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Title Page:

Sino Longitudinal Study on Cognitive Decline (SILCODE): study design of a longitudinal observational study

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ABSTRACT

Introduction Understanding the biological mechanism of subjective cognitive decline (SCD) in preclinical Alzheimer's disease (AD) and identifying those who will convert to mild cognitive impairment (MCI) in short time are critical for developing appropriate strategies for early diagnosis and intervention of AD. We aim to present the study protocol of Sino Longitudinal Study on Cognitive Decline (SILCODE), a longitudinal observational study focusing on SCD in the context of AD.

Methods and analysis Within SILCODE, participants will undergo extensive assessment, including clinical and neuropsychological assessments, blood and urine sample collection, glucose metabolism and amyloid positron emission tomography as well as multimodal MRI scans, with each subject followed up every 15-months for five years. The primary outcome measure is clinical progression.

Ethics and dissemination The medical research ethics committee and institutional review board of the XuanWu Hospital of Capital Medical University approved this study protocol (ID: [2017]046). The results will be published in peer-reviewed journals and presented at national and international scientific meetings.

Trial registration NCT03370744; pre-results.

Article Summary

Strengths and limitations of this study

- This is the first study to establish models predicting SCD conversion with different combination in China, which will provide references for clinical applications of SCD.
- This longitudinal study will use multi-modal MRI to investigate the brain structural and functional progressing pattern from SCD to MCI and explore the underlying mechanism.
- Besides neuropsychological and clinical information, blood and urine biomarker, multi-modal MRI, glucose metabolism and amyloid positron emission tomography will be used in this study.
- The study results will be limited by the participant's age (older than 55 years old) and study setting (not communicate based, participants from advertisement or referrals).

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease and one of the greatest healthcare challenges of the 21st century.[1] It has been revealed that pathophysiology starts many years before the diagnosis of AD. Several recent clinical trials of Aβ-lowering therapies in the mild or moderate dementia stage have failed, which further encouraged researchers to shift their focus to the preclinical stage of AD.[2]

Subjective cognitive decline (SCD) refers to self-perceived cognitive decline relative to a previously normal status, without impaired performance on standardized neuropsychological tests.[3, 4] Evidence from recent research suggests that SCD may be one of the first symptomatic manifestation of AD.[3, 5] Previous studies have found higher rate and shorter time of conversion to mild cognitive impairment (MCI) and dementia in SCD individuals as compared to cognitively normal controls (NC). [6] Further, the number of individuals with SCD presenting to the healthcare system (e.g. memory clinics) is increasing. So, persons with SCD are ideal for early diagnosis and intervention trails.

However, the heterogeneous nature of SCD limited its value in clinical application and SCD with different features presented different conversion rates. AD is characterized by the aggregation of β-amyloid (Aβ) deposition and studies have found higher Aβ in SCD predicting rapid cognitive decline.[7, 8] As to neuroimaging, thinner cortex in temporal, frontal and occipital areas is associated with steeper decline in cognition.[9, 10] Besides, neuropsychologic tests such as long-term verbal memory and life style may also have impact on SCD progression.[11] In aspect of SCD reports, studies found that fulfilling SCD-plus[12], reporting stable worries about cognitive decline[13, 14] and older at onset of symptoms[15] showed higher risk progressing to MCI and dementia. Though studies have focused on features that could predict conversion in SCD, there are little research building predicting models from multi dimensions. And some noninvasive and convenient biomarkers of AD have not been identified in SCD individuals such as plasma Aβ and urine AD7c-NTP.

The Alliance for preclinical AD of China, belonging to the National Clinical Research Center for Geriatric Disorders, is a national multicenter research platform dedicated to development of clinical, neuroimaging, genetic, and biochemical biomarkers for the early detection and tracking of AD. Its sites include more than 180 hospital/research institute members all over

China. The alliance is led by professor Han and focuses on early diagnose of SCD due to preclinical AD, which also develops and provides standard operation procedures and quality control for harmonized data and material acquisition and storage across all sites.

Sino Longitudinal Study on Cognitive Decline (SILCODE) is a longitudinal observational study based on the multicenter research platform of the Alliance for preclinical AD of China, focusing on SCD in the context of AD. The main aims of SILCODE are: 1) establishing models for predicting cognitive decline/conversion in SCD individuals. Furthermore, we would like to build models based on various combinations of information since not all hospitals in China are able to acquire PET data and some are even not able to acquire multi-modal MRI; 2) revealing brain structural and functional progressing pattern in SCD individuals and exploring underlying mechanism based on multi-modal neuroimaging. Herein, we report the study design of SILCODE.

METHODS

Overall study design

This is an observational longitudinal study in China recruiting SCD. Participants are consecutively recruited from standardized public advertisement, referrals from general physicians or memory clinic or informants. During the baseline period, all participants will receive clinical and neuropsychological examinations, blood tests, urine tests and multi-modal MRI including structural MRI, diffusion tensor imaging (DTI) and functional MRI (fMRI). Glucose metabolism and amyloid positron emission tomography (PET) are optional based on individual's agreement. The follow-up scheme is every 15 months and the follow-up time is at least 5 years for every individual. In the follow-up period, all evaluations are the same as the baseline except for the amyloid PET. At each follow-up visit, diagnoses are reevaluated under supervision of a neurologist. The figure 1 provides the overall study outline.

Sample size

This project will use sub-distribution hazard function model, one kind of competing risk model, with nearly 50 independent variables before screening. After screening, we plan to choose at most the top 10 variables for each model. So, appropriately 100 (10 time more than independent

variables) SCD-convertors are needed in our project. According to a previous meta-analysis,[16] the annual conversion rate to MCI in SCD is 6.6% and the rate of follow up is about 90%. Finally, about 340 individuals with SCD are needed at baseline.

Inclusion/exclusion criteria

All eligible subjects are right-handed (the Edinburgh handedness scale score >40 points), older than 55 years old, and Han Chinese subjects. Written informed consent will be acquired from each subject before enrollment.

The inclusion criteria for SCD are as following: (1) presence of self-perceived continuous cognitive decline compared to previous normal status and unrelated to an acute event; and (2) concerns (worries) associated with memory complaint; and (3) failure to meet the following criteria for MCI. MCI are defined by an actuarial neuropsychological method proposed by Jak and Bondi.[17] Participants are considered to have MCI if any one of the following three criteria are met with a total Clinical Dementia Rating (CDR) score of 0.5 as well as failure to meet the criteria for dementia: (1) having impaired scores (defined as >1 SD below the age-corrected normative mean) on both measures within at least one cognitive domain (i.e., memory, language, or speed/executive function); (2) having impaired scores in each of the three cognitive domains sampled; (3) the Functional Activities Questionnaire (FAQ) ≥9. The diagnosis of AD syndrome is based on the diagnostic guidelines for dementia due to AD delivered by the National Institute on Aging–Alzheimer's Association workgroups (NIA-AA)[18] with a total CDR score of 1.

Exclusion criteria for all participants are: (1) history of stroke; (2) severe depression (Hamilton Depression Rating Scale score > 24); (3) other neurological conditions which could cause cognitive decline (e.g., brain tumors, Parkinson's disease, encephalitis, or epilepsy) rather than AD spectrum disorders; (4) other diseases which could cause cognitive decline (e.g., thyroid dysfunction, severe anemia, syphilis, or HIV); (5) history of psychosis or congenital mental growth retardation; (6) cognitive decline caused by traumatic brain injury; (7) those who could not complete the study protocol or with contraindications for MRI.

Clinical, risk factor and neuropsychological assessments

The clinical assessments include a structured medical history, family history, physical examination, routine laboratory tests (e.g. blood routine test, biochemical test, thyroid function test, Vitamin B12, folic acid, treponema pallidum specific antibody and aids antibody) and MRI (T1 and Flair) for medial temporal atrophy scale and Fazekas score.

For risk factors of AD, all subjects and their informants provide the following information: vascular risk factors, occupation/retirement, socioeconomic status, nicotine and alcohol uses. Additionally, Pittsburgh sleep quality index (PSQI),[19] Rapid Eye Movement (REM) sleep Behavior Disorder Screening Questionnaire (RBDSQ)[20] and the Epworth Sleepiness Scale (ESS)[21] will be used for sleep quality assessment and the Semi-quantitative Food Frequency Questionnaire (SFFQ) will be used for nutrition intakes assessment.

The neuropsychological tests are selected for comparability with other similar ongoing studies (e.g., DELCODE).[22] The cognitive testing will be performed by trained neuropsychologists. The neuropsychological battery measure different cognitive domain including episodic memory (Auditory Verbal Learning Test-HuaShan version [AVLT-H]),[23] language (Animal Fluency Test, 30-item Boston Naming Test[24]) and speed/executive function (Shape Trails Test Parts A and B).[25] Besides, the SILCODE will implement tests for global cognition, daily life ability and neuropsychiatric assessment including the Mini Mental State Examination (MMSE), the Montreal Cognitive Assessment-basic (MoCA-B),[26] the memory and executive screening (MES),[27] the CDR, FAQ, the 15-item short form of the Geriatric Depression Scale (GDS), the Hamilton Anxiety Scale (HAMA), the Hamilton Depression Scale (HAMD) and the Neuropsychiatric Inventory (NPI).

Assessments of subjective cognitive functioning

A semi-structured interview used by the DELCODE study is employed in this project to evaluate the details of SCD.[22] It includes information about the onset time, concerns, comparison with others and so on in not only memory domain but also language, attention and execute capacity. We also will require informant reports in evaluation of the self-reported information as suggested by previous studies.[4] In early stages of cognitive decline, self-report may provide more information whereas in later stages of SCD, informant-report may be more effective.[5]

For quantitative assessment of SCD, we will use a newly developed SCD questionnaire including nine reliable SCD items, which are characterized by different domains, such as global memory functioning and daily activities ability.[28] In addition, the 12-item Everyday Cognition questionnaire will also be applied to all participants to measure SCD severity.

Blood tests

APOE genotype

SNPs rs7412 and rs429358 form the APOE ε2/ε3/ε4 haplotype. APOE will be genotyped using the standard Sanger sequencing method (Sangon, Shanghai, China) using the following primers: 5′-ACGCGGGCACGGCTGTCCAAGG-3′ (forward) and 5′-GGCGCTCGCGGATGGCGCTGA-3′ (reverse). APOE will be amplified using the following conditions: 1 cycle of 98 °C for 10 s, 35 cycles of 72 °C for 5 s, 1 cycle of 72 °C for 5 min. PCR was performed in a final volume of 30 μl, containing 10 pmol of forward and reverse primers and 50 ng of genomic DNA template, using PrimeSTAR HS DNA Polymerase with GC Buffer (Takara Bio).

Plasma β-amyloid

Recently, some research found that plasma amyloid level was correlated with cognitive capacity and cerebrospinal fluid amyloid protein.[29, 30] Especially, when predicting brain amyloid deposition, the accuracy of the composite biomarker (amyloid-β precursor protein 669-711/ Aβ1-42 and Aβ1-40/Aβ1-42 ratios) could approximately equal to 90%.[31] So, we would like to identify if the plasma β-amyloid is also associated with cognitive decline in SCD individuals. Plasma Aβ will be determined using commercially available kit, V-PLEX Aβ Peptide Panel 1 (6E10) Kit (K15200E) (Mesoscale Diagnostics LLC, Rockville, USA). Aβ peptide levels from each blood draw will be measured in duplicate using the same aliquot.

Urine tests

Ten ml of first morning urine will be collected from each subject and then immediately refrigerated. An enzyme-linked immunosorbent assay kit will be used to detect the protein level

of AD7c-NTP in the urine specimens.

Imaging protocol

MRI data will be acquired using an integrated simultaneous 3.0 Tesla TOF PET/MR (SIGNA PET/MR, GE Healthcare, Milwaukee, WI, USA) at the XuanWu Hospital of Capital Medical University. For each participant, simultaneous PET and 3.0 T MRI data will be obtained. Brain MR images will be inspected by an experienced neuroradiologist (with more than 5 years' experience).

Positron emission tomography: All participants will be invited for optional [^{18}F] florbetapir (AV-45) and [^{18}F] fluordesoxyglucose (FDG) in 3-dimensional acquisition mode with interval between 3-90 days. For FDG-PET, each subject will be instructed to fast for at least 6 h and has a confirmed serum glucose level below 8 mmol/L; 35 minutes dynamic scan is acquired about 40 minutes after an intravenous injection of 3.7 MBq/kg of 18F-FDG. For Aβ-PET, 35 minutes dynamic scan is acquired about 40 minutes after an intravenous injection of 7-10mCi [^{18}F] florbetapir. The PET data are acquired using a TOF-OSEM algorithm (time-of-flight ordered subset expectation maximization) with the following parameters: 8 iterations, 32 subsets matrix = 192×192, field of view (FOV)= 350×350, half-width height = 3.

Structural MRI: Parameters for T1-weighted 3D brain structural images are as following: SPGR sequence, FOV=256×256 mm^2, matrix=256×256, slice thickness=1 mm, gap=0, slice number=192, repetition time (TR) =6.9 ms, echo time (TE)=2.98 ms, inversion time (TI)=450 ms, flip angle =12°, voxel size = 1×1×1 mm^3.

DTI: DTI data are obtained with Single-shot spin-echo diffusion-weighted EPI sequence with following parameters: FOV=224×224mm^2, data matrix=112×112, slice thickness=2mm, gap=0, slice number=70, slice order=interleaved, TR=16500 ms, TE=95.6 ms, 30 gradient directions and 5 b0 images (b=1000s/mm^2), voxel size =2×2×2 mm^3.

Resting state fMRI: Single-shot gradient-echo EPI sequence is used for rs-fMRI with following parameters: FOV=224×224mm^2, data matrix=64×64, slice thickness=4.0 mm, gap=1.0 mm, slice number=28, slice order=interleaved, TR=2000ms, TE=30 ms, FA=90°, voxel size =3.5×3.5×4 mm^3.

Task based fMRI: We will also conduct an optional block design fMRI paradigm to measure

somatosensory mismatch negativity (MMN), which could be elicited by discriminable change in the acoustic environment. The specific cognitive function links to three stages including sensory register, short-term memory and long-term memory. Whereas most studies were concentrated on short-term and long-term memory, few studies focus on somatosensory MMN. So, this project will use a self-designed air jet pressure stimulation device combined with fMRI to test the somatosensory MMN. For the MMN condition, the switch trial stimuli are a 'deviant' and has proven to elicits robust MMNs. For the matched control condition, the same two stimuli were alternated sequentially to form a regular pattern, such that no MMN would be elicited.

Imaging data analysis

For analysis of the structural MRI, morphological features like regional cortical thickness and gray matter volume will be analyzed based on the T1 weighted images. DTI data will be analyzed by tract-based spatial statistics (TBSS) regarding the fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AxD). Resting-state functional MRI data will be analyzed by several different strategies including the amplitude of low-frequency fluctuations (ALFF), regional homogeneity (ReHo), and functional connectivity derived from the independent component analysis (ICA). Further, structural, white matter and functional network will be constructed and topographic characteristic such as modality, rich-club will be calculated.

Analysis of PET data will be performed based on standardized uptake volume ratios (SUVR) with pre-specified cut-offs. In addition, voxel-based and ROI-based methods will be used in both FDG-PET and Aβ-PET.

Statistical analysis

All data (demographic information, clinical data, risk factors, neuropsychologic tests, blood and urine biomarker, multi-modal MRI biomarker, glucose mentalism and amyloid deposition) will be compared between SCD convertors and SCD non-convertors with two sample t-test for continuous variables and chi-square test for categorical variables.

Four models will be established in this project. Model 1 will simply take demographic data, lifestyle, clinical assessment, neuropsychological assessments and SCD report into account.

Model 2 will add APOE genotype, plasma Aβ and urine AD7c-NTP on the basis of model 1. Model 3 will take neuroimaging data into account on the basis of model 2 and model 4 will include amyloid-PET and FDG-PET on the basis of model 3. Detailed descriptions of independent variables are provided in table 1. The primary outcome is defined as converting to MCI.

Table 1 independent variable list

Demographic data	age; education level; family history of dementia; socioeconomic information
Lifestyle	nicotine and alcohol use; sleep quality (PSQI, RBDSQ, ESS); nutrition style
Clinical assessment	vascular risk factors (hypertension, hyperlipemia, diabetes, coronary heart disease, Fazekas score); medial temporal lobe atrophy scale; HAMA; HAMD; GDS
Neuropsychological assessment	AVLT-D; AVLT-R; STT-A; STT-B; BNT; AFT
SCD report	onset time; comparison with others; SCD-Q9; Ecog; consistency of SCD; inform report
Blood tests	APOE genotype; Plasma β-amyloid
Urine tests	level of AD7c-NTP
Neuroimage data	cortical thickness; gray matter volume; FA; MD; RD; AxD; ALFF; ReHo; functional connectivity; network characteristic (rich-club, modality)
PET data	global SUVR of FDG-PET and Aβ-PET

HAMA, he Hamilton Anxiety Scale; HAMD, the Hamilton Depression Scale; GDS, Geriatric Depression Scale; AVLT, Auditory Verbal Learning Test; STT, Shape Trails Test; BNT, Boston naming test; AFT, animal fluency test; SCD, subjective cognitive decline; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; AxD, axial diffusivity; ALFF, amplitude of low-frequency fluctuations; ReHo: regional homogeneity; SUVR, standardized uptake volume ratios

The least absolute shrinkage and selection operator (LASSO) logistic regression model with

penalty parameter tuning will be used for variable screening. Then, using the sub-distribution hazard function model, we will analyze the effect of variables screening by LASSO on the time to conversion to MCI in five-years follow-up. The hazard ratio (HRs) with 95% confidence intervals are determined. All statistical tests were performed using R statistical software. The "glmnet" package will be used for the LASSO logistic regression model analysis and the "cmprsk" package will be performed for sub-distribution hazard function model.

To revealing progressing structural and functional pattern in SCD, one-way analysis of variance (ANOVA) will be used in those who convert to MCI and AD dementia in five years with Bonferroni for post-hoc test. The structural parameters (global and regional gray matter volume, cerebral cortex thickness), white matter parameters (regional FA, MD, RD, AxD), functional characteristic (global and regional ALFF, ReHo, functional connectivity) and network characteristics will be compared in this part.

DISCUSSION

The current study evaluates characteristics of SCD those presenting cognitive decline within five years and furthermore we will construct risk forecast models based on different combination of potential predictors to achieve clinical practice of SCD.

Considering the long preclinical stage of AD and compensated cognitive function in SCD stage, the application of a refined SCD approach as an enrichment strategy for clinical trials focusing on preclinical AD shows great promise. The SILCODE is a longitudinal study and we will focus on features of SCD convertors with multi-perspective analysis. Especially, we would like to establish an integrated diagnostic system for early detection and prediction of SCD progressing at the individual level. We aim to provide scientific evidence for more effective diagnosis of SCD due to AD and explore the underlying mechanism of SCD.

In this project, plasma Aβ, urine AD7c-NTP and multi-modal MRI will also be included in the predicting models. These examinations are non-invasive and feasible in clinical practice. Previous studies have found their diagnostic efficiency in MCI and dementia,[31, 32] but in SCD individuals, there are few evidences. So, this project also focuses these new biomarkers and try to identify their usage in clinical scene. Additionally, through this project, we would like to reveal the structural and functional patterns of involved brain area, especially the temporal and

spatial sequences, which may provide basis for us to understand the relationship between structural and functional changes during disease progression.

At last, cultural factors may impact on SCD relate to language, social stigmatization of cognitive problems, styles of responding on self-reported measures, tolerance of slowly progressive cognitive decline in aging and others,[33] which indicating the need for SCD research in China. SILCODE is a longitudinal study in China and the implement of SILCODE would provide characteristics of SCD in China, which contribute to the harmonization of the SCD concept across cultural borders.

Author Contributions

Xuanyu Li and Xiaoni Wang are joint first authors. All authors contributed to the overall design and implementation of the study. Xuanyu Li and Xiaoni Wang drafted the manuscript. Ying Han is supervising the project and made critical revision of the manuscript for important intellectual content. All authors contributed to the drafting of the manuscript and approved the final manuscript.

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Competing interests

None declared.

Patient consent

All participation is based on written informed consent and the participants will be able to withdraw from the study at any time.

Ethics approval

Ethical approval of this has been obtained from the medical research ethics committee and institutional review board of the XuanWu Hospital, Capital Medical University (ID: [2017]046).

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Figure legend

Figure 1 Flow chart of SILCODE. SCD, subjective cognitive decline; sMRI, structural MRI; DTI, diffusion tensor imaging; fMRI, functional MRI; FDG-PET, fluordesoxyglucose positron emission tomography; Aβ-PET, β-amyloid positron emission tomography

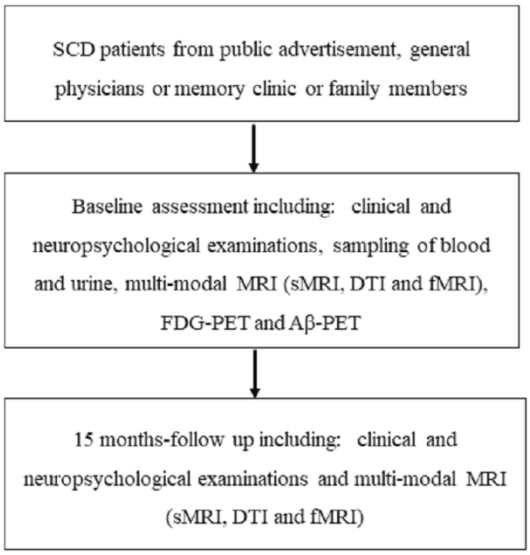

Figure 1 Flow chart of SILCODE

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Sino Longitudinal Study on Cognitive Decline (SILCODE): protocol for a Chinese longitudinal observational study to develop risk prediction models of conversion to mild cognitive impairment in individuals with subjective cognitive decline

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Sino Longitudinal Study on Cognitive Decline (SILCODE): protocol for a Chinese longitudinal observational study to develop risk prediction models of conversion to mild cognitive impairment in individuals with subjective cognitive decline

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ABSTRACT

Introduction Understanding the biological mechanism of subjective cognitive decline (SCD) in preclinical Alzheimer's disease (AD) and identifying those who will convert to mild cognitive impairment (MCI) in a short time are critical for developing appropriate strategies for early diagnosis and intervention of AD. We aim to present the study protocol of the Sino Longitudinal Study on Cognitive Decline (SILCODE), a longitudinal observational study focusing on SCD in the context of AD.

Methods and analysis Within SILCODE, approximately 800 subjects between the ages of 50-79 years old with SCD will be recruited from standardized public advertisement or memory clinics. They will undergo extensive assessment, including clinical and neuropsychological assessments, blood samples collection for plasma beta-amyloid and ApoE genotype, urine samples collection for AD7c-NTP, multimodal MRI scans (structural MRI, diffusion tensor imaging, resting-state functional MRI and optional task-based functional MRI) as well as optional glucose metabolism and amyloid positron emission tomography. Subjects will be contacted by telephone every 3 months and be interviewed average every 15 months for five years. The study endpoint is the development of mild cognitive impairment or dementia. Jak & Bondi's actuarial neuropsychological method will be used for diagnosis of MCI. The least absolute shrinkage and selection operator (LASSO) logistic regression model followed by the sub-distribution hazard function model with death as a competing risk will be constructed to establish risk prediction models.

Ethics and dissemination The medical research ethics committee and institutional review board of the Xuanwu Hospital of Capital Medical University approved this study protocol (ID: [2017]046). The results will be published in peer-reviewed journals and presented at national and international scientific conferences.

Trial registration NCT03370744; pre-results.

Article Summary

Strengths and limitations of this study

- A strength of this study is the use of multiple markers (clinical, blood, urine and imaging markers) to establish models predicting SCD conversion to MCI, which will provide

references for clinical applications of SCD.

- This longitudinal study could enrich the understanding of SCD based on a large-scale Chinese population, since cultural factors have an impact on SCD.
- The relatively short follow-up duration (5 years) of this study makes longitudinal data will be only analyzed in an exploratory way.
- The study results will be limited by the research environment that individuals will be recruited from memory clinics and volunteer samples without community-based samples or population-based samples.

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease and one of the greatest healthcare challenges of the 21st century.[1] It has been revealed that pathophysiology starts many years before the onset of clinical symptoms.[2] Several recent clinical trials of Aβ-lowering therapies in the mild or moderate dementia stage and even mild cognitive impairment stage have failed,[3 4] which further encouraged researchers to shift their focus to the preclinical stage of AD for the target of new treatments.[5]

Subjective cognitive decline (SCD) refers to self-perceived cognitive decline relative to a previously normal status, without impaired performance on standardized neuropsychological tests.[6 7] Evidences from recent researches suggest that SCD may be one of the earliest symptomatic manifestations of AD.[6 8] Previous studies have found higher rate and shorter time of conversion to mild cognitive impairment (MCI) and dementia in SCD individuals as compared to normal controls (NC).[9-12] SCD may be an early trigger for help-seeking[13] and we have observed that the number of individuals with SCD presenting to the healthcare system (e.g. memory clinics) is increasing in our clinical practice as the rising awareness of AD dementia in China. Individuals with SCD are ideal for ultra-early diagnosis and intervention trials.

However, the heterogeneous nature of SCD limited its value in the clinical application. Research to date has focused on factors for predicting risk for MCI/AD progression in SCD.[14-17] Though some longitudinal and observational studies of SCD are ongoing,[18-20] there is a lack of data from China even Asian. However, cultural factors impacting on SCD relate to language,

social stigmatization of cognitive problems, styles of responding on self-reported measures (e.g. extremity of answers), tolerance of slowly progressive cognitive decline in aging and others.[21] Association between objective cognitive performance and SCD is different across races.[22] Our group reported that the prevalence of SCD was 14.4% in the Shunyi District of Beijing, China,[23] which is different from prevalence of SCD reported in western countries.[24 25] Thus there is a need for more findings from longitudinal studies based on large-scale Chinese population.

The Alliance for preclinical AD in China, belonging to the National Clinical Research Center for Geriatric Disorders, is a national multicenter research platform, which dedicates to the development of clinical, neuroimaging, genetic, and biochemical biomarkers for the early detection and tracking of AD. Its sites include more than 180 hospitals/research institutes members all over China. The alliance is led by professor Han and focuses on the early detection of SCD during preclinical AD, which also develops and provides standard operating procedures and quality control for harmonized data and material acquisition and storage across all sites.

In this protocol, we introduce in detail the design of the Sino Longitudinal Study on Cognitive Decline (SILCODE) study. This is an ongoing longitudinal observational study based on the multicenter research platform of the Alliance for preclinical AD in China, focusing on SCD in the context of AD. The main aim of SILCODE is to estimate cognitive decline and establish statistical prediction models in SCD in Chinese elderly people.

METHODS

Overall study design

This is an observational longitudinal study in China recruiting SCD. During the baseline period, all eligible subjects with SCD will be invited to receive clinical and neuropsychological examinations, blood tests, urine tests and multi-modal MRI including structural MRI (sMRI), diffusion tensor imaging (DTI) and resting-state functional MRI (rs-fMRI) sequentially as shown in figure 1. Glucose metabolism and amyloid positron emission tomography (PET) and task-based fMRI (t-fMRI) will be optional based on the individual's agreement. A semi-structured interview[18] as wll as the SCD questionnaire[26] and Everyday Cognition questionnaire (ECog)[27] will be used to assess the SCD. The duration between neuropsychological tests and other data collection will be within three months. Subjects will be contacted by telephone every

3-months and be interviewed average every 15 months for five years (4 time points). the Montreal Cognitive Assessment-basic (MoCA-B)[28] subtests (language, delayed recall and orientation) will be taken during the call. Those expressing significantly cognitive decline on the telephone will be interviewed ahead of time for the next follow-up. During the follow-up visits with interview, evaluations including clinical and neuropsychological examinations, blood tests, urine tests, and multi-modal MRI as well as optional glucose metabolism PET and t-fMRI will be conducted average every 15 months. At each follow-up visit, diagnoses will be reevaluated under the supervision of two neurologists. Figure 1 provides the overall study outline.

Patient and Public Involvement

Patients and public will not be involved in the development of the research question or the design of the study. Patients will not be involved in the recruitment and conduct of the study. The general results will be disseminated to participants through public education activities.

Sample size calculation

The formula proposed by Hsieh and Lavori[29] was used to estimate the sample size in our study with PASS software (version 15.0.5). According to a previous meta-analysis,[9] the cumulative conversion proportion was 24.4% over a mean of 4.1 years follow-up. We assumed the power (1-β) was 80%, α was 0.05 and the rate of loss to follow-up was 20%. Due to the lack of statistical data of SCD, we calculated the sample size with data reported by Licher et al[30] and Pinto et al[31] (Hazard ratio= 1.03, standard deviation=8.2, R^2 =0.127) from cognitively normal elderly subjects for the association between age and cognitive decline. This calculation rendered a total sample size of 762. Considering the internal validation, we will recruit at least 800 subjects.

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Recruitment of participants

Participants will be consecutively recruited from standardized public advertisements, referrals from general physicians or memory clinics or informants. Residents who meet the inclusion criteria will be recruited. Written informed consent will be acquired from each subject before

enrollment.

SCD, MCI and dementia diagnostic criteria

SCD is defined by the research criteria for pre-MCI (SCD) proposed by Jessen et al in 2014[6]: (1) Self-experienced persistent decline in cognitive capacity in comparison with a previously normal status and unrelated to an acute event; (2) Normal age-, gender-, and education-adjusted performance on standardized cognitive tests.

MCI is defined by an actuarial neuropsychological method proposed by Jak and Bondi.[32] Participants are considered to have MCI if any one of the following three criteria are met as well as failure to meet the criteria for dementia: (1) having impaired scores (defined as >1 SD below the age-corrected normative means) on both measures within at least one cognitive domain (memory, language, or speed/executive function); (2) having impaired scores in each of the three cognitive domains sampled (memory, language, or speed/executive function); (3) the Functional Activities Questionnaire (FAQ) ≥9. The normative means in our study are from Guo and his team in a Chinese population. [33-36] Measures and normative means are shown in table 1.

The diagnosis of AD dementia is based on the Diagnostic and Statistical Manual of Mental Disorders, fifth edition and the diagnostic guidelines for dementia due to AD delivered by the National Institute on Aging–Alzheimer's Association workgroups (NIA-AA),[37] who have a total CDR score ⩾ 1.

Table 1 Cognitive domains, neuropsychological tests and normative means

Cognitive domains	Neuropsychological tests	Normative means
Memory	Auditory Verbal Learning Test-long delayed memory[33]	50-59 years old: 5; 60-69 years old: 4; 70-79 years old: 3
	Auditory Verbal Learning Test-recognition[33]	50-59 years old: 20; 60-69 years old: 19; 70-79 years old: 18
Language	Animal Fluency Test[35]	junior middle school: 12; high school: 13; college: 14
	30-item Boston Naming Test[34]	junior middle school: 20; high school: 21;

		college: 22
speed/executive	Shape Trails Test Parts A[36]	50-59 years old: 70s; 60-69 years old: 80s; 70-79 years old: 100s
	Shape Trails Test Parts B[36]	50-59 years old: 180s; 60-69 years old: 200s; 70-79 years old: 240s

Inclusion/exclusion criteria

The inclusion criteria are as follows: (1) older than 50 years old and Mandarin-speaking subjects; (2) presence of self-perceived continuous cognitive decline compared to previous normal status and unrelated to an acute event; and (3) concerns (worries) associated with memory complaint; and (4) failure to meet the criteria for MCI or dementia.

Exclusion criteria are as follows: (1) history of stroke; (2) current major psychiatric diagnoses such as severe depression and anxiety. When mild and moderate symptoms of psychiatric diagnosis are suspected, patients will be not be excluded[38]. They will be evaluated by a psychiatrist to clear if the psychiatric diagnoses are the cause of SCD; (3) other neurological conditions which could cause cognitive decline (e.g., brain tumors, Parkinson's disease, encephalitis, or epilepsy) rather than AD spectrum disorders; (4) other diseases which could cause cognitive decline (e.g., thyroid dysfunction, severe anemia, syphilis, or HIV); (5) history of psychosis or congenital mental growth retardation; (6) cognitive decline caused by traumatic brain injury; (7) those who could not complete the study protocol or with contraindications for MRI.

Clinical progression

At each follow-up visit, diagnoses will be reevaluated under the supervision of two neurologists. The main outcome measure of clinical progression in our study is converting to MCI or AD dementia. Time to event is the date difference between the baseline neuropsychological tests and the date at which the MCI is first diagnosed. For those who miss the diagnosis of MCI during follow-up, the time between the baseline neuropsychological tests and dementia first diagnosed is defined as the time to event in our study.

Assessments of subjective cognitive functioning

A semi-structured interview used by the DELCODE study will be employed in this project to evaluate the details of SCD.[18] It includes information about the onset time, concerns, comparison with others, and the history of visiting a physician not only memory domain but also language, attention, and executive. We also will require informant reports in the evaluation of the self-reported information as suggested by Subjective Cognitive Decline Initiative Working Group.[7 39]

For quantitative assessment of the severity of SCD, we will use a newly developed SCD questionnaire including nine reliable SCD items (table 2), which are characterized by different domains, such as global memory functioning and daily activities ability.[23 26] In addition, the 12-item ECog[27] will also be applied to all participants to measure SCD severity. These questionnaires will not be considered as inclusion criteria but only for statistical analysis.

Table 2 items included in SCD questionnaire[26]

Items
Do you think you have problems with your memory?
Do you have difficulty remembering a conversation from a few days ago?
Do you have complaints about your memory in the last 2 years?
How often is the following a problem for you: Personal dates
How often is the following a problem for you: Phone numbers you use frequently
On a whole, do you think that you have problems remembering things that you want to do or say?
How often is the following a problem for you: Going to the store and forgetting what you wanted to buy
Do you think that your memory is worse than 5 years ago?
Do you feel you are forgetting where things were placed?

Clinical, risk factor and neuropsychological assessments

The clinical assessments include a structured medical history, physical examination, routine laboratory tests (including blood routine test, biochemical test, thyroid function test, Vitamin B12, folic acid, treponema pallidum specific antibody and HIV antibody) and MRI (T1 and Flair) for medial temporal atrophy scale and Fazekas score.

For risk factors of AD, all subjects and their informants will provide the following information: family history, vascular risk factors, occupation/retirement, socioeconomic status, cigarettes, and alcohol uses, and nutrition style. Additionally, Pittsburgh sleep quality index (PSQI)[40], Rapid Eye Movement (REM) sleep Behavior Disorder Screening Questionnaire (RBDSQ)[41] and the Epworth Sleepiness Scale (ESS)[42] will be used for sleep quality assessment

The neuropsychological tests are selected for comparability with other similar ongoing studies (e.g., DELCODE).[18] To avoid deviations in the evaluation, the neuropsychologists performing the cognitive tests will be trained with standard guidelines. Kappa coefficient (Fleiss' kappa) will be used to measure the assessment agreement among the neuropsychologists. Neuropsychological results will be double-checked. The neuropsychological battery (table 1) measures different cognitive domains including episodic memory (Auditory Verbal Learning Test-HuaShan version [AVLT-H]),[33] language (Animal Fluency Test,[35] 30-item Boston Naming Test[34] and speed/executive function (Shape Trails Test Parts A and B[36]). Besides, the SILCODE will implement tests for global cognition, daily life ability and neuropsychiatric assessment including MoCA-B,[28] the memory and executive screening (MES),[43] the CDR, FAQ, the 15-item short form of the Geriatric Depression Scale (GDS), the Hamilton Anxiety Scale (HAMA), the Hamilton Depression Scale (HAMD) and the Neuropsychiatric Inventory (NPI).

Blood tests

ApoE genotype

SNPs rs7412 and rs429358 form the ApoE ε2/ε3/ε4 haplotype. ApoE will be genotyped using the standard Sanger sequencing method (Sangon, Shanghai, China) using the following primers: 5′-ACGCGGGCACGGCTGTCCAAGG-3′ (forward) and 5′-GGCGCTCGCGGATGGCGCTGA-3′ (reverse). ApoE will be amplified using the following conditions: 1 cycle of 98 °C for 10 s, 35 cycles of 72 °C for 5 s, 1 cycle of 72 °C for 5 min. PCR was performed in a final volume of 30 μl, containing 10 pmol of forward and reverse primers and 50 ng of genomic DNA template, using PrimeSTAR HS DNA Polymerase with GC Buffer (Takara Bio).

Plasma β-amyloid

Recently, some research found that plasma amyloid level was correlated with cognitive capacity and cerebrospinal fluid amyloid protein.[44 45] Especially, when predicting brain amyloid deposition, the accuracy of the composite biomarker (amyloid-β precursor protein 669-711/ Aβ1-42 and Aβ1-40/Aβ1-42 ratios) could approximately equal to 90%.[46] So, we would like to identify if the plasma β-amyloid is also associated with cognitive decline in SCD individuals. Plasma Aβ will be determined using commercially available kit, V-PLEX Aβ Peptide Panel 1 (6E10) Kit (K15200E) (Mesoscale Diagnostics LLC, Rockville, USA). Aβ peptide levels from each blood draw will be measured in duplicate using the same aliquot.

Urine tests

Ten ml of first-morning urine will be collected from each subject and then immediately refrigerated. An enzyme-linked immunosorbent assay kit will be used to detect the protein level of AD7c-NTP in the urine specimens.

Imaging protocol

MRI data will be acquired using an integrated simultaneous 3.0 Tesla TOF PET/MR (SIGNA PET/MR, GE Healthcare, Milwaukee, WI, USA) at the Xuanwu Hospital of Capital Medical University. For each participant, simultaneous PET and 3.0 T MRI data will be obtained. Brain MR images will be inspected by an experienced neuroradiologist.

Positron emission tomography: All participants will be invited for optional [^{18}F] florbetapir (AV-45) and [^{18}F] fluordesoxyglucose (FDG) PET in 3-dimensional acquisition mode. The time duration between the FDG-PET and AV-45 PET is 3 days at least to eliminate the effect of the first tracer. For FDG-PET, each subject will be instructed to fast for at least 6 h and will have a confirmed serum glucose level below 8 mmol/L; 35 minutes dynamic scan is acquired about 40 minutes after an intravenous injection of 3.7 MBq/kg of 18F-FDG. For Aβ-PET, 35 minutes dynamic scan is acquired about 40 minutes after an intravenous injection of 7-10mCi [^{18}F] florbetapir. The PET data are acquired using a TOF-OSEM algorithm (time-of-flight ordered subset expectation maximization) with the following parameters: 8 iterations, 32 subsets matrix = 192×192, field of view (FOV)= 350×350, half-width height = 3.

Structural MRI: Parameters for T1-weighted 3D brain structural images are as following: SPGR sequence, FOV=256×256 mm^2, matrix=256×256, slice thickness=1 mm, gap=0, slice number=192, repetition time (TR) =6.9 ms, echo time (TE)=2.98 ms, inversion time (TI)=450 ms, flip angle =12°, voxel size = 1×1×1 mm^3.

DTI: DTI data are obtained with Single-shot spin-echo diffusion-weighted EPI sequence with following parameters: FOV=224×224mm^2, data matrix=112×112, slice thickness=2mm, gap=0, slice number=70, slice order=interleaved, TR=16500 ms, TE=95.6 ms, 30 gradient directions and 5 b0 images (b=1000s/mm^2), voxel size =2×2×2 mm^3.

Rs-fMRI: Single-shot gradient-echo EPI sequence is used for rs-fMRI with following parameters: scan duration=8 minutes, FOV=224×224mm^2, data matrix=64×64, slice thickness=4.0 mm, gap=1.0 mm, slice number=28, slice order=interleaved, TR=2000ms, TE=30 ms, FA=90°, voxel size =3.5×3.5×4 mm^3.

T-fMRI: T-fMRI: All participants will also be invited for a block design fMRI paradigm to measure somatosensory mismatch negativity (MMN). We will use a self-designed air jet pressure stimulation device, which could carry out 5.0 bar pressure somatosensory stimulation in right index and middle finger respectively. The index and middle finger of the right hand will be fixed by two adhesive tapes to restrict any movement during the experiment. The length of the two air pipes from the air compressor to the apertures will be 6.0 meters, and the diameter of the two stimulation apertures will be 1.0 millimeter. In addition, the distance between the aperture and the center of the finger pad will be fixed at 5.0 millimeters for all subjects. Subjects will be instructed to watch a video (without sound) presented on a custom designed screen and to ignore stimulation to the fingers.

The functional MRI paradigm will have a total 9 min duration. In a block design, 8 control blocks with 32s duration and 8 MMN blocks (also 32s duration). A constant stimulus onset asynchrony of 500 ms will be employed for both the MMN and control conditions resulting in 40 stimuli per block. In devising the MMN and control blocks, we will take advantage of an MMN paradigm in which two stimuli will be presented equiprobably and their presentation order will be varied. In MMN blocks, stimulation will be arranged to compose alternating ‘mini-sequences’ of stimulus 1 (index finger, 5.0 bar, 300ms) and 2 (middle finger, 5.0 bar, 300ms). Mini-sequences will be 2, 3, or 4 repetitions of a single stimulus (each sequence length

is represented equiprobably) followed by a mini-sequence of the other stimuli, and so on. The number of trials in a given mini-sequence will be various such that the occurrence of a switch from stimulus 1 to stimulus 2 (and vice-versa) is irregular. As such, the switch trial stimuli will be designated as deviants and will elicit the MMN. This basic stimulation paradigm, which has proven to elicit robust MMNs, will be used for the MMN condition.[47 48] For the matched control condition to the aforementioned MMN condition, the same two stimuli will be alternated sequentially to form a regular pattern (e.g. stimulus 1, stimulus 2, stimulus 1, stimulus 2, etc.).

Imaging data analysis

PET data analysis. The AV-45 and FDG PET scans will be preprocessed by the Statistical Parametric Mapping version 12 (SPM 12; https://www.fil.ion.ucl.ac.uk/spm/software/spm12). The structural images will be individually registered to the averaged PET images. The unified segmentation method[49] will be then applied to all coregistered structural images. The PET images will be spatially normalized to Montreal Neurological Institute (MNI) standard space by using the forward parameters estimated during the unified segmentation and smoothed with a Gaussian kernel of 8-mm full-width half-maximum (FWHM). Finally, voxel-wise AV-45 and FDG PET standardized uptake value ratio (SUVR) will be normalized by the whole cerebellum and pons as the reference regions, respectively. A global AV-45 PET SUVR value will be estimated using a composite of the prefrontal, orbitofrontal, parietal, temporal, anterior cingulate, and posterior cingulate and precuneus cortices.

SMRI data analysis. The cortical thickness analysis will be performed using the FreeSurfer image analysis suite, version 5.3 (http://surfer.nmr.mgh.harvard.edu). We will construct models of the boundaries between grey matter and white matter as well as pial surface.[50] Cortical thickness measures will be obtained by calculating the distance between these surfaces. Automated reconstruction and labeling will be performed using the default "recon-all" command line. All generated images will be visually inspected for image and segmentation quality and corrected manually if necessary. Subsequently, "lobesStrict" will be performed to obtain a lobar annotation and consecutively imported for further statistical analyses. For the voxel-based morphometry (VBM) analysis, structural images will be segmented into grey matter (GM) tissue using the new segment function within SPM12. The diffeomorphic

anatomical registration using the exponentiated lie algebra (DARTEL) toolbox will be used to generate a reference template object of the sample which will be warped into a standard MNI space. The generated flow fields of each subject will be calculated and normalization parameters will be then implemented to normalize the GM maps in the native space to the MNI space. During the normalization process, the modulated images (local native amount of grey matter) will be preserved. Images will be spatially smoothed with an 8 mm FWHM Gaussian kernel. Finally, for each individual, we will obtain a smoothed GM volumetric map. Additionally, the total intracranial volume (TIV) for each individual will be estimated by summing the segmented GM, white matter, and cerebral spinal fluid (CSF). Then the smoothed GM images of every individual will be used for subsequent statistical analysis.

DTI data analysis. Raw DTI data will be processed by using the Oxford Centre for Functional MRI of the Brain (FMRIB) Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl).[51-53] Initially, the EddyCorrect tool will be used for correcting eddy current distortions and motion artifacts by fine registration of the DTI images to a reference image (b0 image). The Brain Extraction Tool (BET)[53] will be used for creating brain masks of all subjects and then a diffusion tensor will be modeled at each voxel by using the least- squares algorithm fitting tensor model within the DTI-FIT Tool.[54] Fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AxD) values of each voxel will be calculated based on the eigenvalues of the tensor. Voxel-wise statistical analysis of the FA, MD, RD, and AxD data will be performed using Tract-Based Spatial Statistics (TBSS).[55] All subjects' FA maps will be nonlinearly coregistrated to FMRIB58_FA template with FSL's nonlinear image registration algorithm. Then, the mean FA image will be obtained and thinned to create a mean FA skeleton representing the center of all tracts common to all subjects. Each subject's aligned FA data will be then projected onto the FA skeleton to obtain their FA skeletons and deformation matrixes. With the deformation matrixes, the skeletonized AxD, MD, and RD maps will be created for every individual by the tbss_non_FA tool. These maps will be used for subsequent statistical analysis.

Rs-fMRI data analysis. Resting-state functional MR imaging will be preprocessed by using the Data Processing Assistant for Resting-state fMRI (DPASF; http://www.rfmri. org/DPARSF).[56] The first 10 volumes will be discarded for image stabilization and participant's to adaptation to

the scanning. The remaining functional sequences will be first corrected for timing differences between each slice and motion effects. Next, the structural image will be co-registered to the mean functional image. Then the transformed structural images will be segmented to grey matter, white matter, and CSF. The motion-corrected functional volumes will be spatially normalized to MNI 152 standard space and resampled to 3 mm × 3 mm × 3 mm cubic voxels by using the normalization parameters estimated during unified segmentation. The resulting images will further undergo spatial smoothing (Gaussian kernel with an 8mm FWHM), linear detrending and temporal filtering (0.01-0.08 Hz). Note, in order to avoid overestimating regional homogeneity (ReHo) values, spatial smoothing will be conducted for individual ReHo maps rather than during data preprocessing. Finally, nuisance signals (including Friston 24-head motion parameters, the white matter, and CSF) will be extracted and regressed out from the data to reduce the residual effects of nonneuronal factors. For the amplitude of low frequency fluctuation (ALFF) analysis[57], the time series of each voxel will be transformed into the frequency domain using a fast Fourier Transform. The square root of the power spectrum will be calculated and averaged across 0.01-0.08 Hz. This averaged square root will be taken as the ALFF value for this voxel. For the ReHo analysis[58], Kendall's coefficient of concordance (KCC) will be computed on the ranked time series of a given voxel with its 26 nearest neighbors. The resultant KCC will be taken as the ReHo values. The generated ALFF and ReHo images will be used for statistical analysis.

Network analysis. We will construct structural cortical network based on grey matter volumes.[59] The nodes will be defined as brain regions corresponding to automated anatomic labeling (AAL) areas. The structural connections will be defined as statistical correlations between pairs of average grey matter volumes in our study. A structural connection will be considered to be existing if the correlation coefficient is statistically significant. Before the correlation analysis, the effects of age, sex and total grey matter volume on grey matter volume of regions will be adjusted. We will calculate Pearson correlation coefficients across individuals between the average grey matter of every pair of regions and then an interregional correlation matrix will be obtained for every individual. We will construct white matter network based on fiber number. The network nodes will be defined as the 90 regions of interest corresponding to AAL template. The weight of the network edge will be defined as the number of connected

fibers with two regions. The fiber number will be calculated through diffusion-tensor imaging tractography as reported in our previous study.[60] To avoid spurious connections, a minimum threshold of fiber number (weight of the edge=10) will be used. We will construct functional network based on the average time sequence. The network nodes will be defined as the 90 regions of interest corresponding to AAL template. The network edge will be defined as the partial correlation coefficients between the average time sequence of two regions and we will obtain an incidence matrix. Non-significant correlations will be excluded. The network analyses will be performed by the GRETNA toolbox (http://www.nitrc.org/projects/ gretna/).[61] The 'rich club coefficient' is defined as the density of connections between rich club nodes and rich club regions in our study will be defined the top 13 brain regions with the highest degree as reported in our previous study.[62] A module is defined as a subset of nodes connected to the other nodes in the same module than those outside the module.[63] We would like to use Newman's metric to measure the modularity[64] and maximize the modularity parameter Q by the algorithm proposed by Clauset et al.[65] The two parameters will be calculated for the structural cortical network, white matter network and functional network constructed for each individual. The values of the two measures will be calculated for every individual and be took into the models construction.

T-fMRI data analysis. We will use SPM12 to process and analyze the t-fMRI data. The first 10 volumes will be discarded due to unsteady magnetization. First, the functional images from each run will be realigned. The structural image will be then coregistered to the first scan in the functional image, and the resulting coregistered structural image will be normalized to MNI 152 standard space. Finally, these spatially normalized functional images will be smoothed (Gaussian kernel with an 8mm FWHM).

Statistical analysis

Baseline comparison between SCD convertors and SCD non-convertors

All data (demographic information, clinical data, risk factors, neuropsychologic tests, blood and urine biomarkers, multi-modal MRI biomarkers, glucose mentalism, and amyloid deposition) will be described. The Shapiro-Wilk test and Q-Q plots will be used to confirm the normality. All normally distributed continuous variables will be reported as the mean ± SD.

Compared at baseline between SCD convertors and SCD non-convertors will be done with two-sample t-test for continuous variables and chi-square test for categorical variables. Dunnett's multiple comparison tests will be performed for comparison.

Statistical analyses of t-fMRI data will be subjected to a general linear model analysis. The MMN condition will be served as the predictor of interest and will be modeled with a boxcar function convolved with the canonical haemodynamic response function; the Control condition will be served as a baseline. The beta estimates from the individual general linear models enter a second level random effects analysis. Contrast maps will be created by applying paired t-tests comparing the MMN versus control condition for each group separately as well as a two-sample t-test for the between-groups comparison. Activation maps will be corrected ($P<0.001$) by the false discovery rate approach implemented in SPM.

Longitudinal patterns in SCD-converters

The longitudinal analysis in neuropsychological, plasma, urine and MRI variables in SCD-converters will be assessed. We will use the general linear mixed effects model to estimate the individual's change in each variable.

Statistical prediction models

We will use the competing risk regression model to detect the association between possible risk factors and endpoint. The endpoint event of our study is converting to MCI. Death before conversion to MCI is considered as a competing risk in our study. Time to event is the date difference between the baseline neuropsychological tests and the date at which the MCI or dementia is first diagnosed.

Independent variables are listed in table 3 including features of baseline clinical characteristics, blood, urine, MRI and PET biomarkers. considering the overfitting effect when establishing the model, the least absolute shrinkage and selection operator (LASSO) model with penalty parameter tuning will be used for variable screening. It is a data mining method for shrinkage estimation and dimensionality reduction, overcoming processing difficulties caused by high-dimensional data, and estimating the parameters more accurately. Then, we will perform the sub-distribution hazard function model, which could evaluate hazards for the endpoint (MCI or dementia) and competing (death) events, to establish the final multivariate models. Ten-fold cross-validation is used to perform internal verification of the established models. The optimism-

corrected C-statistics will be used to evaluate the performance of risk prediction models.

To better clarify the relationship between possible risk factors and endpoint events, some confounding factors will be adjusted. Four models will be established in our study. Model 1 will simply take demographic data, lifestyle, clinical assessment, neuropsychological assessments, and SCD report into account. Model 2 will add ApoE genotype, plasma Aβ, and urine AD7c-NTP on the basis of model 1. Model 3 will take neuroimaging data into account on the basis of model 2 and model 4 will include amyloid-PET and FDG-PET on the basis of model 3.

The hazard ratio (HRs) with 95% confidence intervals are determined. A two-sided $p<0.05$ is defined as statistical significance. All statistical tests were performed using R statistical software. The "glmnet" package will be used for the LASSO model analysis and the "cmprsk" package will be performed for the sub-distribution hazard function model.

Table 3 independent variable list

Types	
Demographic data	age; education level; family history of dementia; socioeconomic information
Lifestyle	nicotine and alcohol use; sleep quality (PSQI, RBDSQ, ESS); nutrition style
Clinical assessment	vascular risk factors (hypertension, hyperlipemia, diabetes, coronary heart disease, Fazekas score); medial temporal lobe atrophy scale; HAMA; HAMD; GDS
Neuropsychological assessment	AVLT-D; AVLT-R; STT-A; STT-B; BNT; AFT
SCD report	onset time; comparison with others; SCD-Q9; Ecog; consistency of SCD; inform report
Blood tests	ApoE genotype; Plasma β-amyloid
Urine tests	level of AD7c-NTP
MRI data	cortical thickness; grey matter volume; FA; MD; RD; AxD; ALFF; ReHo; network characteristic (rich-club coefficient, modality); MMN-related haemodynamic responses
PET data	global SUVR of FDG-PET and Aβ-PET

HAMA, he Hamilton Anxiety Scale; HAMD, the Hamilton Depression Scale; GDS, Geriatric Depression Scale; AVLT, Auditory Verbal Learning Test; STT, Shape Trails Test; BNT, Boston naming test; AFT, animal fluency test; SCD, subjective cognitive decline; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; AxD, axial diffusivity; ALFF, amplitude of low-frequency fluctuations; ReHo: regional homogeneity; SUVR, standardized uptake volume ratios; MMN: mismatch negativity

DISCUSSION

The current study evaluates characteristics of SCD those presenting cognitive decline within five years and furthermore, we will construct risk forecast models based on different combinations of potential predictors achieve early diagnosis in the preclinical stage of AD.

Considering the long preclinical stage of AD and compensated cognitive function in the SCD stage, the application of a refined SCD approach as an enrichment strategy for clinical trials focusing on preclinical AD shows great promise. The SILCODE is a longitudinal study and we will focus on features of SCD convertors with multi-perspective analysis. Especially, we would like to establish an integrated diagnostic system for early detection and prediction of SCD progressing at the individual level. We aim to provide scientific evidence for a more effective diagnosis of SCD due to AD and explore the underlying mechanism.

In this project, plasma Aβ, urine AD7c-NTP, and multi-modal MRI will also be included in the predicting models. Plasma and urine biomarkers are non-invasive and feasible in clinical practice. Previous studies have found their diagnostic efficiency in MCI and dementia,[46 66] but in SCD individuals, there are few pieces of evidence. So, this project also focuses on new biomarkers and tries to identify their usage in the clinical scene. Additionally, through this project, we would like to reveal the longitudinal patterns of involved clinical and MRI biomarkers, especially the temporal sequences, which may provide basis for us to understand the changes during disease progression.

The stage model theory proposes that human memory can be divided into three stages: sensory memory, short-term memory, and long-term memory.[67] The MMN has been proposed as an objective measure of the existence of auditory (visual, somatosensory) sensory memory traces.[68] Previous studies have suggested that MMN may have the potential to measure the age-related changes[47] or improve the diagnostic value for the early diagnosis of AD.[69] Although much more SCD studies were concentrated on the short-term memory and long-term memory,[18]

[70 71] there were quite few literatures about the sensory memory stage. SILCODE will investigate the changes of the initial memory stage at the SCD population and the predictive value of somatosensory MMN for disease progression in an exploratory way.

At last, cultural factors were found to impact on SCD by a cross-cultural comparison between the US and China[21], which indicates the need for SCD research in China. SILCODE is a longitudinal study in China and the implement of SILCODE would provide characteristics of SCD in China, which contribute to the harmonization of the SCD concept across cultural borders.

Author Contributions

Xuanyu Li and Xiaoni Wang are joint first authors. Xuanyu Li and Xiaoni Wang are responsible for the implementation of the trial, and manuscript draft and revision. Li Su is responsible for the design and implement of imaging data analysis. Xiaochen Hu is responsible for the design of SCD and clinical assessments. Ying Han is overall responsible for the study design, implementation of the trial, manuscript draft and revision, and funding. All authors contributed to the drafting of the manuscript and approved the final manuscript.

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Competing interests

None declared.

Patient consent

All participation is based on written informed consent and the participants will be able to withdraw from the study at any time.

Ethics approval

Ethical approval of this has been obtained from the medical research ethics committee and institutional review board of the Xuanwu Hospital, Capital Medical University (ID: [2017]046).

Data sharing statement

Individual participant data that underlie future results published will not be publicly available but be available from our lab after deidentification. Proposals for access should be sent to hanying@xwh.ccmu.edu.cn. The date will be the beginning 9 months and ending 36 months following future article publication with investigators whose proposed use of the data has been approved by an independent review committee identified for individual participant data meta-analysis.

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Figure legend

Figure 1 Flow chart of SILCODE. SCD, subjective cognitive decline; fMRI, functional MRI; FDG-PET, fluordesoxyglucose positron emission tomography; Aβ-PET, β-amyloid positron emission tomography; MoCA: Montreal Cognitive Assessment-basic

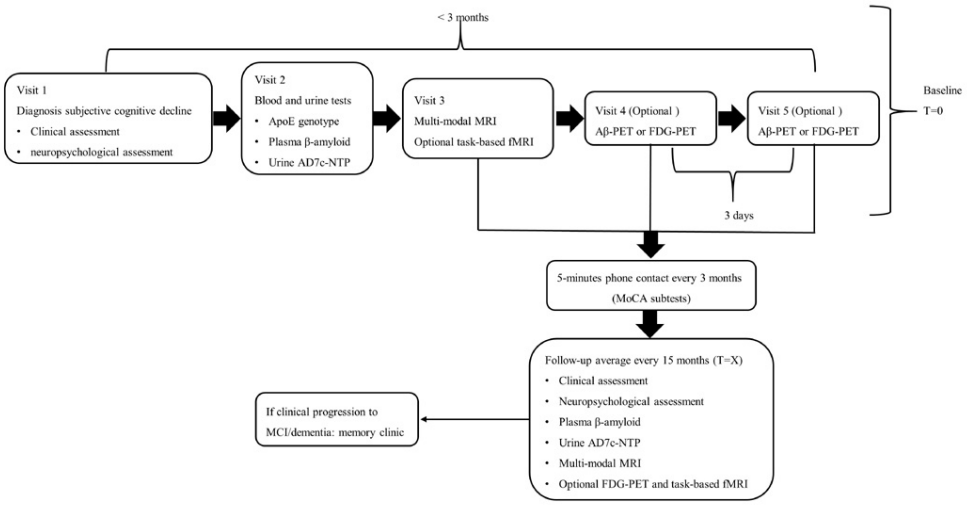

Figure 1 Flow chart of SILCODE. SCD, subjective cognitive decline; fMRI, functional MRI; FDG-PET, fluordesoxyglucose positron emission tomography; Aβ-PET, β-amyloid positron emission tomography; MoCA: Montreal Cognitive Assessment-basic

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Sino Longitudinal Study on Cognitive Decline (SILCODE): protocol for a Chinese longitudinal observational study to develop risk prediction models of conversion to mild cognitive impairment in individuals with subjective cognitive decline

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ABSTRACT

Introduction Understanding the biological mechanism of subjective cognitive decline (SCD) in preclinical Alzheimer's disease (AD) and identifying those who will soon convert to mild cognitive impairment (MCI) are critical for developing appropriate strategies for early diagnosis and intervention of AD. We present the study protocol of the Sino Longitudinal Study on Cognitive Decline (SILCODE), a longitudinal observational study focusing on SCD in the context of AD.

Methods and analysis Within SILCODE, approximately 800 subjects with SCD who are between 50 and 79 years old will be recruited through standardized public advertisements or memory clinics. They will undergo extensive assessment, including clinical and neuropsychological assessments, blood sample collection for plasma beta-amyloid and ApoE genotype, urine samples collection for AD7c-NTP, and multimodal MRI scans (structural MRI, diffusion tensor imaging, resting-state functional MRI and optional task-based functional MRI) as well as optional glucose metabolism and amyloid positron emission tomography. Subjects will be contacted by telephone every 3 months and interviewed, on average, every 15 months for five years. The study endpoint is the development of mild cognitive impairment or dementia. Jak & Bondi's actuarial neuropsychological method will be used for diagnosis of MCI. The least absolute shrinkage and selection operator (LASSO) logistic regression model followed by the sub-distribution hazard function model with death as a competing risk will be constructed to establish risk prediction models.

Ethics and dissemination The ethics committee of the Xuanwu Hospital of Capital Medical University has approved this study protocol (ID: [2017]046). The results will be published in peer-reviewed journals and presented at national and international scientific conferences.

Trial registration NCT03370744; pre-results.

Article summary

Strengths and limitations of this study

- A strength of this study is the use of multiple markers (clinical, blood, urine and imaging markers) to establish models predicting conversion from SCD to MCI, which will provide references for clinical applications of SCD.

- This longitudinal study could enrich the understanding of SCD by examining a large-scale Chinese population because cultural factors have an impact on SCD.
- The relatively short follow-up duration (5 years) of this study allows longitudinal data to be analyzed only in an exploratory way.
- The study results will be limited by the research environment in that individuals will be recruited from memory clinics and volunteer samples, without community-based or population-based samples.

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease and one of the greatest healthcare challenges of the 21st century.[1] It has been revealed that pathophysiology starts many years before the onset of clinical symptoms.[2] Several recent clinical trials of Aβ-lowering therapies in the mild or moderate dementia stage and even mild cognitive impairment stage have failed,[3 4] further encouraging researchers to shift their focus to the preclinical stage of AD as a target for new treatments.[5]

Subjective cognitive decline (SCD) refers to self-perceived cognitive decline relative to a previously normal status, without impaired performance on standardized neuropsychological tests.[6 7] Evidence from recent studies suggests that SCD may be one of the earliest symptomatic manifestations of AD.[6 8] Previous studies have found that individuals with SCD have a higher conversion rate and shorter conversion time to mild cognitive impairment (MCI) and dementia than normal controls (NC).[9-12] SCD may be an early trigger for help-seeking[13], and we have observed that the number of individuals with SCD presenting to the healthcare system (e.g., memory clinics) is increasing in our clinical practice as awareness of AD dementia increases in China. Individuals with SCD are ideal for ultra-early diagnosis and intervention trials.

However, the heterogeneous nature of SCD limits its value in clinical applications. Research to date has focused on factors for predicting the risk of MCI/AD progression in SCD.[14-17] Though some longitudinal and observational studies of SCD are ongoing,[18-20] there are few data available from China or anywhere else in Asia. However, SCD is subject to influence by various cultural factors including factors relate to language, social stigmatization of cognitive problems, styles of responding on self-reported measures (e.g., extremity of answers), tolerance of slowly

progressive cognitive decline in ageing and others.[21] The association between objective cognitive performance and SCD is different across races.[22] Our group reported that the prevalence of SCD was 14.4% in the Shunyi District of Beijing, China,[23] which is different from the prevalence of SCD reported in Western countries.[24] [25] Thus, there is a need for additional findings from longitudinal studies based on large-scale Chinese populations.

The Alliance for Preclinical AD in China, belonging to the National Clinical Research Center for Geriatric Disorders, is a national multicenter research platform that is dedicated to the development of clinical, neuroimaging, genetic, and biochemical biomarkers for the early detection and tracking of AD. Its sites include more than 180 member hospitals/research institutes across China. The alliance is led by Professor Han and focuses on the early detection of SCD during preclinical AD; it also develops and provides standard operating procedures and quality control for harmonized data and material acquisition and storage across all sites.

In this protocol, we introduce in detail the design of the Sino Longitudinal Study on Cognitive Decline (SILCODE) study. This is an ongoing longitudinal observational study based on the multicenter research platform of the Alliance for Preclinical AD in China and focusing on SCD in the context of AD. The main aim of SILCODE is to estimate cognitive decline and establish statistical prediction models for the outcomes of SCD in elderly Chinese people.

METHODS

Overall study design

This observational longitudinal study will take place in China and will recruit people with SCD. During the baseline period, all eligible subjects with SCD will be invited to receive clinical and neuropsychological examinations, blood tests, urine tests and multimodal MRI [including structural MRI (sMRI), diffusion tensor imaging (DTI) and resting-state functional MRI (rs-fMRI)] sequentially as shown in figure 1. Glucose metabolism and amyloid positron emission tomography (PET) and task-based fMRI (t-fMRI) will be performed as well if the subject agrees to them. A semi-structured interview[18] as well as the SCD questionnaire[26] and the Measurement of Everyday Cognition (ECog)[27] will be used to assess SCD. The maximum interval between neuropsychological tests and other data collection will be within three months. Subjects will be contacted by telephone every 3-months and will be interviewed, on average, every 15 months

for five years (4 time points). The Montreal Cognitive Assessment-Basic (MoCA-B)[28] subtests (language, delayed recall and orientation) will be administered during the call. Participants expressing significant cognitive decline on the telephone will be interviewed ahead of time for the next follow-up. During the follow-up visits with interviews, evaluations including clinical and neuropsychological examinations, blood tests, urine tests, and multimodal MRI as well as optional glucose metabolism PET and t-fMRI will be conducted, on average, every 15 months. At each follow-up visit, diagnoses will be re-evaluated under the supervision of two neurologists. Figure 1 provides the overall study outline.

Patient and public involvement

Patients and the public will not be involved in the development of the research question or the design of the study. Patients will not be involved in the recruitment of participants or the conduct of the study. The general results will be disseminated to participants through public education activities.

Sample size calculation

The formula proposed by Hsieh and Lavori[29] was used to estimate the sample size in our study with the software PASS (version 15.0.5). According to a previous meta-analysis,[9] the cumulative conversion proportion was 24.4% over a mean follow-up period of 4.1 years. We assumed that the power (1-β) was 80%, α was 0.05 and the rate of loss to follow-up was 20%. Due to the lack of statistical data on SCD, we calculated the sample size with data reported by Licher et al[30] and Pinto et al[31] (hazard ratio= 1.03, standard deviation=8.2, R^2 =0.127) from cognitively normal elderly subjects for the association between age and cognitive decline. This calculation rendered a total sample size of 762. Considering the need for internal validation, we will recruit at least 800 subjects.

Recruitment of participants

Participants will be consecutively recruited through standardized public advertisements and through referrals from general physicians, memory clinics, or informants. Residents who meet the inclusion criteria will be recruited. Written informed consent will be acquired from each

subject before enrolment.

Diagnostic criteria for SCD, MCI and dementia

SCD is defined by the research criteria for pre-MCI (SCD) proposed by Jessen et al in 2014[6]: (1) Self-experienced persistent decline in cognitive capacity in comparison with a previously normal status and unrelated to an acute event; (2) Normal age-, gender-, and education-adjusted performance on standardized cognitive tests.

MCI is defined by an actuarial neuropsychological method proposed by Jak and Bondi.[32] Participants are considered to have MCI if they meet any one of the following three criteria and fail to meet the criteria for dementia: (1) having impaired scores (defined as >1 SD below the age-corrected normative means) on both measures in at least one cognitive domain (memory, language, or speed/executive function); (2) having impaired scores in each of the three cognitive domains sampled (memory, language, or speed/executive function); (3) the Functional Activities Questionnaire (FAQ) ≥9. The normative means in our study are from Guo and his team in a Chinese population. [33-36] Measures and normative means are shown in table 1.

The diagnosis of AD dementia is based on the Diagnostic and Statistical Manual of Mental Disorders, fifth edition, and the diagnostic guidelines for dementia due to AD delivered by the National Institute on Aging–Alzheimer's Association workgroups (NIA-AA),[37] and a total CDR score ≥ 1.

Table 1 Cognitive domains, neuropsychological tests and normative means

Cognitive domains	Neuropsychological tests	Normative means
Memory	Auditory Verbal Learning Test-long delayed memory[33]	50-59 years old: 5; 60-69 years old: 4; 70-79 years old: 3
	Auditory Verbal Learning Test-recognition[33]	50-59 years old: 20; 60-69 years old: 19; 70-79 years old: 18
Language	Animal Fluency Test[35]	junior middle school: 12; high school: 13; college: 14
	30-item Boston Naming Test[34]	junior middle school: 20; high school: 21;

		college: 22
speed/executive	Shape Trail Test Part A[36]	50-59 years old: 70s; 60-69 years old: 80s; 70-79 years old: 100s
	Shape Trail Test Part B[36]	50-59 years old: 180s; 60-69 years old: 200s; 70-79 years old: 240s

Inclusion/exclusion criteria

The inclusion criteria are as follows: (1) 50-79 years old, right-handed and Mandarin-speaking subjects; (2) presence of self-perceived continuous cognitive decline compared to previous normal status and unrelated to an acute event; (3) concerns (worries) associated with memory complaint; and (4) failure to meet the criteria for MCI or dementia.

The exclusion criteria are as follows: (1) History of stroke. (2) Current major psychiatric diagnoses such as severe depression and anxiety. When mild or moderate symptoms of psychiatric disorders are suspected, patients will not be excluded[38]. They will be evaluated by a psychiatrist to determine whether the psychiatric diagnoses are the cause of SCD. (3) Other neurological conditions that could cause cognitive decline (e.g., brain tumours, Parkinson's disease, encephalitis, or epilepsy) rather than AD spectrum disorders. (4) Other diseases that could cause cognitive decline (e.g., thyroid dysfunction, severe anaemia, syphilis, or HIV); (5) history of psychosis or congenital mental developmental delay. (6) Cognitive decline caused by traumatic brain injury. (7) Inability to complete the study protocol or presence of contraindications for MRI.

Clinical progression

At each follow-up visit, diagnoses will be re-evaluated under the supervision of two neurologists. The main outcome measure of clinical progression in our study is conversion to MCI or AD dementia. The time to event is the interval between the baseline neuropsychological tests and the date at which the MCI is first diagnosed. For those who bypass the diagnosis of MCI during follow-up, the time between the baseline neuropsychological tests and first diagnosis of dementia is defined as the time to event in our study.

Assessments of subjective cognitive functioning

A semi-structured interview used by the DELCODE study will be employed in this project to evaluate the details of SCD.[18] It includes information about the onset time, concerns, comparison with others, and the history of visiting a physician not only for the memory domain but also for language, attention, and executive control. We will also require informant reports in the evaluation of the self-reported information as suggested by the Subjective Cognitive Decline Initiative Working Group.[7 39]

For quantitative assessment of the severity of SCD, we will use a newly developed SCD questionnaire including nine reliable SCD items (table 2), which are characterized by different domains, such as global memory functioning and daily activities ability.[23 26] In addition, the 12-item ECog[27] will also be applied to all participants to measure SCD severity. These questionnaires will not be inclusion criteria and will be considered only for statistical analysis.

Table 2 items included in SCD questionnaire[26]

Items
Do you think you have problems with your memory?
Do you have difficulty remembering a conversation from a few days ago?
Do you have complaints about your memory in the last 2 years?
How often is the following a problem for you: Personal dates
How often is the following a problem for you: Phone numbers you use frequently
On a whole, do you think that you have problems remembering things that you want to do or say?
How often is the following a problem for you: Going to the store and forgetting what you wanted to buy
Do you think that your memory is worse than 5 years ago?
Do you feel you are forgetting where things were placed?

Clinical, risk factor and neuropsychological assessments

The clinical assessments include a structured medical history, physical examination, routine laboratory tests (including blood tests, biochemical tests, thyroid function tests, vitamin B12 status, folic acid status, *Treponema pallidum*-specific antibodies and HIV antibodies) and MRI (T1 and FLAIR) for medial temporal atrophy scale and Fazekas score.

For risk factors of AD, all subjects and their informants will provide the following information: family history, vascular risk factors, occupation/retirement, socioeconomic status, cigarettes, and alcohol use, and nutrition style. Additionally, the Pittsburgh sleep quality index (PSQI)[40], Rapid Eye Movement (REM) Sleep Behaviour Disorder Screening Questionnaire (RBDSQ)[41] and the Epworth Sleepiness Scale (ESS)[42] will be used for sleep quality assessment

The neuropsychological tests are selected for comparability with other similar ongoing studies (e.g., DELCODE).[18] To avoid deviations in the evaluation, the neuropsychologists performing the cognitive tests will be trained according to standard guidelines. The kappa coefficient (Fleiss' kappa) will be used to measure the assessment agreement among the neuropsychologists. Neuropsychological results will be double-checked. The neuropsychological battery (table 1) measures different cognitive domains including episodic memory (Auditory Verbal Learning Test-HuaShan version [AVLT-H]),[33] language (Animal Fluency Test,[35] 30-item Boston Naming Test[34] and speed/executive function (Shape Trail Test Parts A and B[36]). In addition, the SILCODE study will implement tests for global cognition, daily life ability and neuropsychiatric assessment, including MoCA-B,[28] the memory and executive screening (MES),[43] the CDR, FAQ, the 15-item short form of the Geriatric Depression Scale (GDS), the Hamilton Anxiety Scale (HAMA), the Hamilton Depression Scale (HAMD) and the Neuropsychiatric Inventory (NPI).

Blood tests

ApoE genotype

SNPs rs7412 and rs429358 form the ApoE ε2/ε3/ε4 haplotype. ApoE will be genotyped using the standard Sanger sequencing method (Sangon, Shanghai, China) using the following primers: 5′-ACGCGGGCACGGCTGTCCAAGG-3′ (forward) and 5′-GGCGCTCGCGGATGGCGCTGA-3′ (reverse). ApoE will be amplified using the following conditions: 1 cycle of 98 °C for 10 s, 35 cycles of 72 °C for 5 s, 1 cycle of 72 °C for 5 minutes. PCR was performed in a final volume of 30 μl, containing 10 pmol of forward and reverse primers and 50 ng of genomic DNA template, using PrimeSTAR HS DNA Polymerase with GC Buffer (Takara Bio).

Plasma β-amyloid

Recently, some studies found that plasma amyloid level was correlated with cognitive capacity and cerebrospinal fluid amyloid protein.[44] [45] In particular, when predicting brain amyloid deposition, the accuracy of the composite biomarker (amyloid-β precursor protein 669-711/Aβ1-42 and Aβ1-40/Aβ1-42 ratios) could be approximately equal to 90%.[46] Therefore, we would like to identify whether the plasma β-amyloid is also associated with cognitive decline in SCD individuals. Plasma Aβ will be determined using a commercially available kit, V-PLEX Aβ Peptide Panel 1 (6E10) Kit (K15200E) (Mesoscale Diagnostics LLC, Rockville, USA). Aβ peptide levels from each blood draw will be measured in duplicate using the same aliquot.

Urine tests

Ten millilitres of first morning voided urine will be collected from each subject and then immediately refrigerated. An enzyme-linked immunosorbent assay kit will be used to detect the protein level of AD7c-NTP in the urine specimens.

Imaging protocol

MRI data will be acquired using an integrated simultaneous 3.0 Tesla TOF PET/MR (SIGNA PET/MR, GE Healthcare, Milwaukee, WI, USA) at the Xuanwu Hospital of Capital Medical University. For each participant, simultaneous PET and 3.0 T MRI data will be obtained. Brain MR images will be inspected by an experienced neuroradiologist.

Positron emission tomography: All participants will be invited for optional [^{18}F] florbetapir (AV-45) and [^{18}F] fluorodeoxyglucose (FDG) PET in 3-dimensional acquisition mode. The time duration between the FDG-PET and AV-45 PET is at least 3 days to eliminate the effect of the first tracer. For FDG-PET, each subject will be instructed to fast for at least 6 h and must have a confirmed serum glucose level below 8 mmol/L; 35 minutes dynamic scan is acquired approximately 40 minutes after an intravenous injection of 3.7 MBq/kg of 18F-FDG. For Aβ-PET, a 35-minute dynamic scan is acquired approximately 40 minutes after an intravenous injection of 7-10 mCi [^{18}F] florbetapir. The PET data are acquired using a TOF-OSEM algorithm (time-of-flight ordered subset expectation maximization) with the following parameters: 8 iterations, 32 subsets matrix = 192×192, field of view (FOV)= 350×350, half-

width height = 3.

Structural MRI: Parameters for T1-weighted 3D brain structural images are as follows: SPGR sequence, FOV=256×256 mm^2, matrix=256×256, slice thickness=1 mm, gap=0, slice number=192, repetition time (TR) =6.9 ms, echo time (TE)=2.98 ms, inversion time (TI)=450 ms, flip angle =12°, voxel size = 1×1×1 mm^3.

DTI: DTI data are obtained with a single-shot spin-echo diffusion-weighted EPI sequence with the following parameters: FOV=224×224mm^2, data matrix=112×112, slice thickness=2 mm, gap=0, slice number=70, slice order=interleaved, TR=16500 ms, TE=95.6 ms, 30 gradient directions and 5 b0 images (b=1000 s/mm^2), voxel size =2×2×2 mm^3.

Rs-fMRI: **A** single-shot gradient-echo EPI sequence is used for rs-fMRI with the following parameters: scan duration=8 minutes, FOV=224×224mm^2, data matrix=64×64, slice thickness=4.0 mm, gap=1.0 mm, slice number=28, slice order=interleaved, TR=2000 ms, TE=30 ms, FA=90°, voxel size =3.5×3.5×4 mm^3.

T-fMRI: All participants will also be invited for a block design fMRI paradigm to measure somatosensory mismatch negativity (MMN). We will use a custom-designed air jet pressure stimulation device, which can apply 5.0-bar pressure somatosensory stimulation to the right index or middle finger. The index and middle finger of the right hand will be fixed in place with two pieces of adhesive tapes to restrict any movement during the experiment. The length of the two air pipes from the air compressor to the apertures will be 6.0 metres, and the diameter of the two stimulation apertures will be 1.0 millimetres. In addition, the distance between the aperture and the centre of the finger pad will be fixed at 5.0 millimetres for all subjects. Subjects will be instructed to watch a video (without sound) presented on a custom designed screen and to ignore stimulation to the fingers.

The functional MRI paradigm will have a total duration of 9 minutes. In a block design, 8 control blocks with 32 s duration and 8 MMN blocks (also 32 s duration) will be used. A constant stimulus onset asynchrony of 500 ms will be employed for both the MMN and control conditions resulting in 40 stimuli per block. In devising the MMN and control blocks, we will take advantage of an MMN paradigm in which two stimuli will be presented equiprobably and their presentation order will be varied. In MMN blocks, stimulation will be arranged to compose alternating ‘mini-sequences’ of stimulus 1 (index finger, 5.0 bar, 300 ms) and 2 (middle finger,

5.0 bar, 300 ms). Mini-sequences will be 2, 3, or 4 repetitions of a single stimulus (each sequence length is represented equiprobably), followed by a mini-sequence of the other stimuli, and so on. The number of trials in a given mini-sequence will vary such that the occurrence of a switch from stimulus 1 to stimulus 2 (and vice-versa) is irregular. As such, the switch trial stimuli will be designated as deviants and will elicit the MMN. This basic stimulation paradigm, which has proven to elicit robust MMNs, will be used for the MMN condition.[47 48] For the matched control condition to the aforementioned MMN condition, the same two stimuli will be alternated sequentially to form a regular pattern (e.g., stimulus 1, stimulus 2, stimulus 1, stimulus 2, etc.).

Imaging data analysis

PET data analysis. The AV-45 and FDG-PET scans will be preprocessed by Statistical Parametric Mapping version 12 (SPM 12; https://www.fil.ion.ucl.ac.uk/spm/software/spm12). The structural images will be individually registered to the averaged PET images. The unified segmentation method[49] will then be applied to all coregistered structural images. The PET images will be spatially normalized to Montreal Neurological Institute (MNI) standard space by using the forward parameters estimated during the unified segmentation and smoothed with an 8-mm full width at half maximum (FWHM) Gaussian kernel. Finally, the voxel-wise AV-45 and FDG-PET standardized uptake value ratio (SUVR) will be normalized by the whole cerebellum and pons as the reference regions, respectively. A global AV-45 PET SUVR value will be estimated using a composite of the prefrontal, orbitofrontal, parietal, temporal, anterior cingulate, and posterior cingulate and precuneus cortices.

SMRI data analysis. The cortical thickness analysis will be performed using the FreeSurfer image analysis suite, version 5.3 (http://surfer.nmr.mgh.harvard.edu). We will construct models of the boundaries between the grey matter and the white matter as well as the pial surface.[50] Cortical thickness measures will be obtained by calculating the distance between these surfaces. Automated reconstruction and labelling will be performed using the default "recon-all" command line. All generated images will be visually inspected for image and segmentation quality and corrected manually if necessary. Subsequently, "lobesStrict" will be performed to obtain a lobar annotation and consecutively imported for further statistical analyses. For the

voxel-based morphometry (VBM) analysis, structural images will be segmented into grey matter (GM) tissue using the new segment function within SPM12. The diffeomorphic anatomical registration through the exponentiated Lie algebra (DARTEL) toolbox will be used to generate a reference template object of the sample that will be warped into a standard MNI space. The generated flow fields of each subject will be calculated and normalization parameters will be then implemented to normalize the GM maps in the native space to the MNI space. During the normalization process, the modulated images (local native amount of grey matter) will be preserved. Images will be spatially smoothed with an 8 mm FWHM Gaussian kernel. Finally, for each individual, we will obtain a smoothed GM volumetric map. Additionally, the total intracranial volume (TIV) for each individual will be estimated by summing the segmented GM, white matter, and cerebral spinal fluid (CSF). Then, the smoothed GM images of every individual will be used for subsequent statistical analysis.

DTI data analysis. Raw DTI data will be processed using the Oxford Centre for Functional MRI of the Brain (FMRIB) Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl).[51-53] Initially, the EddyCorrect tool will be used to correct eddy current distortions and motion artefacts by fine registration of the DTI images to a reference image (b0 image). The Brain Extraction Tool (BET)[53] will be used for creating brain masks of all subjects, and then a diffusion tensor will be modelled at each voxel by using the least- squares algorithm fitting tensor model within the DTI-FIT Tool.[54] Fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AxD) values of each voxel will be calculated based on the eigenvalues of the tensor. Voxel-wise statistical analysis of the FA, MD, RD, and AxD data will be performed using tract-based spatial statistics (TBSS).[55] All subjects' FA maps will be nonlinearly coregistered to the FMRIB58_FA template with FSL's nonlinear image registration algorithm. Then, the mean FA image will be obtained and thinned to create a mean FA skeleton representing the centre of all tracts common to all subjects. Each subject's aligned FA data will then be projected onto the FA skeleton to obtain their FA skeletons and deformation matrixes. With the deformation matrixes, the skeletonized AxD, MD, and RD maps will be created for every individual by the tbss_non_FA tool. These maps will be used for subsequent statistical analysis.

Rs-fMRI data analysis. Resting-state functional MR imaging will be preprocessed using the

Data Processing Assistant for Resting-state fMRI (DPASF; http://www.rfmri. org/DPARSF).[56] The first 10 volumes will be discarded for image stabilization and the participant's to adaptation to the scanning. The remaining functional sequences will be first corrected for timing differences between each slice and motion effects. Next, the structural image will be coregistered to the mean functional image. Then, the transformed structural images will be segmented into grey matter, white matter, and CSF. The motion-corrected functional volumes will be spatially normalized to MNI 152 standard space and resampled to 3 mm × 3 mm × 3 mm cubic voxels by using the normalization parameters estimated during unified segmentation. The resulting images will further undergo spatial smoothing (Gaussian kernel with 8 mm FWHM), linear detrending and temporal filtering (0.01-0.08 Hz). To avoid overestimating regional homogeneity (ReHo) values, spatial smoothing will be conducted for individual ReHo maps rather than during data preprocessing. Finally, nuisance signals (including Friston 24-head motion parameters, the white matter, and CSF) will be extracted and regressed out from the data to reduce the residual effects of nonneuronal factors. For the amplitude of low frequency fluctuation (ALFF) analysis[57], the time series of each voxel will be transformed into the frequency domain using a fast Fourier transform. The square root of the power spectrum will be calculated and averaged across 0.01-0.08 Hz. This averaged square root will be taken as the ALFF value for this voxel. For the ReHo analysis[58], Kendall's coefficient of concordance (KCC) will be computed on the ranked time series of a given voxel with its 26 nearest neighbours. The resultant KCC will be taken as the ReHo values. The generated ALFF and ReHo images will be used for statistical analysis.

Network analysis. We will construct a structural cortical network based on grey matter volumes.[59] The nodes will be defined as brain regions corresponding to automated anatomic labelling (AAL) areas. The structural connections will be defined as statistical correlations between pairs of average grey matter volumes in our study. A structural connection will be considered to exist if the correlation coefficient is statistically significant. Before the correlation analysis, the effects of age, sex and total grey matter volume on the grey matter volume of regions will be adjusted. We will calculate Pearson correlation coefficients across individuals between the average grey matter of every pair of regions, and then an interregional correlation matrix will be obtained for every individual. We will construct a white matter network based

on fibre number. The network nodes will be defined as the 90 regions of interest corresponding to the AAL template. The weight of the network edge will be defined as the number of connected fibres with two regions. The fibre number will be calculated through diffusion tensor imaging tractography as reported in our previous study.[60] To avoid spurious connections, a minimum threshold of fibre number (weight of the edge=10) will be used. We will construct a functional network based on the average time sequence. The network nodes will be defined as the 90 regions of interest corresponding to the AAL template. The network edge will be defined as the partial correlation coefficients between the average time sequence of two regions, and we will obtain an incidence matrix. Non-significant correlations will be excluded. The network analyses will be performed with the GRETNA toolbox (http://www.nitrc.org/projects/gretna/).[61] The 'rich-club coefficient' is defined as the density of connections between rich-club nodes and rich-club regions in our study will be defined the top 13 brain regions with the highest degree as reported in our previous study.[62] A module is defined as a subset of nodes connected to the other nodes in the same module other than those outside the module.[63] We plan to use Newman's metric to measure the modularity[64] and maximize the modularity parameter Q by the algorithm proposed by Clauset et al.[65] The two parameters will be calculated for the structural cortical network, white matter network and functional network constructed for each individual. The values of the two measures will be calculated for every individual and used in the model construction.

T-fMRI data analysis. We will use SPM12 to process and analyse the t-fMRI data. The first 10 volumes will be discarded due to unsteady magnetization. First, the functional images from each run will be realigned. The structural image will then be coregistered to the first scan in the functional image, and the resulting coregistered structural image will be normalized to MNI 152 standard space. Finally, these spatially normalized functional images will be smoothed (Gaussian kernel with an 8 mm FWHM).

Statistical analysis

Baseline comparison between SCD convertors and SCD non-convertors

All data (demographic information, clinical data, risk factors, neuropsychologic tests, blood and urine biomarkers, multimodal MRI biomarkers, glucose mentalism, and amyloid deposition)

will be described. The Shapiro-Wilk test and Q-Q plots will be used to confirm the normality. All normally distributed continuous variables will be reported as the mean ± SD. A comparison of the baseline between SCD convertors and SCD non-convertors will be performed with a two-sample t-test for continuous variables and a chi-squared test for categorical variables. Dunnett's multiple comparison tests will be performed for comparison.

Statistical analyses of t-fMRI data will be subjected to a general linear model analysis. The MMN condition will serve as the predictor of interest and will be modelled with a boxcar function convolved with the canonical haemodynamic response function; the Control condition will serve as a baseline. The beta estimates from the individual general linear models enter a second level random effects analysis. Contrast maps will be created by applying paired t-tests comparing the MMN versus control condition for each group separately as well as a two-sample t-test for the between-groups comparison. Activation maps will be corrected ($P<0.001$) by the false discovery rate approach implemented in SPM.

Longitudinal patterns in SCD converters

The longitudinal analysis in neuropsychological, plasma, urine and MRI variables in SCD-converters will be assessed. We will use the general linear mixed effects model to estimate the individual's change in each variable.

Statistical prediction models

We will use the competing risk regression model to detect the association between possible risk factors and endpoint. The endpoint event of our study is converting to MCI. Death before conversion to MCI is considered a competing risk in our study. The time to event is the date difference between the baseline neuropsychological tests and the date at which the MCI or dementia is first diagnosed.

Independent variables are listed in table 3, including features of baseline clinical characteristics, blood, urine, MRI and PET biomarkers. Considering the overfitting effect when establishing the model, the least absolute shrinkage and selection operator (LASSO) model with penalty parameter tuning will be used for variable screening. It is a data mining method for shrinkage estimation and dimensionality reduction, overcoming processing difficulties caused by high-dimensional data, and estimating the parameters more accurately. Then, we will perform the sub-distribution hazard function model, which could evaluate hazards for the endpoint (MCI or

dementia) and competing (death) events, to establish the final multivariate models. Ten-fold cross-validation is used to perform internal verification of the established models. The optimism-corrected C-statistics will be used to evaluate the performance of risk prediction models.

To better clarify the relationship between possible risk factors and endpoint events, some confounding factors will be adjusted. Four models will be established in our study. Model 1 will simply take demographic data, lifestyle, clinical assessment, neuropsychological assessments, and SCD report into account. Model 2 will add the ApoE genotype, plasma Aβ, and urine AD7c-NTP on the basis of model 1. Model 3 will take neuroimaging data into account on the basis of model 2 and model 4 will include amyloid-PET and FDG-PET on the basis of model 3.

The hazard ratio (HRs) with 95% confidence intervals are determined. A two-sided $p<0.05$ is defined as statistical significance. All statistical tests were performed using R statistical software. The "glmnet" package will be used for the LASSO model analysis, and the "cmprsk" package will be performed for the sub-distribution hazard function model.

Table 3 independent variable list

Types	
Demographic data	age; education level; family history of dementia; socioeconomic information
Lifestyle	nicotine and alcohol use; sleep quality (PSQI, RBDSQ, ESS); nutrition style
Clinical assessment	vascular risk factors (hypertension, hyperlipemia, diabetes, coronary heart disease, Fazekas score); medial temporal lobe atrophy scale; HAMA; HAMD; GDS
Neuropsychological assessment	AVLT-D; AVLT-R; STT-A; STT-B; BNT; AFT
SCD report	onset time; comparison with others; SCD-Q9; Ecog; consistency of SCD; inform report
Blood tests	ApoE genotype; Plasma β-amyloid
Urine tests	level of AD7c-NTP
MRI data	cortical thickness; grey matter volume; FA; MD; RD; AxD; ALFF; ReHo; network characteristic (rich-club coefficient, modality); MMN-related haemodynamic

	responses
PET data	global SUVR of FDG-PET and Aβ-PET

HAMA, he Hamilton Anxiety Scale; HAMD, the Hamilton Depression Scale; GDS, Geriatric Depression Scale; AVLT, Auditory Verbal Learning Test; STT, Shape Trails Test; BNT, Boston naming test; AFT, animal fluency test; SCD, subjective cognitive decline; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; AxD, axial diffusivity; ALFF, amplitude of low-frequency fluctuations; ReHo: regional homogeneity; SUVR, standardized uptake volume ratios; MMN: mismatch negativity

DISCUSSION

The current study evaluates the characteristics of SCD patients presenting cognitive decline within five years. Furthermore, we will construct risk forecast models based on different combinations of potential predictors to achieve early diagnosis in the preclinical stage of AD.

Considering the long preclinical stage of AD and compensated cognitive function in the SCD stage, the application of a refined SCD approach as an enrichment strategy for clinical trials focusing on preclinical AD shows great promise. SILCODE is a longitudinal study, and we will focus on features of SCD converters with multi-perspective analysis. In particular, we would like to establish an integrated diagnostic system for the early detection and prediction of SCD progressing at the individual level. We aim to provide scientific evidence for a more effective diagnosis of SCD due to AD and explore the underlying mechanism.

In this project, plasma Aβ, urine AD7c-NTP, and multimodal MRI will also be included in the predicting models. Plasma and urine biomarkers are non-invasive and feasible in clinical practice. Previous studies have found their diagnostic efficiency in MCI and dementia,[46 66] but in SCD individuals, there are few pieces of evidence. Therefore, this project also focuses on new biomarkers and tries to identify their usage in the clinical scene. Additionally, through this project, we would like to reveal the longitudinal patterns of involved clinical and MRI biomarkers, especially the temporal sequences, which may provide a basis for us to understand the changes during disease progression.

The stage model theory proposes that human memory can be divided into three stages: sensory memory, short-term memory, and long-term memory.[67] The MMN has been proposed as an objective measure of the existence of auditory (visual, somatosensory) sensory memory traces.[68] Previous studies have suggested that MMN may have the potential to measure the age-

related changes[47] or improve the diagnostic value for the early diagnosis of AD.[69] Although many more SCD studies have concentrated on short-term memory and long-term memory,[18 70 71] there are very few published studies on the sensory memory stage. SILCODE will investigate the changes in the initial memory stage in the SCD population and the predictive value of somatosensory MMN for disease progression in an exploratory way.

Finally, cultural factors were found to impact SCD by a cross-cultural comparison between the US and China,[21] which indicates the need for SCD research in China. SILCODE is a longitudinal study in China, and the implementation of SILCODE would provide characteristics of SCD in China, which contribute to the harmonization of the SCD concept across cultural borders.

Author contributions

Xuanyu Li and Xiaoni Wang are joint first authors. Xuanyu Li and Xiaoni Wang are responsible for the implementation of the trial and manuscript draft and revision. Li Su is responsible for the design and implementation of imaging data analysis. Xiaochen Hu is responsible for the design of SCD and clinical assessments. Ying Han is responsible for the study design, implementation of the trial, manuscript draft and revision, and funding. All authors contributed to the drafting of the manuscript and approved the final manuscript.

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and The Lewy Body Society.

Competing interests

None declared.

Patient consent

All participation is based on written informed consent and the participants will be able to withdraw from the study at any time.

Ethics approval

Ethical approval of this has been obtained from the medical research ethics committee and institutional review board of the Xuanwu Hospital, Capital Medical University (ID: [2017]046).

Data sharing statement

Individual participant data that underlie future results published will not be publicly available but will be available from our laboratory after deidentification. Proposals for access should be sent to hanying@xwh.ccmu.edu.cn. The date will be the beginning 9 months and ending 36 months following future article publication with investigators whose proposed use of the data has been approved by an independent review committee identified for individual participant data meta-analysis.

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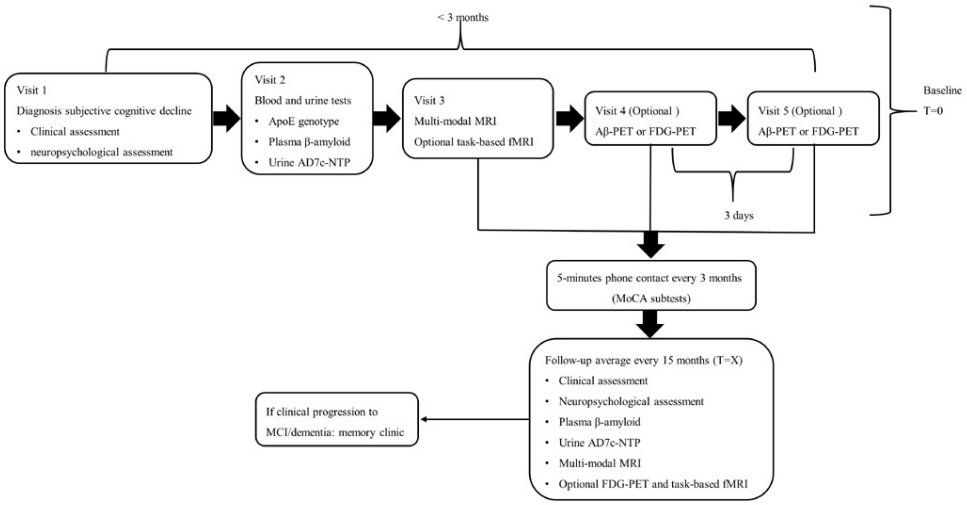
Figure legend

Figure 1 Flow chart of SILCODE. SCD, subjective cognitive decline; fMRI, functional MRI; FDG-PET, fluordesoxyglucose positron emission tomography; Aβ-PET, β-amyloid positron emission tomography; MoCA: Montreal Cognitive Assessment-basic



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