next, the motion corrected functional volumes will be specially normalized to the group template using the transfer parameter estimated by DARTEL segmentation and resampled to 3 mm isotropic voxels. Further, the functional images will be spatially smoothed with a 4 mm Gaussian kernel. The linear detrend and temporal bandpass filtering (0.01 – 0.08 Hz) will be performed to reduce the influences of low frequency drift and high-frequency physiological noise. Finally, several nuisance signals will be regressed out from the data, including the six motion parameters, the global, the white matter, and the cerebrospinal fluid signals. Functional connectivity (FC) analysis using DPARSF software (http://www.restfmri.net/forum/DPARSF).

DTI data analysis

DTI data processing will be carried out using FSL software (FMRIB Software Library, http:// www.fmrib.ox.ac.uk/fsl). Initially, eddy current correction will be run to correct gradient-coil distortions and small-head motions using affine registration to a reference image (b0 volume). The brain voxels of DTI data will be extracted using the Brain Extraction Tool (BET). The maps of diffusion tensor parameters including fractional anisotropy (FA), axial diffusivity (DA), radial diffusivity (DR), and mean diffusivity (MD) will be calculated using DTI-FIT tool, which fits a diffusion tensor model to diffusion-weighted images for each voxel. Voxel-wise statistical analysis of the FA, DA, DR, and MD data will be performed for regional differences using tract-based spatial statistics (TBSS).

Association between neuroimage and neuropsychological performance

Analysis the interaction between the parameters acquired by multimodal magnetic resonance and cognition measures.

The scheme of the current prospective trial is described in Fig. 1

Discussion

In the published paper works, the researchers usually focus on the differences between patients and normal elders, little pays attention to the differences between the similar diseases. At the stage of dementia, the

white matter microstructure has been detected different between VD and AD[18]. While it is uncertain that the differences took place in the stage of dementia or earlier. A study has demonstrated that the hippocampus structure is different in aMCI and VaMCI[36]. But what about other parts of gray matters hasn't been detected. And by using neuropsychological tests, previous study has observed the mode of memory defict in aMCI and VaMCI is also different[8]. However, there is lack of evidence to compare the relationship between those abnormal cognitive behaviors and brain structure. So it is meaningful to use multimodal magnetic resonance to observe the neuroimage in the two subtypes of MCI.

Some limitations have to be took into account about this study. First, the trial is just an observational study without follow up so whether would they convert to AD or VD in the future is uncertain. Therefore, we will plan to apply a follow-up to track the dynamic evolution of these patients. Second, our hospital is a primary hospital, we don't have a PET to raise the precision of diagnosis. But the diagnosis of the two groups follow the criteria for the diseases critically to make the diagnosis as precisely as possible. And we will plan to gather the blood and cerebrospinal fluid in our future designs.

The purpose for this study is to find out the differences in neuroimaging between aMCI and VaMCI and exploring the possible mechanism of the cognitive disorders in the two diseases. Moreover, to provide scientific

diagnosis evidence for different subtypes of MCI.

Ethics approval and consent to participate

Ethical approval of this has been obtained from the medical research ethics committee and institutional review board of Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. All participation is based on written informed consent and the participants will be able to withdraw from the study at any time.

Consent for publication

Written consent is obtained from each subject before publishing in this study.

Availability of data and material

Not applicable.

Funding

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Author Contributions

All authors participated in the design of the study. YY drafted the manuscript. YCH is supervising the project and made critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

Competing interests

All authors report no competing interests.

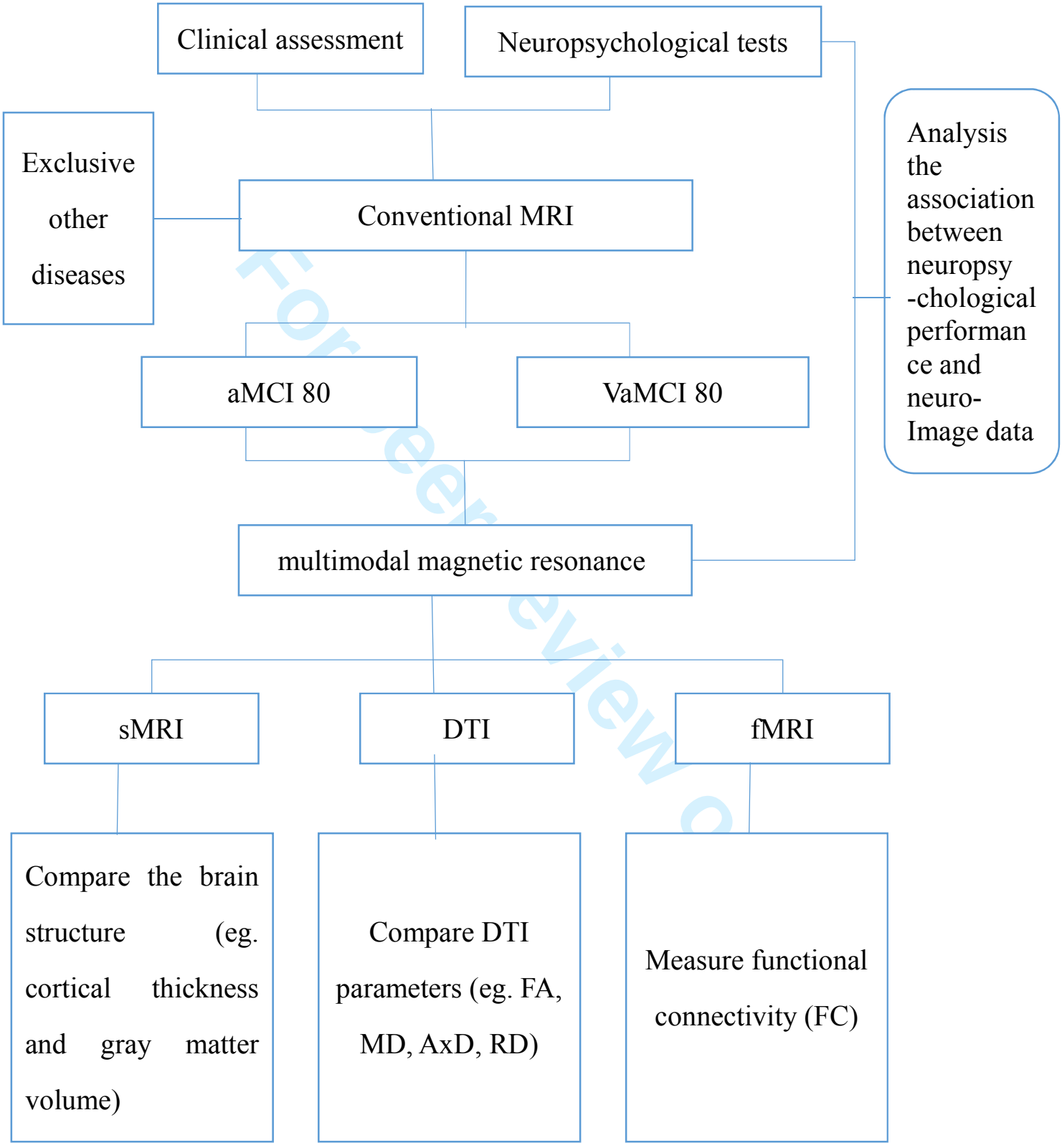

Fig 1 Flowchart of the current prospective diagnostic trial

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Abstract

Introduction:

Amnestic mild cognitive impairment (aMCI) and vascular mild cognitive impairment (VaMCI) are the two main types of mild cognitive

impairment (MCI), one would probably progress to Alzheimer's disease (AD) and the other is likely to be vascular dementia (VD). The brain structure and function of MCI are different from normal elders. However, whether this two MCI are different in brain structure hasn't been detected. This study is designed to analysis the brain neuroimage in VaMCI and aMCI with multi-modality magnetic resonance imaging (structural MRI, function MRI and diffusion tensor imaging).

Methods and analysis:

In this study, 80 subjects who are diagnosed as aMCI and 80 VaMCI and 80 age, gender and education matched normal controls (NC) will be recruited at the Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. All subjects will undergo the neuroimaging and neuropsychological evaluation. The primary outcome measures are 1) Microstructural alterations revealed with multimodal MRI scans including structure MRI (sMRI), resting state functional MRI (rs-fMRI), diffusion tensor imaging (DTI); 2) neuropsychological evaluation, including mini-mental state examination (MMSE), Montreal Cognitive Assessment (MoCA), Auditory Verbal Learning Test (AVLT), Memory and Executive Screening (MES), trail making test(TMT), the Stroop color naming condition, Clinical Dementia Rating scale (CDR), for the purpose of evaluating the global cognition, memory function, attention, visuospatial skills, processing speed, executive function, and emotion,

respectively.

Trial Registration:

Protocol Registered on ClinicalTrials.gov (NCT02706210)

Keywords: Amnestic mild cognitive impairment, Vascular mild cognitive impairment, Neuropsychological, Neuroimaging techniques,

Strengths and limitations of this study

Compare different subtypes of MCI.

Using a combination of multi-modal imaging.

Lack of Clinicopathologic diagnosis.

Introduction

With the prolongation of life span, the incidence of chronic diseases has been increasing. Dementia is a progressive brain disease, which is one of the main chronic non-communicable diseases associated with disability and mortality among elderly individuals. Dementia resulting from Alzheimer's disease (AD) has catapulted into the public's consciousness while dementia has many causes—not just Alzheimer's, vascular dementia (VD) is also considered to be the main types of dementia. Mild cognitive impairment (MCI) constitutes an intermediate stage between normal aging and dementia[1]. A widely shared view is that future treatment strategies need to focus on treatment of the MCI.

Amnestic MCI (aMCI), the rate of this MCI subtype conversion to AD

has been reported to be 10% to 15% annually, also the research spotlight has turned its to be the predementia stage of AD, so it is likely to be the clinical transition stage of AD[2 3], and vascular MCI (VaMCI) is considered to be the early stage of VD[4 5]. The pathogenesis of the two diseases are different, the aMCI is considered to be neurodegenerative disease caused by the endogenous neuronal factors such as tau and amyloid pathology[6] while VaMCI is caused by vascular diseases such as infarcts or profuse white matter (WM) disease [4 7]. A study has demonstrated that the hippocampus structure is different in aMCI and VaMCI[8]. But what about other parts of the brain hasn't been detected. Both VaMCI and aMCI are associated with deficits in multiple cognitive domains, with the same chief complaints in memory deficits[9 10]. And by using neuropsychological tests, previous study has observed the mode of memory defict in aMCI and VaMCI is also different[10]. A considerable body of literature has concluded that patients with VaMCI generally shows more impairment in semantic memory, executive/attentional functioning, and visual-spatial and perceptual skills, whereas the clinical picture of AD is characterized by deficits in episodic memory[4 11 12]. Under some specific neuropsychological tests, they may have different performances. However, few studies have examined the relationship between these two kinds of MCI in their brain structural and cognitive behavior. If it can be clear which will turn into what type of dementia in

patients with cognitive impairment stage, it may be helpful to the understanding of the mechanism of the two MCI.

According to previous publications, aMCI and VaMCI are considered to be a kind of "disconnection syndrome"[13 14]. By applying the structural magnetic resonance imaging (sMRI), resting state functional magnetic resonance (fMRI) and diffusion tensor magnetic resonance imaging (DTI) technology, we could comprehensive analysis comparison of brain structure and function in patients with aMCI and VaMCI directly and noninvasively. Previous studies have detected the atrophy of brain volume and abnormal functional connectivity in those MCI patients[15-19]. Previous studies also have detected that the brain structures impaired in VD seem not consistent with AD,especially in the corpus callosum[20 21], but whether they are different in MCI hasn't been detected. Moreover, the changes in brain structural and function are not completely similar[22-24]. Unlike using a single technique, by using multimodal magnetic resonance, the brain structure and function can be analyzed omni-directionally and multi-angularly. By using multimodal magnetic resonance could also consolidate our understanding of how functional networks interact with their structural substrates.

This project in order to reveal the cognitive impairment disease neural circuits in the development of the network connection and its change rule. People can further understand the pathogenesis of cognitive impairment,

discover the relationship between brain structure, function and cognitive behavior.

Methods

Subject

A total of 240 right-handed subjects (80 aMCI and 80 VaMCI and 80 normal controls, with age, sex and education matched) will be recruited from the Neurology Department of Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. All participants will receive baseline evaluation, including complete sociodemographic and clinical data collection. Patient histories will be collected from informants, usually from their spouses or children. The diagnosis for the participants will be performed by two experienced neurologists, respectively.

Ethical approval of this has been obtained from the medical research ethics committee and institutional review board of Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. The study will be conducted in accordance with the approved guidelines. Written informed consent will be obtained from all participants. All participation is based on written informed consent and the participants will be able to withdraw from the study at any time.

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Diagnosis of aMCI was made based on the recent international consensus criteria, which were adapted as follows[1 4 25-27]: 1) subjective cognitive complaints reported by the informant; 2) objective cognitive impairments that do not meet the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-R, fourth-revised edition) criteria for dementia; 3) normal or near-normal performance of general cognitive functioning and no or minimum impairments in daily life activities; 4) abnormal memory function, documented by extensive neuropsychological evaluation; normal general cognitive function, Clinical Dementia Rating Scale (CDR) score = 0.5; 5) Neuropsychological testing included a Hanchinski ischemic (HIS) score (HIS score ≤ to 4) and Montreal cognitive assessment score (MoCA, Beijing Version)[28].

Criteria for VaMCI

Diagnosis of VaMCI depended on the following criteria[1 4 19 28]: 1) subjective cognitive complaints reported by the participant or his/her caregiver; 2) insufficient cognitive impairment to meet the DSM-IV-R criteria for dementia; vascular etiology as follows: cognitive impairment due to subcortical small vessel disease (SIVD) was defined as moderate WM changes (at least 1 region score <2 according to the Wahlund rating scale), and/or multiple lacunar infarcts (<2) on brain imaging. And may or not be suggested by minor neurological signs.

Neuropsychological testings for aMCI, the MoCA scoring, and HIS determination (HIS ≥ 7) were also performed.

Exclusion Criteria

Participants were excluded if they had the following: 1) psychiatric disease (eg, depression, Hamilton depression rating scale >20, Center for Epidemiologic Studies Depression Scale >21), systemic disease or other neurological disorder, 2) visual or auditory abnormalities, severe aphasia or palsy that made clinical assessments infeasible; 3) any medical or psychological conditions that might affect cognitive functioning, including tranquilizers, anti-anxiolytics, hypnotics, nootropics, and cholinomimetic agents, 4) inability to undergo brain MRI, 5) insufficient Mandarin language abilities to complete the assessment.

The exclusive criteria for VaMCI also included the following: signs of large vascular disease, such as cortical, and/or cortico-subcortical, or non-lacunar territorial infarcts and watershed infarcts or hemorrhages.

Neuropsychological Evaluations

All participants received a battery of neuropsychological tests to assess general mental status and other cognitive domains. These tests included the CDR scale[1 27], the Mini-Mental State Examination (MMSE)[29], the Montreal Cognitive Assessment (MoCA)[28], and the Auditory Verbal Learning Test (AVLT)[30], Memory and Executive Screening (MES)[31], trail

making test(TMT)[32], the Stroop color naming condition[33], Clinical Dementia Rating scale (CDR), and also Hamilton Depression Scale (HAMD)[34]. Those tests are used for the purpose of evaluating the global cognition, memory function, attention, visuospatial skills, processing speed, executive function, and emotion, respectively.

Magnetic resonance brain imaging protocol

For each participant, conventional brain T1-weighted image (T1WI), T2-weighted image (T2WI) will be obtained to exclude serious brain diseases. For inpatients, the data will be collected at Hongqi Hospital of Mudanjiang Medical University while for outpatients, the T1WI and T2WI may be acquired from other hospitals. The imaging data for analyzing will be acquired using a 3.0 T Trio Siemens scanner at Hongqi Hospital of Mudanjiang Medical University.

sMRI

A high-resolution anatomical images will be acquired using a 3D magnetization-prepared rapid gradient echo (MP-RAGE) T1-weighted sequence with the following parameters: TR = 1900 ms, TE = 2.2 ms, inversion time (TI) = 900 ms, FA = 9°, number of slices = 176, slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm^3, and matrix = 256 × 256. Brain MR images were inspected by an experienced neuroradiologist, and no gross abnormalities were observed for any subject.

fMRI

Using an echo-planar imaging sequence (EPI) with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 40 ms, flip angle (FA) = 90°, number of slices = 28, slice thickness = 4 mm, gap = 1 mm, voxel size = 4 × 4 × 4 mm^3, and matrix = 64 × 64. Participants will be asked to lie quietly in the scanner with their eyes closed during data acquisition. Each scan lasted for 478 s.

DTI

Using an EPI sequence in 32 independent, non-collinear directions of a b-value = 1000 s/mm^2, and one additional image with no diffusion weighting (b = 0). TR = 11000 ms, TE = 98 ms, flip angle = 90°, field of view (FOV) = 256 mm × 256 mm, imaging matrix = 128 × 128, number of slices = 60, and slice thickness = 2 mm. Three acquisitions will be averaged to increase the signal-to-noise ratio.

MRI image analysis

sMRI data analysis

Both the cortical reconstruction and morphological features extraction will be obtained by using the FreeSurfer software (http://surfer.nmr.mgh.harvard.edu/) with a standard cortical automatic handling protocol. First, create a gray matter (GM) template and normalize the GM to the template. The data will be registered to a standard anatomical template[35], and the resulting aligned T1 data set will be segmented and converted to Montreal Neurological Institute (MNI)

space atlas MNI152 1-mm brain template using the DARTEL toolbox[36] and corrected for bias-field inhomogeneity. Subsequently, the images will be skull-stripped using a watershed algorithm[37] then segmented into subcortical WM and deep GM volumetric structures[38 39]. The initial tessellation will be constituted by reconstructing the boundary of GM /WM and the outer cortical surface[40 41]. After that, a series of deformable procedures will be performed. All reconstructed surfaces will be visually inspected for gross-anatomical topological defects. Finally, a variety of morphological features at each vertex on the pial surface will be computed. The parameters will include the whole GM volume, cortical thickness, and surface area.

fMRI data analysis

Image preprocessing will be performed by using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) and Data Processing Assistant for Resting-State fMRI[42]. The preprocessing procedures will be performed including removal of the first 10 volumes, slice timing, and head motion correction. All fMRI data will be satisfied the criteria of spatial movement in any direction < 3 mm or 3° and the subjects will be demonstrated no significant group differences in the head motion parameters (i.e, three translation and three rotation parameters). To normalize the fMRI data spatially, the T1 data used in sMRI will be normalized to the mean functional data, and then segmented and

transformed into MNI152 space using the DARTEL toolbox[36 43], and a group template will be generated. Next, the motion corrected functional volumes will be specially normalized to the group template using the transfer parameter estimated by DARTEL segmentation and resampled to 3 mm isotropic voxels. Further, the functional images will be spatially smoothed with a 4 mm Gaussian kernel. The linear detrend and temporal bandpass filtering (0.01 – 0.08 Hz) will be performed to reduce the influences of low frequency drift and high-frequency physiological noise. Finally, several nuisance signals will be regressed out from the data, including the six motion parameters, the global, the WM, and the cerebrospinal fluid signals. Functional connectivity (FC) analysis is using DPARSF software (http://www.restfmri.net/forum/DPARSF).

To perform the whole-brain resting-state FC (rsFC) analysis, Pearson's correlations between the time courses of any pairs of voxels will be computed, resulting in a whole-brain connectivity matrix for each participant. This procedure will be limited within a GM mask, which was usually generated by a specific thresholding (previous cutoff = 0.2) the mean map of all GM maps involving all subjects without cerebellum. These individual correlation matrices will then be transformed as a z-score matrix by using Fisher's r-to-z transformation to improve normality. Then, the FC strength (FCS) as the sum of the connections between a given voxel and all other GM voxels. This computation will be

conservatively restricted to connections with a correlation coefficient, which could eliminate the weak correlations possibly arising from noise.

DTI data analysis

DTI data processing will be carried out using FMRIB Software Library software (FSL, http:// www.fmrib.ox.ac.uk/fsl)[44]. Initially, eddy current correction will be run to correct gradient-coil distortions and small-head motions using affine registration to a reference image (b0 volume). The brain voxels of DTI data will be extracted using the Brain Extraction Tool (BET). The maps of diffusion tensor parameters including fractional anisotropy (FA), axial diffusivity (DA), radial diffusivity (DR), and mean diffusivity (MD) will be calculated using DTI-FIT tool, which fits a diffusion tensor model to diffusion-weighted images for each voxel. Voxel-wise statistical analysis of the FA, DA, DR, and MD data will be performed for regional differences using tract-based spatial statistics (TBSS). TBSS is a whole-brain voxel-wise analysis method. Then, the FSL's nonlinear image registration algorithm will be used, all subjects' FA maps will be aligned into a $1\times1\times1$ mm^3 standard Montreal Neurological MNI 152 space. The target template will be the FMRIB58_FA (http://www.fmrib.ox.ac.uk/fsl/data/FMRIB58_FA). Then a mean FA image will be created by averaging the aligned FA maps. The mean FA image will be thinned to build a mean FA skeleton representing

the center of all tracts common to all participants in the study. Each subject's aligned FA data will be projected onto the FA skeleton to obtain their FA skeletons and deformation matrixes. With the deformation matrixes, the skeletonized DA, DR, and MD maps will be created by the tbss_non_FA tool. The skeletonized FA, DA, DR, and MD map images will be subsequently fed to statistical analysis.

Statistics

Repeated measures analysis of variance (ANOVA) will be performed to compare the global differences across the 3 groups, with follow-up post hoc analyses performed as needed to explain the main effects and interactions.

A multivariate analysis of covariance (MANCOVA) will be conducted with different kinds of MCI as the independent variable, each brain volume as a dependent variable, and with age and gender as covariates to analyze differences in brain volume by different kinds of MCI. In addition, Pearson correlations (two-tailed) will be used to investigate correlations between neuropsychological test scores and GM volume, cortical thickness, and surface area.

A general linear model with multivariate ANOVA (MANOVA) with post hoc test Bonferroni correction was performed to compare the FA, MD, DA, and DR of each tract between the groups. SPSS (version 20; SPSS Inc., Cary, NC) was used for this statistical analysis.

A one-way analysis of covariance (ANCOVA) will also be performed to determine the main effect of groups on FCS, with age and gender as covariates, followed by two-sample t-tests post hoc analyses. The two-sample t-tests post hoc analyses will be fulfilled within the regions showing significant group effects. All the cluster sizes were determined by Monte Carlo simulations[45] using the REST AlphaSim utility[46].

The two-sample t-tests will be performed on the rsFC maps for each seed, with age and gender as covariates. The significant level was set at $P < 0.05$ with a cluster size of 1350 mm^3, corresponding to a corrected $P < 0.05$. The analysis mask will be generated by selecting the voxels that may have a significant positive rsFC in any of the two groups. To investigate the relationship between the brain function, structure and cognitive behavior, a general linear model analysis (dependent variable: for function: FCS; for structure: FA, MD, DA, and DR; independent variable: clinical variables, including MMSE, MoCA, AVLT-immediate recall, AVLT-delayed recall, and AVLT-delayed recognition, MES and TMT) will be calculated separately.

Association between neuroimage and neuropsychological performance

Analysis the interaction between the parameters acquired by multimodal magnetic resonance and cognition measures.

The scheme of the current prospective trial is described in Fig. 1

Discussion

In previous studies, the researchers usually focus on the differences between patients and normal elders, little pays attention to the differences between the similar diseases. At the stage of dementia, the WM microstructure in corpus callosum especially in forceps minor has been detected different between VD and AD[20]. While it is uncertain that the differences took place in the stage of dementia or earlier. In spite that the mode of memory defict in aMCI and VaMCI is also different[10], there is still lack of evidence to compare the relationship between those abnormal cognitive behaviors and brain structure and function. So it is meaningful to use multimodal magnetic resonance to observe the neuroimage in the two subtypes of MCI.

Some limitations have to be took into account about this study. First, the trial is just an observational study without follow up so whether would they convert to AD or VD in the future is uncertain. Therefore, we will plan to apply a follow-up to track the dynamic evolution of these patients. Second, our hospital is a primary hospital, we don't have a PET to raise the precision of diagnosis. But the diagnosis of the two groups follow the criteria for the diseases critically to make the diagnosis as precisely as possible. And we will plan to gather the blood and cerebrospinal fluid in our future designs.

The purpose for this study is to find out the differences in neuroimaging

between aMCI and VaMCI and exploring the possible mechanism of the cognitive disorders in the two diseases. Moreover, to provide scientific diagnosis evidence for different subtypes of MCI.

Consent for publication

Written consent is obtained from each subject before publishing in this study.

Availability of data and material

Not applicable.

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Author Contributions

All authors participated in the design of the study. YY drafted and revised the manuscript. WNZ revised the work critically for important intellectual content. SOL revised the work critically for important intellectual content. YCH is the final approval of the version published. All authors read and approved the final manuscript.

Competing interests

All authors report no competing interests.

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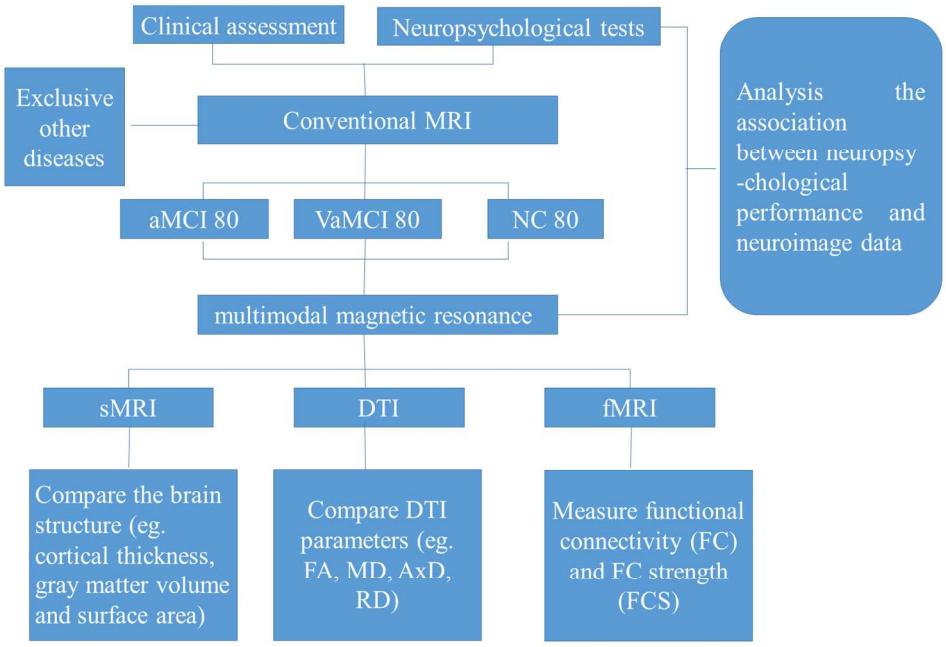
Fig 1 Flowchart of the current prospective diagnostic trial.
80 subjects who are diagnosed as amnestic mild cognitive impairment (aMCI) and 80 vascular mild cognitive impairment (VaMCI) and 80 age, gender and education matched normal controls (NC) will go through a full neuropsychological tests which will include mini-mental state examination (MMSE), Montreal Cognitive Assessment (MoCA), Auditory Verbal Learning Test (AVLT), Memory and Executive Screening (MES), trail making test(TMT), the Stroop color naming condition, Clinical Dementia Rating scale (CDR) and neuroimage tests contain sMRI, DTI and fMRI. FA, fractional anisotropy; axial diffusivity, DA; DR, radial diffusivity; MD, mean diffusivity.



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Magnetic Resonance Imaging-based Comparative Study of Different Mild Cognitive Impairment Subtypes: Protocol for an Observational Case-Control Study

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Magnetic Resonance Imaging-based Comparative Study of Different Mild Cognitive Impairment Subtypes: Protocol for an Observational Case-Control Study

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Abstract

Introduction:

Amnestic mild cognitive impairment (aMCI) and vascular mild cognitive impairment (VaMCI) comprise the two main types of mild cognitive

impairment (MCI). The first condition generally progresses to Alzheimer's disease (AD), whereas the second is likely to develop into vascular dementia (VD). The brain structure and function of MCI patients differ from those of normal elderly individuals. However, whether brain structures or functions differ between these two MCI subtypes has not been studied. This study is designed to analyse brain neuroimages in VaMCI and aMCI patients using multi-modality magnetic resonance imaging (MRI) (structural MRI (sMRI), functional MRI, and diffusion tensor imaging (DTI)).

Methods and analysis:

In this study, 80 subjects diagnosed with aMCI, 80 subjects diagnosed with VaMCI, and 80 age-, gender-, and education-matched normal controls (NC) will be recruited to the Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. All subjects will undergo neuroimaging and neuropsychological evaluations. The primary outcome measures will be 1) microstructural alterations revealed by multimodal MRI scans, including sMRI, resting-state functional MRI (rs-fMRI), and DTI; and 2) a neuropsychological evaluation, including the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Auditory Verbal Learning Test (AVLT), Memory and Executive Screening (MES), trail making test (TMT), Stroop colour naming condition, and Clinical Dementia Rating scale (CDR), to evaluate global

cognition, memory function, attention, visuospatial skills, processing speed, executive function, and emotion, respectively.

Trial registration:

Protocol registered on ClinicalTrials.gov (NCT02706210)

Keywords: Amnestic mild cognitive impairment, Vascular mild cognitive impairment, Neuropsychological, Neuroimaging techniques,

Strengths and limitations of this study

Comparison of different subtypes of MCI.

Use of a combination of multimodal imaging systems.

Lack of clinicopathological diagnosis.

Introduction

Due to the prolongation of the life span, the incidence of chronic diseases has increased. Dementia is a progressive brain disease and is one of the main chronic non-communicable diseases associated with disability and mortality among elderly individuals. Dementia resulting from Alzheimer's disease (AD) has received much public attention. Dementia has many causes in addition to AD. Vascular dementia (VD) is also one of the main types of dementia. Mild cognitive impairment (MCI) constitutes an intermediate stage between normal ageing and dementia[1]. A widely shared view is that future treatment strategies should focus on the treatment of MCI.

The rate of conversion of the amnestic MCI (aMCI) subtype to AD is 10% to 15% annually. Research has focused on the predementia stage of AD, which is likely to be the clinical transition stage of AD[2 3]. In addition, vascular MCI (VaMCI) represents the early stage of VD[4 5]. The pathogenesis of these two diseases differs. aMCI is a neurodegenerative disease caused by endogenous neuronal factors, including tau and amyloid pathology[6], whereas VaMCI is caused by vascular diseases such as infarcts or profuse white matter (WM) disease[4 7]. One study has demonstrated that the structure of the hippocampus is different in aMCI and VaMCI patients[8]. However, differences in other parts of the brain have not been detected. Both VaMCI and aMCI are associated with deficits in multiple cognitive domains, but they result in the same chief complaints of memory deficits[9 10]. Using neuropsychological tests, a previous study indicated that the mode of memory defects in aMCI and VaMCI patients also differs[10]. A considerable body of literature has concluded that VaMCI patients generally exhibit greater impairment of semantic memory, executive/attentional functioning, and visual-spatial and perceptual skills, whereas the clinical condition of AD is characterized by deficits in episodic memory[4 11 12]. Patients with these conditions may exhibit different performances on specific neuropsychological tests. However, few studies have examined the relationship between these two types of MCI regarding brain structure

and cognitive behaviour. Determining the conditions that will result in different types of dementia in patients with cognitive impairment may be helpful for understanding the mechanisms of the two forms of MCI.

According to previous publications, both aMCI and VaMCI are types of a "disconnection syndrome"[13 14]. By applying structural magnetic resonance imaging (sMRI), resting-state functional magnetic resonance (fMRI), and diffusion tensor magnetic resonance imaging (DTI) technology, we can comprehensively analyse brain structure and function in aMCI and VaMCI patients directly and noninvasively. Previous studies have detected atrophy of brain volume and abnormal functional connectivity in MCI patients[15-19], along with brain structure impairment in VD that is not consistent with AD, especially in the corpus callosum[20 21]. However, whether these structures differ in various types of MCI has not been determined. Moreover, the changes in brain structure and function are not completely similar[22-24]. In contrast to using a single technique, the brain structure and function can be analysed in an omni-directional and multi-angular manner using multimodal magnetic resonance. The use of multimodal magnetic resonance could also consolidate our understanding of how functional networks interact with their structural substrates.

This project seeks to reveal neural circuits involved in the development of network connections and changes that occur in the context of diseases involving cognitive impairment. We seek to further understand the

pathogenesis of cognitive impairment and discover the relationship between brain structure, function, and cognitive behaviour.

Methods

Subjects

A total of 240 right-handed subjects (80 aMCI patients, 80 VaMCI patients, and 80 normal age-, sex-, and education-matched controls) will be recruited from the Neurology Department of Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. All participants will undergo a baseline evaluation, including complete sociodemographic and clinical data collection. Patient histories will be collected from informants, typically from their spouses or children. Two experienced neurologists will perform the diagnosis of the participants.

Inclusion Criteria

Criteria for aMCI

The diagnosis of aMCI will be based on the recent international consensus criteria as follows[1 25]: 1) subjective cognitive complaints reported by the informant; 2) objective cognitive impairments that do not meet the Diagnostic and Statistical Manual of Mental Disorders (DSM-□) criteria for dementia; 3) normal or near-normal performance of general cognitive functioning and no or minimum impairments in daily life activities; 4) abnormal memory function documented by an extensive

neuropsychological evaluation, normal general cognitive function, and a Clinical Dementia Rating scale (CDR) score = 0.5[26]; and 5) neuropsychological testing including a Hachinski ischaemic score (HIS) ≤ 4 and Montreal Cognitive Assessment score (MoCA, Beijing Version)[27].

Criteria for VaMCI

The diagnosis of VaMCI will be based on the Diagnostic Criteria for Vascular Cognitive Disorders (VCD)[4 5] and DSM-□ criteria; and vascular aetiology as follows: cognitive impairment due to subcortical small vessel disease (SIVD)[28] defined as moderate WM changes (at least 1 region score < 2 according to the Wahlund rating scale) or multiple lacunar infarcts (< 2) on brain imaging that may or may not be suggested by minor neurological signs. Neuropsychological tests for aMCI, MoCA scoring, and HIS determination (HIS ≥ 7) will also be performed.

Exclusion Criteria

Participants with the following conditions will be excluded: 1) psychiatric disease (e.g., depression, Hamilton depression rating scale (HAMD) > 20, Center for Epidemiologic Studies Depression Scale > 21), systemic disease, or other neurological disorders; 2) visual or auditory abnormalities, severe aphasia, or palsy that renders clinical assessments infeasible; 3) any medical or psychological conditions that might affect

cognitive functioning, including tranquilizers, anti-anxiolytics, hypnotics, nootropics, and cholinomimetic agents; 4) inability to undergo brain MRI, such as claustrophobia; 5) insufficient Mandarin language abilities to complete the assessment; and 6) marked head-motion according to the MRI image.

The exclusion criteria for VaMCI will also include the following: signs of large vascular disease, such as cortical or cortico-subcortical or non-lacunar territorial infarcts and watershed infarcts or haemorrhages.

Neuropsychological Evaluations

All participants will receive a battery of neuropsychological tests to assess general mental status and other cognitive domains. These tests will include the CDR scale[1 26], Mini-Mental State Examination (MMSE)[29], MoCA[27], Auditory Verbal Learning Test (AVLT)[30], Memory and Executive Screening (MES)[31], trail making test (TMT)[32], Stroop colour naming condition[33], CDR, and HAMD[34]. These tests will be used to evaluate global cognition, memory function, attention, visuospatial skills, processing speed, executive function, and emotion, respectively.

Magnetic Resonance Brain Imaging Protocol

For each participant, conventional brain T1-weighted image (T1WI) and T2-weighted image (T2WI) data will be obtained to exclude serious brain diseases. For inpatients, the data will be collected at Hongqi Hospital of

Mudanjiang Medical University. For outpatients, the T1WI and T2WI data may be acquired from other hospitals. The imaging data used for analysis will be acquired using a 3.0 T Trio Siemens scanner (Magnetom Tim Trio; Siemens Medical Solutions, Erlangen, Germany) at Hongqi Hospital of Mudanjiang Medical University.

sMRI

High-resolution anatomical images will be acquired using a 3D magnetization-prepared rapid gradient echo (MP-RAGE) T1-weighted sequence with the following parameters: repetition time (TR) = 1900 ms, echo time (TE) = 2.2 ms, inversion time (TI) = 900 ms, flip angle (FA) = 9°, number of slices = 176, slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm^3, and matrix = 256 × 256. Brain magnetic resonance images will be inspected by an experienced neuroradiologist to determine the presence of gross abnormalities.

fMRI

An echo-planar imaging (EPI) sequence with the following parameters will be used: TR = 2000 ms, TE = 40 ms, FA = 90°, number of slices = 28, slice thickness = 4 mm, gap = 1 mm, voxel size = 4 × 4 × 4 mm^3, and matrix = 64 × 64. Participants will be asked to lie quietly in the scanner with their eyes closed during data acquisition. Each scan will last for 478 s.

DTI

An EPI sequence will be used in 32 independent, non-collinear directions with a b-value of 1000 s/mm^2, and one additional image with no diffusion weighting (b = 0) will be acquired (TR = 11,000 ms, TE = 98 ms, FA = 90°, field of view (FOV) = 256 mm × 256 mm, imaging matrix = 128 × 128, number of slices = 60, and slice thickness = 2 mm). Three acquisitions will be averaged to increase the signal-to-noise ratio.

MRI Data Analysis

sMRI Data Analysis

Both the cortical reconstruction and morphological feature extraction will be conducted using FreeSurfer software (http://surfer.nmr.mgh.harvard.edu/) with a standard cortical automatic handling protocol. First, a grey matter (GM) template will be created, and the GM will be normalized to the template. The data will be registered to a standard anatomical template[35], and the resulting aligned T1 data set will be segmented and converted to a Montreal Neurological Institute (MNI) space atlas MNI152 1-mm brain template using the DARTEL toolbox[36] and corrected for bias-field inhomogeneity. Subsequently, the images will be skull-stripped using a watershed algorithm[37] then segmented into subcortical WM and deep GM volumetric structures[38 39]. The initial tessellation will be conducted anatomical topological defects. Finally, a variety of morphological features at each vertex on the pial surface will be computed. The parameters will include the entire GM

volume, cortical thickness, and surface area.

fMRI Data Analysis

Image pre-processing will be performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) and the Data Processing Assistant for Resting-State fMRI[40]. Pre-processing procedures will be performed, including removal of the first 10 volumes, slice timing, and head motion correction. All fMRI data will satisfy the criteria of spatial movement in any direction < 3 mm or 3°, and the subjects will exhibit no significant group differences in the head motion parameters (i.e., three translation and three rotation parameters). To spatially normalize the fMRI data, the T1 data used in sMRI will be normalized to the mean functional data, segmented, and transformed into MNI152 space using the DARTEL toolbox[36 41], and a group template will be generated. Next, the motion corrected functional volumes will be specifically normalized to the group template using the transfer parameter estimated by DARTEL segmentation and resampled to 3-mm isotropic voxels. Furthermore, the functional images will be spatially smoothed with a 4-mm Gaussian kernel. Linear detrend and temporal bandpass filtering (0.01-0.08 Hz) will be performed to reduce the effects of low-frequency drift and high-frequency physiological noise. Finally, several nuisance signals will be regressed from the data, including the six motion parameters and the global, WM, and cerebrospinal fluid signals. Functional connectivity (FC)

analysis will be performed using DPARSF software (http://www.restfmri.net/forum/DPARSF).

To perform the whole-brain resting-state FC (rsFC) analysis, Pearson's correlations between the time courses of all pairs of voxels will be computed, resulting in a whole-brain connectivity matrix for each participant. This procedure will be limited within a GM mask, which is typically generated by a specific thresholding (previous cut-off = 0.2) of the mean map of all GM maps involving all subjects without cerebella. These individual correlation matrices will then be transformed as a z-score matrix using Fisher's r-to-z transformation to improve normality. Then, the FC strength (FCS) will be computed as the sum of the connections between a given voxel and all other GM voxels. This computation will be conservatively restricted to connections with a correlation coefficient, which could eliminate the weak correlations possibly arising from noise.

DTI Data Analysis

DTI data processing will be performed using software from the FMRIB Software Library (FSL, http:// www.fmrib.ox.ac.uk/fsl)[42]. Initially, eddy current corrections will be performed to correct gradient-coil distortions and small head motions using affine registration to a reference image (b0 volume). The brain voxels of DTI data will be extracted using the Brain Extraction Tool (BET). The maps of diffusion tensor parameters,

including fractional anisotropy (FA), axial diffusivity (DA), radial diffusivity (DR), and mean diffusivity (MD), will be calculated using the DTI-FIT tool, which fits a diffusion tensor model to diffusion-weighted images for each voxel. Voxel-wise statistical analysis of the FA, DA, DR, and MD data will be performed for regional differences using tract-based spatial statistics (TBSS), which is a whole-brain voxel-wise analysis method. Then, FSL nonlinear image registration algorithm will be used. FA maps of all subjects will be aligned into a $1\times1\times1$ mm^3 standard Montreal Neurological MNI 152 space. The target template will be the FMRIB58_FA (http://www.fmrib.ox.ac.uk/fsl/data/FMRIB58_FA). Then, a mean FA image will be created by averaging the aligned FA maps. The mean FA image will be thinned to build a mean FA skeleton representing the centre of all tracts common to all participants in the study. Each subject's aligned FA data will be projected onto the FA skeleton to obtain their FA skeletons and deformation matrixes. Using the deformation matrixes, skeletonized DA, DR, and MD maps will be created by the tbss_non_FA tool. The skeletonized FA, DA, DR, and MD map images will be subsequently subjected to statistical analysis.

Statistical Analysis

Repeated measures analysis of variance (ANOVA) will be performed to compare the global differences across the 3 groups, with follow-up post hoc analyses performed as needed to explain the main effects and

interactions.

Multivariate analysis of covariance (MANCOVA) will be conducted with the MCI type as the independent variable, each brain volume as a dependent variable, and age and gender as covariates to analyse differences in brain volume by different types of MCI. In addition, Pearson correlations (two-tailed) will be used to investigate correlations between neuropsychological test scores and the GM volume, cortical thickness, and surface area.

A general linear model with multivariate ANOVA (MANOVA) and post hoc Bonferroni correction will be performed to compare the FA, MD, DA, and DR of each tract among the groups. SPSS (version 20; SPSS Inc., Cary, NC) will be used for this statistical analysis.

A one-way analysis of covariance (ANCOVA) will also be performed to determine the main effect of groups on FCS using age and gender as covariates followed by two-sample t-tests for post hoc analyses. The two-sample t-tests post hoc analyses will be conducted within regions exhibiting significant group effects. All the cluster sizes will be determined by Monte Carlo simulations[43] using the REST AlphaSim utility[44].

The two-sample t-tests will be performed on the rsFC maps for each seed using age and gender as covariates. The significance level will be set at $P < 0.05$ with a cluster size of 1350 mm^3, corresponding to a corrected $P <$

0.05. The analysis mask will be generated by selecting the voxels that may have a significant positive rsFC in any of the two MCI groups. To investigate the relationship among brain function, structure, and cognitive behaviour, a general linear model analysis (dependent variable for function: FCS; for structure: FA, MD, DA, and DR; independent variables: clinical variables, including MMSE, MoCA, AVLT-immediate recall, AVLT-delayed recall, AVLT-delayed recognition, MES, and TMT scores) will be conducted separately.

Association Between Neuroimaging and Neuropsychological Performance

We will analyse the interaction between the parameters acquired by multimodal magnetic resonance and cognition measures.

A scheme of the current prospective trial is described in Fig. 1

Ethics and dissemination

Ethical approval for this study has been obtained from the Medical Research Ethics Committee and Institutional Review Board of Hongqi Hospital of Mudanjiang Medical University, Heilongjiang, China. The study will be conducted in accordance with the approved guidelines. Written informed consent will be obtained from all participants, and the participants will be able to withdraw from the study at any time.

Discussion

In previous studies, researchers have typically focused on the differences

between patients and normal elderly individuals, and little attention has been given to the differences between similar diseases. In the late stage of dementia, the WM microstructure in the corpus callosum, especially in the forceps minor, exhibits differences in VD and AD patients[20]. However, the differences that occur in the early stages of dementia are uncertain. Although the mode of memory defects in aMCI and VaMCI patients is different[10], comparisons of the relationship between these abnormal cognitive behaviours and brain structure and function are lacking. Thus, use multimodal magnetic resonance to observe neuroimages in the two subtypes of MCI is relevant.

Some limitations of this study should be noted. First, this trial is only an observational study without follow-up; whether these patients will convert to AD or VD in the future is uncertain. Therefore, we plan to conduct follow-up to track the dynamic evolution of these patients. Second, our facility is a primary hospital. We do not have a PET scanner to increase the precision of diagnosis. However, the diagnosis of the two groups will strictly follow the criteria for the diseases to allow the diagnosis to be as precise as possible. We plan to collect blood and cerebrospinal fluid in future studies.

The purpose for this study is to identify differences in neuroimaging results between aMCI and VaMCI patients and to explore the possible mechanisms of cognitive disorders in the two diseases. Moreover, we

seek to provide scientific evidence for the diagnosis of different subtypes of MCI.

Consent for Publication

Written consent will be obtained from each subject before publishing this study.

Availability of Data and Material

Not applicable.

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Author Contributions

All authors participated in the design of the study. YY drafted and revised the manuscript. WNZ and SOL revised the work critically for important intellectual content. YCH provided the final approval of the version published. All authors read and approved the final manuscript.

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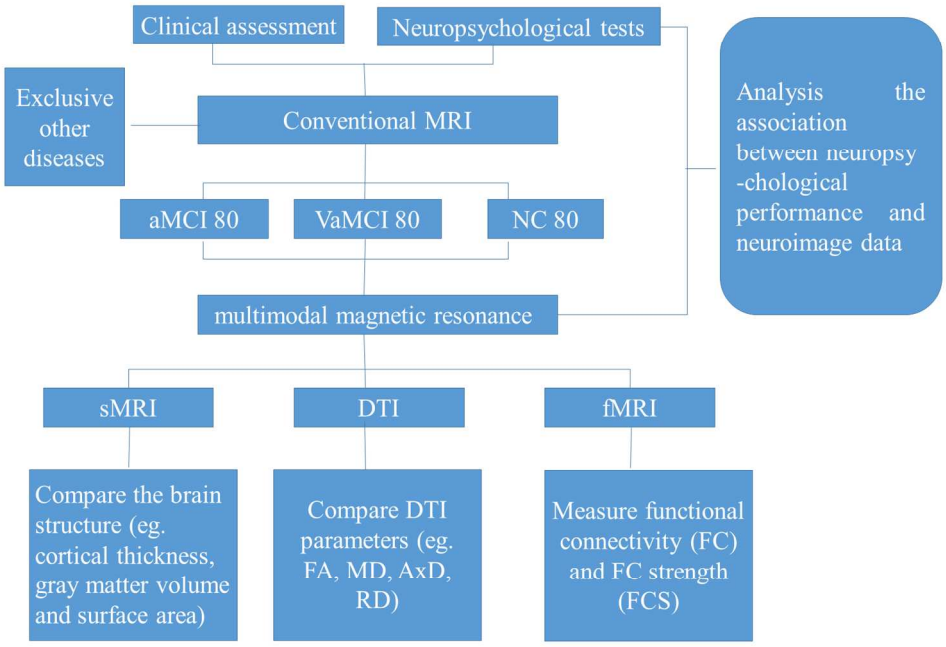
Competing interests

All authors report no competing interests.

Fig. 1. Flowchart of the current prospective diagnostic trial.
Eighty subjects diagnosed with amnestic mild cognitive impairment (aMCI), 80 subjects diagnosed with vascular mild cognitive impairment (VaMCI), and 80 age-, gender-, and education-matched normal controls (NC) will be subjected to full neuropsychological tests, including the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Auditory Verbal Learning Test (AVLT), Memory and Executive Screening (MES), trail making test (TMT), Stroop colour naming condition, Clinical Dementia Rating scale (CDR), and neuroimaging tests, including sMRI, DTI, and fMRI. FA, fractional anisotropy; axial diffusivity, DA; DR, radial diffusivity; MD, mean diffusivity.



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144x108mm (300 x 300 DPI)