Study protocol for the Chinese familial Alzheimer's disease network (CFAN)

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1. Background and significance

Alzheimer's disease (AD) is a common neurodegenerative disorder characterized by decline of episodic memory in the early stages and a loss of daily living ability in the late stages [1]. Currently, the number of patients with AD in China is 8.75 million [2]. This large patient population costs China US $167.74 billion2 per year [3], and thus has an immense social impact and heavy economic burden. AD has become one of the major diseases that seriously endanger the health of the elderly population in China. The early prevention and treatment of AD is an important social problem that needs to be solved urgently.

AD can be categorized as sporadic AD (SAD) or familial AD (FAD) based on family history. FAD requires a patient with AD to have at least one first-degree relative diagnosed with AD or mild cognitive impairment (MCI) due to AD, and was reported to account for 12.50% to 25.00% of all AD cases [4,5]. FAD greatly contributes to AD research. First, it encompasses the physiological and pathological characteristics of all cognitive stages from preclinical to MCI to dementia. Second, it represents an ideal population, with a possible predictable age at onset (AAO), which is relatively young and has minimal complications (such as cerebrovascular disease), and facilitates exploration of the pathogenesis of AD. Third, it promotes the development of transgenic animal models with which anti-dementia drugs are tested.

FAD includes autosomal dominant AD (ADAD) [6], caused by mutations of genes including the amyloid precursor protein gene (*APP*), presenilin 1 gene (*PSEN1*), and presenilin 2 gene (*PSEN2*), which have been targeted by the Dominantly Inherited Alzheimer Network (https://dian.wustl.edu/); the E280A Antioquia cohort study[7]; and FAD cohorts, including those in France[8], England[9], Japan[10], South Korea[11], Finland[12], and so on. To date, 289 *PSEN1*, 48 *PSEN2*, and 58 *APP* mutations have been identified (https://www.alzforum.org/mutations), most of which were first reported in Caucasians. In Chinese, our team reported the first variant (NM_000021.3: c. 289G>T V97L in *PSEN1*) [13]. Several studies have since investigated the genetics of FAD: one study identified four variants in *PSEN1* and one variant in *APP*, and the detection rate of variants in *PSENs/APP* was 18.75% [14].Another identified two variants in *APP*, eight variants in *PSEN1*, and three variants in *PSEN2*, and the detection rate of variants in *PSENs/APP* was 8.80% [15]. However, these cohorts were not multicentric and the sample sizes were relatively small; thus, the findings did not fully represent the mutation spectrum of FAD in China.

As one of the early interventions of AD and dementia, non-pharmacologic treatment (NPT) has been studied more and more in recent years [16]. At present, the NPT research on dementia mainly focuses on improving the lifestyle, physical exercise [17], music [18], cognitive training, risk factor control and

so on. Physical exercise as a common NPT method can improve the cognitive function of MCI patients and slow down the progression of AD [19, 20]. Physical exercise may improve cognitive function through oxidative stress, inflammation, metabolism and other mechanisms [21], and affect the brain structure related to dementia, such as hippocampus/parahippocampal area, anterior cingulate gyrus and prefrontal cortex. Music therapy can improve some cognitive functions of mild AD patients, especially episodic memory, executive function and general cognition, and also has a positive impact on mental and psychological health [22]. Cognitive training can delay the onset of symptoms and improve the Aβ related memory deficit by preventing oxidative stress and changing the plasticity of white matter in the brain [23, 24]. In addition, functional task training [25], lifestyle intervention [26], Mediterranean diet (MeDi) [27], psychosocial intervention [28], and improving sleep quality [29] are also effective ways to improve cognitive function. At present, most of the NPT studies have a single sample, lack of quantitative and objective evaluation criteria, do not exclude other influencing factors (other comorbid diseases, gender, etc.). Furthermore, their neurobiological mechanism is not clear. It is necessary to explore the efficacy and potential neurobiological mechanism of NPT in AD and dementia through well-designed randomized controlled trials, and systematic and effective intervention programs.

Therefore, Jia's team relied on Xuanwu Hospital of Capital Medical University and many research institutes and hospitals aim to establish the Chinese Familial Alzheimer's disease network (CFAN). CFAN is a nationwide, multi-center, large-scale longitudinal observational research cohort. It will help to collect genetic information, clinical characteristics, cognition, neuroimaging, and biological specimen data of FAD to promote research progress on new mutations identification, pathogenesis mechanism and biomarkers.

Recently, in our study, 3330 patients with AD or mild cognitive impairment in 514 pedigrees were enrolled from the CFAN. 13.12% of pedigrees carried *PSENs/APP* missense mutations, 3.71% carried *PSENs/APP* synonymous/untranslated region variants, and 83.17% did not carry *PSENs/APP* mutations. In the present study, 11missensemutations were first identified by the CFAN, including 10 in *PSEN1* and one in *APP*. The present study highlights that the FAD population in China needs extensive investigation, and a concerted effort is necessary to identify the crucial pathological genes underlying the pathogenesis of AD.

2. Introduction

2.1 Overview

CFAN is the world's second largest registry network started by Professor Jianping Jia in 2005. The sponsor of this project is Xuanwu Hospital of Capital Medical University. CFAN is a multicenter observational longitudinal cohort study. The main research population for CFAN are autosomal

dominant inherited AD and unknown gene mutation AD family. All CFAN participants will be assessed longitudinally with comprehensive clinical, cognitive, genetic, imaging, and biomarker examinations, and all data will be collected using a unified standard, and stored into a central database. The purpose of the establishment of CFAN is to advance the research progress of FAD new mutations, pathogenic mechanisms and biomarkers in the Chinese population. At the same time, CFAN has great clinical significance. Through gene screening of the family, asymptomatic gene carriers can be found earlier, early diagnosis and intervention can be performed, and the prognosis can be improved. Long-term follow-up of asymptomatic gene carriers is convenient to further reveal the evolution of AD disease, and to find a breakthrough for early diagnosis and intervention in the population of asymptomatic gene carriers.

2.2 Research sites

There is a total of 68 research centers participating in this project, involving 26 provinces and cities. For details, please refer to Appendix 16.

2.3 Research period

The research period of this project is from February 2005 to January 2038 or longer.

2.4 Sample size

There will be a total of 40,000 subjects participating in this project. The total number of subjects to be included in this hospital is 5,000, and other sites include 400-600 persons per sites.

3. Research aims

This research will establish and continuously improve the FAD research network in conjunction with multi-center institutions nationwide. By collecting information on the family's demography, genetics, neuropsychology, neuroimaging, biomarkers and other information, we can understand the current FAD population in China, clarify the genetic characteristics, pathogenesis, disease characteristics and diagnosis and treatment status of AD in China; which will lay the foundation for ameliorating clinical diagnosis and treatment, establishing a Chinese FAD clinical database and an international cooperative research platform.

3.1 To set up a multi-center, nationwide FAD research network and database platform in China

The network and database include ADAD cohort of the known mutations of *PSEN1*, *PSEN2* and *APP* (mutation carriers and noncarriers; pre-symptomatic and symptomatic) and unknown mutations cohort.

3.2 To clarify the epidemiological characteristics of FAD in China.

Conduct a comprehensive FAD epidemiological survey in China to clarify the impact of different nationalities, regions, gender, age, living environment (rural/urban), education level, etc. on the

occurrence and development of the disease.

3.3 To clarify the genetic characteristics of FAD in China.

This project is to discover new FAD mutation sites，pathogenic genes, to protective genes, to explore the pathogenic and protective mechanism, to analyze the disease development laws of families with different sizes of FAD in China, and to clarify the frequency distribution of mutant genes in the Chinese FAD population.

3.4 To clarify the clinical characteristics and disease development laws of FAD in China.

The project will collect and regularly follow-up the samples (blood, urine and saliva etc.) and data (neuropsychology, imaging etc.) in the cohort. Emphasis is placed on the occurrence and development of asymptomatic mutant gene carriers from asymptomatic to symptomatic periods.

3.5 To discover and verify the early diagnosis biomarkers of AD.

In the FAD family cohort, we will screen high-sensitivity and high-specificity body fluid markers suitable for Chinese people, verify in the SAD cohort, and establish a prediction model of body fluid markers for AD occurrence and disease progression; use structural MRI, dual tracer ^{18}F-FDG PET and ^{11}C-PIB PET multimodal imaging technology, dynamically monitor the dynamic evolution of imaging biomarkers such as brain structure, glucose metabolism and Aβ deposition at various stages of AD progression.

3.6 To establish a genetic counseling model.

We will combine with the genetic characteristics of Chinese FAD to analyze the impact of lifestyle, physical exercise, nootropic drugs, cognitive training, etc. on the disease progression of FAD patients or asymptomatic mutant gene carriers, to establish a genetic counseling model.

4. Research content

4.1 Cohorts

We recruit Pre-MCI, MCI and AD dementia patients and normal cognitive control people to establish horizontal and vertical research cohorts, including ADAD cohort and the unknown mutation carrier cohort. Establish a national FAD research network and electronic database platform to improve the clinical information database and biological sample database.

4.2 Epidemiological characteristics of FAD in China

Investigate the basic information of the cohort, including individual factors such as sociodemography, lifestyle, past medical history, vascular risk factors, occupational exposure, and external environmental factors such as socioeconomic level. Conduct a comprehensive FAD epidemiological survey in China to identify the impact of nationality, region, gender, age, living environment (rural/urban), education level, etc. on the occurrence and development of the disease.

4.3 Genetic characteristics and mechanism of FAD in China

Based on the early recruitment and registration of FAD, the disease development laws of Chinese FAD families of different sizes will be analyzed. Each family must meet the characteristics of co-segregation. Use the latest sequencing technology to find new genes and new mutation sites in the disease-causing genes *(APP, PSEN1*, *PSEN2*) that have been discovered. Explore differences in gene frequency from those reported in the West. Determine the frequency distribution of *APOE* genotypes and *APOE* alleles (*APOEε4*, *APOEε3*, and *APOEε2*), and explore the frequency and incidence of *APOE* genes in FAD population in China. All the above research will provide new evidence for risk assessment and intervention in the early stages of the disease.

4.4 Clinical characteristics, disease development process and laws of FAD in China

Clinical examination including medical history, past history, family history, physical examination and neurological examination will be performed. Neuropsychological assessment usually includes cognitive function assessment (memory assessment, attention/executive function assessment, language function assessment, visual space and structural ability, calculation), non-cognitive assessment (mental behavior change) and evaluation of daily functions (personal life ability, social ability, work ability), etc. It is evaluated by the following unified and standardized neuropsychological scales: Mini-Mental State Examination, MMSE; Rey-Osterrieth Complex Figure Test, ROCFT; Digit Span Backward, DSB; Digit Span Forward, DSF; Verbal Fluency Test, VFT; Trail Making Test A/B, TMT A/B; Boston Naming Test, BNT; Clinical Dementia Rating, CDR; Neuropsychiatric Inventory Questionaire, NPI-Q; Hamilton Anxiety Scale, HAMA; Hamilton Depression Scale, HAMD; Activities of Daily Living, ADL. In addition, Modified Hachinski Ischemic Scale (m-HIS) was used to distinguish AD from VaD.

Through baseline and follow-up observation, we will analyze cognitive function, mental behavior symptoms and daily living ability of asymptomatic mutant gene carriers in FAD families. We will focus on the period from the asymptomatic to the development of symptoms, including subtle neuropsychological, clinical symptoms, changes in imaging, blood, cerebrospinal fluid, urine and saliva.

4.5 Biomarkers of FAD in China

We will conduct neuropsychological tests, clinical examinations, and imaging examinations on members of the FAD family, and conduct regular cerebrospinal fluid and blood sample collections. Use the international standardized electronic patient registration system to establish a large-scale and high-quality clinical information database and biological sample database to provide a foundation and platform for the establishment of the AD diagnosis system and risk prediction model. We will also focus on the asymptomatic mutant gene carriers in FAD families and analyze the changes of various imaging and body fluid markers in normal cognitive, Pre-MCI, MCI, AD and other stages.

4.5.1 Body fluid markers

Firstly, by collecting biological specimens of FAD family members, including blood, cerebrospinal fluid, urine and saliva etc., and referring biological sample banks of people in different clinical stages, the study can effectively identify highly sensitive and highly specific body fluid markers in different clinical stages of AD. Furthermore, longitudinal follow-up of people in different clinical stages of the family, detect AD potential and newly developed biomarkers in body fluids at baseline, and then combined with clinical neuropsychological evaluation, to establish a diagnosis system for AD clinical stages. Moreover, conduct regular follow-up and collection of body fluid samples in the included research cohort to monitor the dynamic changes of body fluid markers of patients as the disease progresses. Finally, combined with individual factors, clinical characteristics, neuropsychological assessment and body fluid markers, a comprehensive risk analysis of the entire disease process of AD occurrence and development was carried out, and body fluid marker prediction models in different stages of AD pathogenesis were established.

4.5.2 Image markers

We follow up the subjects with normal cognitive, Pre-MCI, MCI, AD stages in the FAD family, and test the hippocampus atrophy and cortical thickness of the subjects by MRI. We also detect eight observation brain regions by ^{18}F-FDG PET (bilateral frontal lobe, bilateral temporal lobe, bilateral parietal lobe, bilateral hippocampus). We will obtain accurate registration image data of patients' brain structure, metabolic function, pathological protein molecule deposition at various stages of AD progression. We will determine the quantitative critical thresholds for the early diagnosis of AD by ^{18}F-FDG PET and ^{11}C-PIB PET. Then we will obtain highly sensitive imaging markers for the early diagnosis of AD.

4.6 Research on early intervention and diagnosis and treatment of AD

Regularly follow up the non-onset members of the FAD family members, combined with the genetic characteristics of Chinese FAD, analyze the effects of lifestyle, physical exercise, nootropic drugs, cognitive training, etc. on the disease progression of FAD patients or asymptomatic mutant gene carriers. Investigate the effects of early non-pharmacological intervention on delaying the progression of the disease. Pay close attention to the cognitive situation of carriers of asymptomatic gene mutations. Once symptoms are found, intervene immediately to prevent disease progression. Early diagnosis of AD in the asymptomatic stage will provide a good time window for clinical intervention and treatment, and is expected to reverse and contain the development of dementia.

5. Research Technology Route

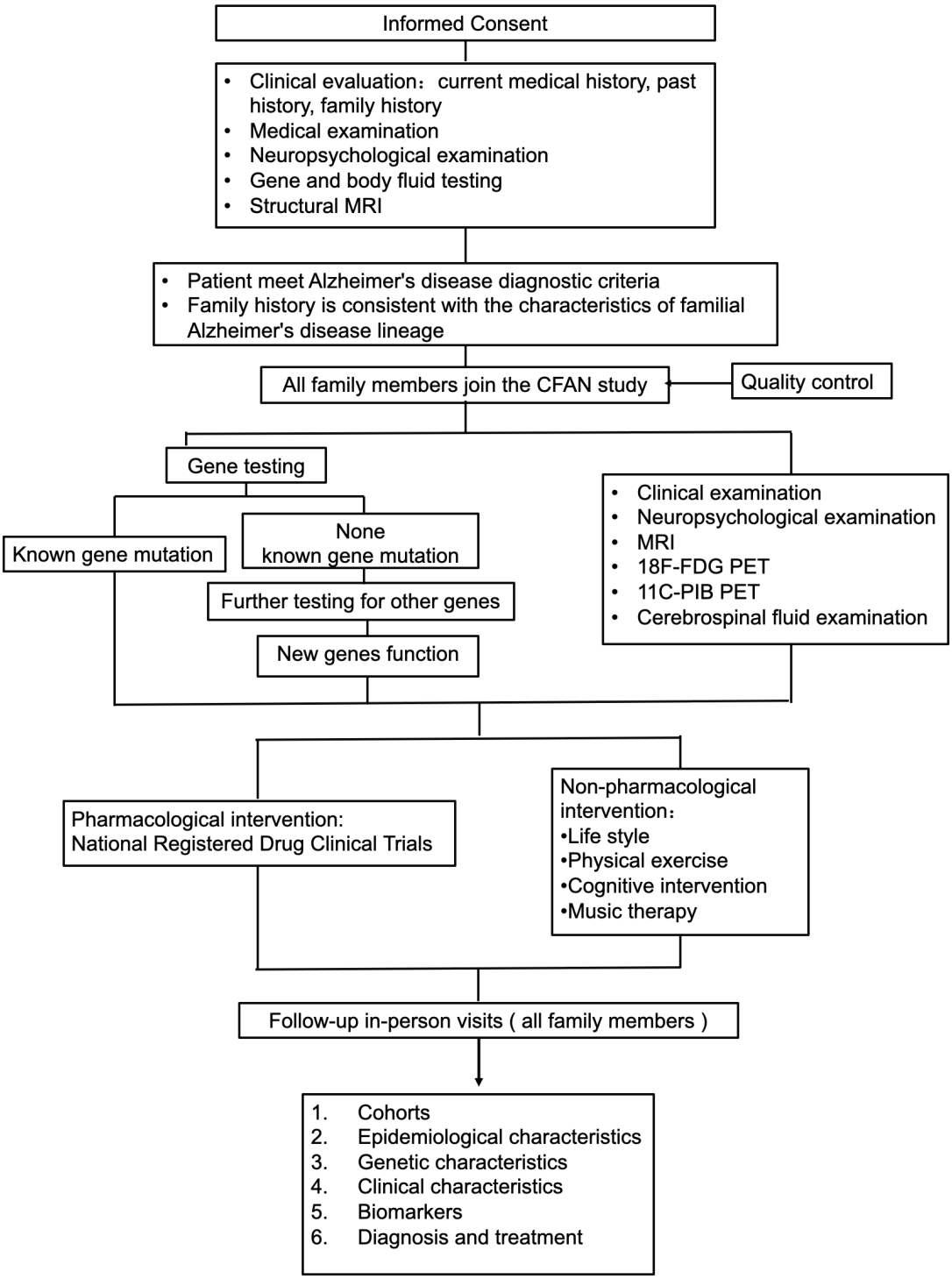

Figure1. Research technology route of CFAN study

6. Research Process

6.1 Participant population

All participants enrolled in CFAN were adults, and at least two first-degree relatives had AD. All participants received clinical assessment and genetic testing at baseline. Based on whether carrying a pathogenic mutation of the *PSEN1*, *PSEN2*, or *APP*, the cohort was further divided into two subgroups: autosomal dominant AD and unknown gene mutation FAD (un-FAD).

Subjects come from three sources:

1) FAD family in the memory clinic of Xuanwu Hospital.

2) FAD family recruited from CFAN subsites, where recruitment was according to the study protocol.

3) Online registration through the CFAN website (www.Chinacfan.org) and submission of basic demographic data and family information to CFAN. The CFAN administrator selects the family that meets inclusion criteria by reviewing the online registration materials, and recommends them to the CFAN subsite or Xuanwu Hospital memory clinic according to the geographical location.

6.2 Inclusion criteria

(1) Written informed consent obtained from the participant or a legal guardian prior to any study-related procedures;

(2) At least two first-degree relatives in a family have AD (clinically or by testing)，and at least 3 out of 2 generations are patients;

(3) At least one family member with normal cognitive function (the age should be greater than the average age of onset of the family);

(4) Pedigrees carrying FAD pathogenic genes (*APP/PSEN1/PSEN2*);

(5) People in this family >18 years old can be recruited;

(6) Participant is cognitively normal or demented but not reaching bedridden level;

(7) Participants are able to provide two reliable informants who can provide clinical information;

(8) Dementia is diagnosed according to the criteria described by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-R [31]);

(9) The diagnosis of AD is made using the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA [32]) or National Institute on Aging and the Alzheimer's Association (NIA-AA) criteria [33];

(10) The diagnosis of MCI is made according to Petersen criteria [34] and the classification is according to the method of Lopez et al [35].

6.3 Exclusion criteria

(1) Dementia caused by other factors such as depression, other psychiatric illnesses, thyroid dysfunction, encephalitis, multiple sclerosis, brain trauma, brain tumor, syphilis, acquired immunodeficiency syndrome (AIDS), Creutzfeldt-Jakob disease and other types of dementias such as vascular dementia (VaD), frontotemporal dementia (FTD[36]), dementia with Lewy bodies (DLB[37]), and Parkinson's dementia (PDD)；

(2) MRI and laboratory tests do not support or rule out a diagnosis of AD；

(3) Severe circulatory, respiratory, urinary, digestive, hematopoietic diseases (such as unstable

angina, uncontrollable asthma, active gastric bleeding) and cancer；

(4) Participant has severe psychiatric illness or severe dementia that would interfere in completing initial and follow-up clinical assessments；

(5) Participant has a history of alcoholism or drug abuse；

(6) Pregnant or lactating women；

(7) No reliable informant；

(8) Lumbar puncture exclusion criteria： coagulation disorders or platelet counts < 100,000 cells/μL, lumbar surgery within the last 6 months prior to lumbar puncture that interferes with anatomy of the inter-vertebral spaces, History of chronic or repeated CSF leakage following previous LP(s);

(9) MRI Exclusion Criteria: electronic and magnetic metal implants such as pacemakers, artificial heart valve, metal prosthesis, metal joint, etc.; metallic foreign body in the eye; aneurysm clips in the brain.

6.4 Study discontinuation

The primary reasons for study discontinuation are:

(1) Protocol violation. The participant fails to meet inclusion criteria, or meets exclusion criteria.

(2) Participant is unwilling or unable to participate.

(3) Consent is withdrawn.

(4) The study is terminated by the project director or the CFAN administration center.

(5) Safety Risk. Any participant who develops safety risk at any time during the visit.

(6) Death.

(7) Others.

6.5 Baseline visit

The baseline study visit includes the following items:

(1) Explain study contents and procedures to participant and sign the informed consent form.

(2) Urine pregnancy test (for women of childbearing potential).

(3) Check inclusion/exclusion criteria.

(4) Blood, urine and saliva samples collection (biomarkers).

(5) Genetic testing to examine risk gene *APOE* and known pathogenic genes *PSEN1*, *PSEN2*, and *APP* for all participants.

(6) Collecting participant and informant demographics.

(7) Collecting family history and draw family tree.

(8) Previous medical, medication history and life style.

(9) Physical exam and vital signs.

(10) Neurological Exam.

(11) Neuropsychological test: Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Neuropsychiatric Inventory Questionaire (NPI-Q), Hamilton Anxiety Scale (HAMA), Hamilton Depression Scale (HAMD), Modified Hachinski Ischemic Scale (m-HIS), the world health organization university of California-Los Angeles auditory verbal learning Test (WHO-UCLA AVLT), Rey-Osterrieth Complex Figure Test (ROCFT), Digit Span Forward (DSF), Digit Span Backward (DSB),Verbal Fluency Test (VFT),Trail Making Test (TMT) A/B, Boston Naming Test (BNT), Clinical Dementia Rating (CDR), Activities of Daily Living (ADL).

(12) MRI includes the following: conventional scan T1 weighted image (T1WI), T2 weighted image (T2WI), T2 FLAIR and hippocampal scan coronary T1-FLAIR sequence.

(13) ^{18}F-FDG PET.

(14) ^{11}C-PIB PET.

(15) Carotid ultrasound.

(16) Lumbar puncture to examine cerebrospinal fluid (after MRI exams) and follow-up phone call (if participate in LP)

(17) After informed consent, non-pharmaceutical interventions are carried out on non-onset gene mutation carriers in the family, including lifestyle changes, increased physical exercise and cognitive training, and nootropic drugs.

6.6 Follow-up in-person visits

The follow-up interval will be determined by the age of the individual in relation to the parent's Average Age of Onset (AAO) or pedigree mean AAO. The affected parent's AAO or pedigree mean AAO is used as the index for the frequency of assessments as follows in **Table 1**. In the years between in-person visits, telephone follow-up will be carried out every six months to inquire about cognitive change. If cognitive changes are reported by the participant and/or the informant during the telephone follow-up, the follow-up plan will be advanced and the participant will have a in-person clinical assessment.

Follow-up study visit includes the following items:

(1) Urine pregnancy test (for women of childbearing potential)

(2) Blood, urine and saliva samples collection (biomarkers)

(3) Family history update

(4) Participant and informant demographics

(5) Medical and concurrent medications history

(6) Physical exam and vital signs

(7) Neurological exam

(8) Neuropsychological test: Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Neuropsychiatric Inventory Questionaire (NPI-Q), Hamilton Anxiety Scale (HAMA), Hamilton Depression Scale (HAMD), Modified Hachinski Ischemic Scale (m-HIS), the world health organization university of California-Los Angeles auditory verbal learning Test (WHO-UCLA AVLT), Rey-Osterrieth Complex Figure Test (ROCFT), Digit Span Forward (DSF), Digit Span Backward (DSB),Verbal Fluency Test (VFT),Trail Making Test (TMT) A/B, Boston Naming Test (BNT), Clinical Dementia Rating (CDR), Activities of Daily Living (ADL).

(9) MRI includes the following: conventional scan T1 weighted image (T1WI), T2 weighted image (T2WI), T2 FLAIR and hippocampal scan coronary T1-FLAIR sequence.

(10) ^{18}F-FDG PET.

(11) ^{11}C-PIB PET.

(12) Carotid ultrasound.

(13) Lumbar puncture to examine cerebrospinal fluid.

Table 1 Follow-up visit plan and frequency

Index: **Individual compared with parent's AAO or pedigree mean AAO**	**In-person assessment interval**
> 10y younger	every 5 years
10y – >3y younger	every 3 years
3y – 0y younger	annual
0y – <3y older	annual
≥ 3y older	once, at 5 years older than parent's AAO
*Documented cognitive decline on telephone follow-up, confirmed by an in-person clinical assessment	every six months

Telephone follow-up will occur as indicated in the schedule of events in order to maintain contact with the family, inquire about cognitive change, and update information about adverse effect.

7. Assessments

7.1 Clinical evaluation

7.1.1 Demographic data (Appendix 1)

The demographic data should be filled in as completely as possible, including date of birth, age, family address, contact information, education level.

7.1.2 Current medical history

The detailed and objective current medical history must be filled out by the physicians. If case report form (CRF) cannot describe the symptoms and characteristics of the subject in detail, fill in the blanks.

Describe the specific manifestations of cognitive impairment, such as the specific manifestations of memory impairment (nearly/distant/ semantic forgetting); the specific manifestations of language barriers (difficulty in finding words, understanding, expressing, naming, writing, etc.); specific manifestations of disorientation (time, place, people, etc.); specific manifestations of personality and mental behavior (apathy, withdrawal, depression, agitation, wandering, abnormal sleep, antisocial behavior such as theft, etc.; the specific forms of hallucinations such as visual hallucinations, auditory hallucinations, etc.); manifestations of dyskinesia (such as ataxia, Parkinson-like dyskinesia, involuntary movements, etc.); implementation of dysfunction; spatial skills decline and other specific performance.

Physicians should describe the extent of decline in each cognitive domain, whether cognitive impairment affects the patient's daily ability and social function, and how it behaves.

7.1.3 Past history

According to the current internationally recognized diagnostic criteria, the previous illness of the subject is diagnosed or confirmed, and the presence or absence of the relevant disease, the time of diagnosis and the course of the disease are recorded in detail. Diseases with significant neurological sequelae, history of general anesthesia, and history of radiotherapy as well as chemotherapy need to be documented in detail. Try to let the subject or family provide the hospital's medical record/diagnostic certificate, etc., or the investigator will retrieve the patient's past medical records for inspection and confirmation.

7.1.4 Medication situation

The subject's medication needs to be documented in detail, including the name of the medication for the treatment of cognitive impairment and other diseases, the frequency of administration, and the adherence of the medication. Try to have the subject present a medical record, drug package or prescription.

7.1.5 Family history and family diagram drawing

For subjects with a family history, the investigator should investigate in detail the incidence of each member of the family, and generally investigate the three generations of relatives of the patient. For cognitive impairment and suspected cognitive impairment in the family, the disease should be recorded in detail (e.g. age of onset, first symptom, progression of disease, involvement of each cognitive domain, age of dementia, diagnosis and treatment, clinical consistency with the proband, etc.). Refer to operation manual for the rules of family diagram drawing.

7.1.6 Physical examination

Physical examinations include general medical examinations and neurological examinations performed by senior neurologists.

7.2 Psychometrics

7.2.1 Mini Mental State Examination (MMSE) [39] (Appendix 2)

MMSE is simple and easy to use, widely used at home and abroad, and is the preferred scale for dementia screening. The scale includes the following seven aspects: time orientation, location orientation, immediate memory, attention and calculations, delayed memory, language, and visual space. There are 30 items. The total scores range from 0 to 30. Test scores are closely related to cultural level.

7.2.2 The Montreal Cognitive Assessment (MoCA) [40] (Appendix 3)

MoCA is designed as a rapid screening instrument for mild cognitive dysfunction. It was created in 1996 by Ziad Nasreddine in Montreal, Quebec. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visual constructional skills, conceptual thinking, calculations, and orientation. Time to complete the MoCA is approximately 10 minutes. The total score is 30; a score of 26 or above is considered normal.

7.2.3 Neuropsychiatric Inventory (NPI-Q) [41] (Appendix 4)

The NPI-Q is a well-validated, reliable, multi-item instrument to assess neuropsychiatric symptoms in AD based on an interview with an informant (caregiver). The interview is relatively brief (<15 minutes). It evaluates both the presence and severity of 12 neuropsychiatric features, including delusions, hallucinations, agitation, dysphoria, anxiety, apathy, irritability, euphoria, disinhibition, aberrant motor behavior, night-time behavior disturbances, and appetite and eating abnormalities. The questionnaire separately evaluates the frequency and severity of each abnormal psychiatric symptom and the degree of distress of the caregiver. The caregiver is asked to rate the frequency of the symptoms of that domain on a scale of 1 to 4 (1 = occasionally, less than once per week; 4 = very frequently, once or more per day or continuously) as well as their severity (1 = mild, 2 = moderate, 3 = severe). The total score for each domain is calculated by multiplying the frequency by the severity. A total score is calculated by adding all the domain scores together. Caregiver distress is rated by the caregiver on a six-point scale from 0 (no distress) to 5 (very severe or extreme distress). Finally, the total score of all the symptoms of the caregiver's distress is obtained separately.

7.2.4 Hamilton Anxiety Rating Scale (HAMA) [42] (Appendix 5)

The HAMA is a psychological questionnaire used by clinicians to rate the severity of a patient's anxiety. The scale [43] consists of 14 items designed to assess the severity of a patient’s anxiety. Each of the 14 items contains a number of symptoms, and each group of symptoms is rated on a scale of 0

to 4, with 4 being the most severe. All of these scores are used to compute an overarching score that indicates a person's anxiety severity [44]. Upon the completion of the evaluation, the clinician compiles a total, composite score based upon the summation of each of the 14 individually rated items. This calculation will yield a comprehensive score in the range of 0 to 56. It has been predetermined that the results of the evaluation can be interpreted as follows: A score of 17 or less indicates mild anxiety severity. A score from 18 to 24 indicates mild to moderate anxiety severity. Lastly, a score of 25 to 30 indicates a moderate to severe anxiety severity.

7.2.5 Hamilton depression scale (HAMD) [45] (Appendix 6)

The HAMD is a multiple item questionnaire used to provide an indication of depression. Max Hamilton originally published the scale in 1960 [45] and revised it in 1966 [46], 1967 [47], 1969[48], and 1980 [49]. The questionnaire is designed for adults and is used to rate the severity of their depression by probing mood, feelings of guilt, suicide ideation, insomnia, agitation or retardation, anxiety, weight loss, and somatic symptoms. The original 1960 version contained 17 items (HDRS-17). Each item on the questionnaire is scored on a 3 or 5 points scale. The total score can be used to evaluate the severity of depression and treatment effect. The milder the symptoms, the lower the total score, and the severer the symptoms, the higher the total score. Assessment time is about 20 minutes.

7.2.6 Modified Hachinski ischemic scale (m-HIS) [50] (Appendix 7)

The m-HIS can be used to identify vascular dementia through clinical findings. The scale has 8 items with a total score of 12 points. If there are step worse, telling physical symptoms, emotional loss, and history of hypertension, score 1 point each; if there are acute onset, history of stroke, focal symptoms of the nervous system, focal signs of the nervous system, score 2 points each. The total score ranges from 0 to 12. The higher the score, the greater the likelihood of vascular dementia; those with a score of 4 or less are AD; those with a score of 7 or more are vascular dementia.

7.2.7 The world health organization university of California-Los Angeles auditory verbal learning Test (WHO-UCLA AVLT) [51] (Appendix 8)

This test was originally developed by the World Health Organization to be used in international research. The WHO-UCLA AVLT includes immediate recall, delayed recall, and delayed recognition. The process is as follows: The tester reads 15 words while informing the subject that they need to immediately recall after listening. Learning and recalling 3 times, after about 30 minutes interval, delayed recall test of 15 words without prompting. Then the tester reads 30 words (including 15 learned words and 15 interference options). The subject is asked to judge whether it is a learned word, and the number of correct words is recorded. This is a recognition test. The scale examines the ability to enter, store, extract, pay attention, and vocabulary of memory, and the score increases with increasing educational level.

7.2.8 Rey–Osterrieth complex figure test [52] (Appendix 9)

The Rey–Osterrieth complex figure test (ROCF) is a neuropsychological assessment in which examinees are asked to reproduce a complicated line drawing, first by copying it freehand (recognition), and then drawing from memory (recall). Many different cognitive abilities are needed for a correct performance, and the test therefore permits the evaluation of different functions, such as visuospatial abilities, memory, attention, planning, and working memory (executive functions). First proposed by Swiss psychologist André Rey in 1941[53] and further standardized by Paul-Alexandre Osterrieth in 1944 [54], it is frequently used to further explain any secondary effect of brain injury in neurological patients, to test for the presence of dementia.

7.2.9 Digit Span Test- Forward and Backward [55, 56] (Appendix 10)

A digit-span task is used to measure working memory's number storage capacity. The tester reads a series of numbers of different lengths and asks the subject to immediately repeat in the forward or reverse direction. The digit span gradually increases from 3 to 10 in the forward test, and gradually increases from 2 to 8 in the reverse test. Each digit span includes two tests. The test was terminated when both of the two tests were performed incorrectly by the subject. The longest digit length that a subject can speak is its digit span.

7.2.10 Verbal Fluency Tests [57] (Appendix 11)

The test is mainly sensitive to frontal dysfunction and mild semantic memory impairment. Subjects are asked to speak as many words as possible within a given time (usually 1 minute), such as the name of an animal, fruit or vegetable. Record the correct number of words, repetitions, series and conversions. The correct number of words is usually used as a scoring indicator, but other factors such as concatenation and conversion can sometimes be used as scoring indicators. The number of series is the items listed consecutively in each sub-category, counting from the second item of each sub-category until the subject is converted to another sub-category, and the series of each sub-category are added to obtain the total series. The total number of series, mainly reflecting the semantic memory function. The number of conversions is the times the subject switches between subcategories, mainly reflecting the frontal lobe execution function.

7.2.11 Trail Making Test: Parts A and B [58] (Appendix 12)

Part A consists of 25 circles, numbered 1 through 25, and the tester instructs the participant to draw lines as fast as possible in increasing numerical order. Part B is also composed of 25 circles, but these circles are white circles (1-12) and black circles (1-13). Subjects need to alternately connect circles of different colors in numerical order. The completion performance of the subject is determined based on the number of correctly completed lines and the time required to complete. Although both the A test and the B test rely on visual motion and perceptual scanning skills, trail making test B requires

considerable cognitive flexibility to complete the black and white transition connection under time pressure.

7.2.12 Boston Naming Test (BNT) [59] (Appendix 13)

The BNT requires the participant to name objects depicted in outline drawings. In our modification of the full BNT, only 30 items are presented. The drawings are graded in difficulty, with the easiest drawings presented first. If a participant encounters difficulty in naming an object, a stimulus cue and/or a phonemic cue is provided. Record the correct number of independent initial naming, clue naming, and option naming.

7.2.13 Activities of daily living (ADL) [60] (Appendix 14)

The ADL scale can evaluate the daily activities of the subject comprehensively, accurately and quickly. It consists of the physical life self-care scale and the instrumental ADL. There are 14 items, including two parts: first, the physical life self-care scale has a total of 6 items, including toilet, eating, dressing, grooming, walking and bathing; second, instrumental ADL has a total of 8 items, including call, shop, prepare meals, do housework, laundry, use transportation, take medicine and financing abilities. The higher the subject scores, the worse the ability of daily living.

7.2.14 Clinical Dementia Rating (CDR) [61] (Appendix 15)

The CDR is a refinement of information that the doctor obtains through conversations with the patient and his/her family, and to assess the degree of impairment of the patient's cognitive function, and then to quickly assess the severity of the patient's condition. The assessment of cognitive domains consists of six items: memory, orientation, judgment and problem solving, work and social ability, family life and hobbies, and independent living ability. The judgment is in five levels, "normal CDR=0, suspected dementia CDR=0.5, mild dementia CDR=1, moderate dementia CDR=2, severe dementia CDR = 3". The CDR-SOB (CDR sum of box) total score is a simple sum of the scores of the six items. CDR sum of boxes adds up the 6 items, and the total score ranges from 0 to 18. As long as the score is greater than 0, the subject has at least one cognitive impairment. Generally, the change value of CDR sum of boxes can be used in clinical trials to obtain more accurate cognitive evaluation[62]. CDR is a widely used measure of the severity of dementia.

7.3 Procedures for genetic studies

7.3.1 Gene testing

The goal of the genetic study of CFAN is the genetic information and useful biological materials (DNA, blood, cerebrospinal fluid, urine, saliva, etc.) provided by cognitive normal and dementia in the ADAD cohort and unknown gene mutation cohort. It is anticipated that collection of these data will facilitate clinical and basic science investigations of the pathogenesis of dementia.

7.3.2 Detection of pathogenic genes and risk genes

After informed consent of the study subjects, we extract 3 ml of the peripheral venous blood of the participants. Peripheral blood genomic DNA is extracted using whole blood genomic DNA extraction kit. *ApoE*, *PSEN1*, *PSEN2* and *APP* gene primer design are referenced. *ApoE*, *PSEN1*, *PSEN2*, and *APP* gene are screened by polymerase chain reaction (PCR). The PCR products above are subjected to Sanger sequencing. The sequencing results are analyzed by DNASTAR (vension7.1) software. The single nucleotide polymorphism gene mutation screening was performed with reference to the PubMed/GeneBank database.

7.3.3 Gene risk locus detection

We performed whole exon sequencing and whole genome sequencing on some families. Case-control methods were used to discover, study and analyze risk-related risk sites, providing genetic basis for disease prevention and control.

The peripheral blood DNA is extracted, and the genomic DNA is interrupted by ultrasound, and sequenced after the process of building a library, hybridization capture, and quality control. SNPs were annotated with CCDS, human genome database, and dbSNP information to determine the genes, coordinates, mRNA sites, amino acid changes, and SNP functions (missense mutations/nonsense mutations/variable cleavage sites) at which mutation sites occur, SIFT predicts that SNP affects protein function prediction and so on.

7.3.4 Body fluid testing

Detection of cerebrospinal fluid, blood, urine and saliva biomarkers in each group, including routine testing items such as blood, urine routine, biochemistry, electrolytes, coagulation function, cerebrospinal fluid routine, biochemical and other indicators. Also including special indicators cerebrospinal fluid and blood $A\beta_{40}$, $A\beta_{42}$, t-tau, p-tau and neurogranin. We conduct body fluid testing to study and explore early diagnostic markers of disease, and predictors of risk progression.

7.3.5 Blood specimens

Blood was taken on an empty stomach in the morning. Each subject received 8 ml of peripheral blood, 5 ml was injected into the purple head anticoagulation tube, and 3 ml was injected into the red head non-anticoagulated tube. The blood collection tube was marked with the name and number, and centrifuged within 2 hours. The purple head anticoagulation tube was centrifuged at 2000 rpm for 10 minutes to aspirate the upper layer of plasma; the remaining specimens were retained in the anticoagulation tube. After the red head non-anticoagulation tube was centrifuged at 2000 rpm for 10 minutes, the serum was aspirated and placed in two 1.5 ml centrifuge tubes, about 500 μl/tube, and the non-anticoagulant tube was discarded. The name and number of the specimen and the type of specimen (the serum is labeled Q and the plasma is J) are indicated on the centrifuge tube. The centrifuge tube was immediately stored at -80 ° C, and the anticoagulation tube was stored in a refrigerator at -20°C.

7.3.6 Cerebrospinal fluid specimens

Lumbar puncture needs to be performed in the morning. To eliminate the impact of puncture bleeding on the quality of cerebrospinal fluid specimens, the first 1-2 ml of CSF should be discarded. 10 ml of cerebrospinal fluid was collected, centrifuged within 2 hours after collection (2000 rpm / 10 minutes of centrifugation), and the supernatant was aspirated and placed in 10 1.5 ml centrifuge tubes, 1 ml/tube. Ten centrifuge tubes were immediately stored in a -80°C freezer.

Approximately 1 ml each of CSF and plasma samples will be utilized for assessment of the various defined measures. CSF will be analyzed for $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, tau and ptau181 measured via ELISA-based methods. Plasma will be analyzed for $A\beta_{1\text{-}40}$, $A\beta_{x\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{x\text{-}42}$. The remaining aliquots will be banked at – 80℃.

7.4 Imaging

A model of how different AD biomarkers change during the development of the AD proposed that biomarkers became abnormal in the following order:

(1) β-amyloid (indicating deposition of amyloid in plaques outside the cell, measured in CSF and by amyloid PET)

(2) Tau (indicating the formation of tau fibrils with the neurons)

(3) Glucose metabolism (measured on PET, indicating damage to neurons)

(4) Structural MRI (indicating damage to brain structure)

(5) Cognitive impairment

At the time of each assessment, participants will undergo scanning with MRI and PET. The PET imaging will include both FDG and PIB scans to measure metabolism and Aβ deposition, respectively.

Xuanwu Hospital and each sub-center have advanced imaging equipment, including cyclotron RDS111 (Siemens) and GE's PET/MR full body scanner. It has the license for the use of radioactive drugs by the National Food and Drug Administration (the fourth category) and has the qualification to prepare new drugs (tracer) for research and development.

7.4.1 Magnetic resonance imaging (MRI)

MRI is a medical imaging technique used in radiology to form pictures of the anatomy and the physiological processes of the body in both health and disease. MRI scanners use strong magnetic fields, magnetic field gradients, and radio waves to generate images of the organs in the body. MRI does not involve X-rays or the use of ionizing radiation. MRI is widely used in hospitals and clinics for medical diagnosis, staging of disease and follow-up without exposing the body to radiation. The contrast provided between grey and white matter makes MRI the best choice for many conditions of the central nervous system, including demyelinating diseases, dementia, cerebrovascular disease, infectious diseases, Alzheimer's disease and epilepsy.

All MRIs should be scheduled prior to the lumbar puncture procedure. All participants will be screened for standard MRI contraindications immediately before the MRI. Scanning will start with placement of stereotactic marker on participant's right temple and proper orientation of head in coil. All participants will then undergo the following imaging: T1 weighted image (T1WI), T2 weighted image (T2WI), blood oxygenation level dependent (BOLD), Diffusion tensor imaging (DTI), arterial spin labeling (ASL), magnetic resonance angiography (MRA).

7.4.2 Positron-emission tomography (PET) imaging

7.4.2.1 11C-PIB PET imaging

Amyloid tracer [N-methyl-11C]2-[4'-(methylamino) phenyl]-6-hydroxybenzothiazole (11C-PIB) is the mostly studied and relatively mature Phenothiazine Aβ imaging agent, which is a 11C-labeled derivative of Thioflavin-T. It can specifically bind to Aβ plaques in the brain of AD patients and is used for positioning and quantitative analysis of Aβ in brain. 11C-PIB PET can differentiate AD and normal patients in the early and middle stages of the disease, reflect the pathological changes of brain tissue, provide more objective data for early diagnosis. Further combined with clinical history, laboratory tests, neuropsychology scale and structural imaging results, it can help improve the accuracy of the diagnosis to guide early treatment. Image acquisition is completed by radiology department of each center, stored and transmitted in DICOM format, and then image processing is carried out to obtain whole brain and regional standard uptake value ratio (SUVR), and used as location and quantitative indicators of Aβ deposition.

7.4.2.2 FDG-PET imaging

FDG-PET can detect abnormal brain metabolism in patients with dementia, and should be performed within 1 month before or after neuropsychological examination (with no new vascular disease event). Fasting for at least 6 hours before examination, and fasting blood glucose was measured. Blood glucose levels should be <140 mg / dL (7.8 mmol/L). Image acquisition is completed by radiology department of each center, stored and transmitted in DICOM format, and then image processing is carried out to obtain whole brain and regional standard uptake value ratio (SUVR), and used as location and quantitative indicators of brain metabolic function.

7.4.3 Carotid ultrasound

Carotid ultrasound can clearly demonstrate the vascular intima-media thickness, the location and size of plaque, the degree of vascular stenosis, and analyze hemodynamics. It plays a key role in the assessment and diagnosis of vascular-related diseases caused by atherosclerosis. Carotid ultrasound examination should be performed within 1 month before or after neuropsychological examination (with no new vascular disease event). An experienced ultrasound physician is required to perform the operation according to "Guidelines for Carotid Ultrasound Examination". Longitudinal and transverse

section scanning are performed from frontal and lateral directions. Observe and record the peak systolic velocity (PSV), end diastolic velocity (EDV), intima-media thickness (IMT) and plaque (diameter, number, acoustic properties) of internal carotid artery, common carotid artery and carotid bifurcation.

8. Statistical analysis

Periodic review of the accumulating data will be made to safeguard the statistical quality of the data and to ascertain whether they conform to the expectations of the original statistical design. At the same time, the statistician will perform statistical quality control of FAD data, focusing on outlier and missing data detection and comparison among sites. Then, the statistician will participate in statistical data analyses, consultations and reporting using R software and other statistical software, such as SAS and SPSS for specialized statistical analyses. The sample size is calculated using the paired sample t test, and the statistical test level is set to 0.05.

The normality and homogeneity of variance tests were performed on continuous variables. Data that passed these two tests were presented as mean ± standard deviation，then underwent t-testing for comparisons between two groups and one-way analysis of variance (ANOVA) testing for comparisons among three or more groups. After ANOVA analysis, a Dunnett's multiple comparison test was used for post hoc testing. Data that did not pass the normality test were presented as quartiles, and underwent nonparametric testing, using the Kruskal-Wallis test and Dunn's multiple comparison test for comparisons among three or more groups. The significance of differences was assessed by the $\chi 2$ test for categorical variables. The correlation between variables were calculated using Pearson or Spearman method.

For the longitudinal repeated data, to explore the correlation between different markers and AD, a Bayesian linear mixed model was used to model each marker as a function of estimated years from expected symptom onset (EYO) and mutation status (carrier or noncarrier). The fixed effects included sex, age, follow-up time, mutation status, EYO, and their two-way interaction.

9. Data management

9.1 Establishment of electronic database

The sponsor will (or entrusting other companies) establish a standard EDC in line with international management practices. During the collection of patient data, a paper version and scanned CRF should be generated when the electronic CRF is uploaded.

9.2 Data entry

The investigators or data management staffs will synchronically enter the data using double-entry method. Data entry will be implemented in two separate web-based systems. Image data and raw data will be uploaded and entered into the CFAN Central Archive (CCA). Coded participant identifiers will

be generated by Xuanwu consisting of a project code, a site number, and an individual number at the time of participant registration. If there are any questions during the data entry process, they will be answered by investigators.

9.3 Data auditing

All items of the data, including clinical, demographic, genetic and neuropsychologic data will be entered into CFAN data platform, and be checked by quality control committee. CFAN has rigorous quality system, and the requirement for the data is clear, correct and complete. Before generating the data base, through the way of integrating computer and manual quality control, examine and confirm the inclusion and exclusion criteria, make sure the losing or out of range data, recognize any repeated item, evaluate the consistency of follow up, and check the follow up status of the subjects. Any data problem found should be required to modify and verify by sub-centers.

9.4 Data access permissions

Data will be made accessible to CFAN personnel in a staged manner. Prior to the completion of quality control procedures, data will be held in a virtual “quarantine” and made accessible only to managerial staff and those responsible for completing Quality control (QC) procedures. After QC is complete, data will be released from quarantine and made accessible to approved users. All access to CFAN data, except that required for data entry, validation, and quality control at the Xuanwu, will be through the CCA. The CCA will be accessible via a secure password-protected web portal and web services. User accounts to the CCA will be approved by the Informatics Core leader, who will deliver regular updates to the CFAN.

9.5 Data modification record

The change trace in the database will be automatically recorded. The record content includes the data change name, the change reason, the original and modified data, the date of change, and the name of the modifier. Only a few people are authorized to change these records, and the modification history will be completely kept in the database.

10. Quality control

The sponsor and investigators should adopt standard operating procedures to ensure the implementation of clinical trial quality control and quality assurance systems. During the clinical trial, all observation results and findings should be verified to safeguard data reliability and ensure that all clinical trial conclusions are derived from the source data. Quality control must be implemented at each data processing stage to ensure that all data are reliable and processed correctly.

10.1 The writing of investigator’s case report form

Case report form (CRF) refers to a type of document defined and designed according to the trial

protocol to record the data of each subject during the trial process. Investigator's brochure and case report form are co-authored by principal investigator and sponsor. Requirements for data entry by investigators:

(1) The investigators should ensure truthful, accurate, complete and timely entry of trial data into the CRF and upload to the EDC.

(2) For all subjects who have filled out the informed consent form and are determined to be eligible for inclusion in this trial, all observed data should be recorded in the CRF in truthful and detailed manner without leaving blanks or missing items.

(3) For all subjects who have filled out the informed consent form and are determined to be eligible for inclusion in this trial, all data in the CRF should be checked against the original medical records to ensure consistency.

(4) When any correction is made in the CRF, only streaking and side notes are allowed, and the investigators should sign and date the changes.

(5) The original laboratory test reports (or photocopies) and imaging reports (or photocopies) should be pasted to the laboratory report paste area in the study medical records.

(6) All data that are significantly higher or lower or beyond the clinically acceptable range should be verified or reviewed and explained by the investigators as necessary.

10.2 Investigator training

Before the clinical trial is initiated, the sponsor organizes and funds centralized training of investigators uniformly at each trial center to help investigators understand and familiarize with the trial protocol, investigator's brochure, case report form and various scales.

Clinical evaluation is conducted by respectively independent evaluators, including physicians, nurses and caregivers. The compilation, implementation, score assessment and result interpretation of all neuropsychological tests are standardized and unified; in addition, centralized training of the evaluators is provided to achieve consistence standards in scale grading. The testing environment and sequence also need to be consistent to reduce the impact of human or environmental factors.

10.3 Subject's compliance

(1) Signing informed consent forms;

(2) The investigators should conscientiously implement the informed consent so that the subjects can fully understand the trial requirements and cooperate with the trial. The sponsor provides trial drugs free of charge and cover the laboratory examination costs.

11. Ethics

11.1 Human subjects, ethical and regulatory considerations

This study will be conducted according to Good Clinical Practice guidelines. Written informed consent for the study must be obtained from all participants and/or authorized representatives and the study partners before protocol-specific procedures are carried out.

11.2 Research ethics boards (REB)

Before the study is initiated, the protocol shall be reviewed and approved by the ethics committee prior to implementation. Verification of REB unconditional approval of the protocol and the written informed consent statement with written information to be given to the participants and/or their authorized representatives. This approval must refer to the study by exact protocol title and number, identify documents reviewed, and state the date of review. During the study period, any modifications of the protocol shall be approved by the ethics committee prior to implementation.

11.3 Informed consent

The principles of informed consent in the current edition of the Declaration of Helsinki and will be implemented before protocol procedures are carried out. Information should be given in both oral and written form and deemed appropriate by the REB.

Participants, their relatives, guardians or authorized representatives and study partners must be given ample opportunity to inquire about details of the study. The consent form generated by the investigator must be approved, along with the protocol. Consent forms must be in a language fully comprehensible to the prospective participants and/or their authorized representatives. Informed consent will be documented by the use of a written consent form approved by the REB and signed by the participant and/or an authorized representative. The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will also comply with local regulations. Consent must be documented by the dated signature of the participant and/or authorized representative. In either case the signature confirms that the consent is based on information that has been understood. Informed consent signed by each participant must be kept on file by the investigator for possible review by regulatory authorities and/or monitors.

11.4 Informed consent for biomarkers, genetic material, and imaging data

The informed consent will not only cover consent for the trial itself, but for the genetic research, biomarker studies, biological sample storage and imaging scans as well. The consent for storage will include consent to access stored data, biological samples, and imaging data for secondary analyses. The informed consent will specify that the CFAN will receive and store all research data. Consent forms will specify that DNA and biomarker samples are for research purposes only; the research tests are not diagnostic in nature and participants will not be informed of their test results.

11.5 Procedures to maintain confidentiality

11.5.1 Genetic research and storage of genetic material

Each sample will contain the following information: sample ID#(to preserve confidentiality), date of collection and processing, total initial volume collected, sample type (plasma, serum, CSF), volume, aliquot number, freezer, shelf, rack, box, location in the box. DNA and RNA will be prepared at the central laboratory for AD. The only information that will be maintained in this database is an Individual number (to preserve confidentiality), DNA/RNA number (barcode #), type of sample received, date drawn, date received, initial volume collected for each tube type, time of draw, year of birth and gender.

The samples banked in a locked freezer dedicated to the CFAN study at the central laboratory of neurodegenerative diseases, Xuanwu hospital of capital medical university. The samples are without a link to identity of the participant from whom the sample came. All samples are bar coded and identified by a bar code.

11.5.2 Biomarker Samples and Research

Biomarker fluid samples will be labeled by bar coding samples. Samples will be stored by bar code number and no other identifying information will be provided.

12. Research implementation conditions and researchers

12.1 Research implementation conditions

The research team relies on Xuanwu Hospital of Capital Medical University. The research team recruited appropriate patients by enrolling in the memory clinic and neurodegenerative and cognitive disorders. Memory Clinic has long been devoted to providing standardized diagnosis and treatment for cognitive impairment caused by various causes and dementia patients of various subtypes, and providing counseling and help to caregivers. The memory clinic has been insisting on systematically collecting clinical data and neuropsychological evaluation results of various types of elderly cognitive impairment and dementia patients, and conducting free APOE genetic testing to strive for early detection, early diagnosis and diagnosis of cognitive impairment diseases. For early treatment, the memory clinic is suitable for patients including memory loss and other cognitive decline patients, such as language ability, orientation, computing power, thinking and judgment ability, etc., including some cognitive impairments accompanied by emotional, psychological or sleep problems Of patients. Neurodegenerative and cognitive impairment diseases are divided into 4 main groups, 56 beds. This will help us to actively carry out the research and development and research of clinical new technologies for early diagnosis of neurodegenerative diseases and cognitive disorders, further improve the screening scale for neurodegenerative diseases and cognitive disorders, develop genetic early diagnosis technology and early diagnosis markers of cerebrospinal fluid To study the sensitivity and specificity of early diagnostic techniques in imaging.

At present, the laboratory has a nerve tissue section room, a microscope room, a histochemical

staining room, a molecular biology laboratory, and a tissue cell culture room. The operating room and clean animal rooms (rats, mice) are rented from the laboratory of Capital Medical University. It can independently complete many experiments such as molecular biology, cytology, experimental zoology and so on.

12.2 The capabilities of researchers

Jianping Jia has been devoted to AD research for more than 20 years. (1) An AD family carrying a presenilin 1 gene mutation (PS1 V97L) in China was reported for the first time, which opened the first of FAD research in China. It also reported for the first time 53 genetic mutation sites in Chinese, and based on this, 7 transgenic mouse models were constructed. Studies have found that Aβ oligomers and APP (precursor of Aβ protein) palmitoylation are affected by related gene mutations, which are two important ways to cause AD. In animal models, preliminary verification has shown that these two ways can be prevented or cut by Chinese medicine monomers such as honokiol. (2) They reported first that there are 31 million patients with mild cognitive impairment and 10 million dementia patients in China. The prevalence rates are 20.8% and 5.14%, respectively; the annual cost of dementia in China is more than 1.100 billion-yuan, accounting for 1/4 of the total cost of dementia in the world. These data provide an important basis for China to formulate a national prevention and control strategy for dementia. (3) Optimize AD clinical trials and continuously introduce new drug researches. He has chaired 8 domestic and 6 international multi-center clinical trials. It is proved that the new drug butylphthalide and the Chinese medicine Selotonone have significant effects on vascular dementia, breaking the dilemma of no available vascular dementia drugs in the world. The safety and effectiveness of donepezil in Chinese patients with severe AD have been verified, and new treatment options have been provided for patients. In collaboration with British experts, the world's first clinical trial of new anti-tau dementia drugs has brought new hope for AD treatment. (4) It was confirmed that Aβ, T-tau, and P-tau in peripheral blood exosomes can effectively reflect pathological changes in the brain and have clinical diagnostic value equivalent to cerebrospinal fluid. (5) Design and develop peripheral blood Aβ protein detectors for early diagnosis of AD patients. At present, preliminary distinction can be made between AD and normal controls. (6) Presided over the development of two editions of "Guidelines for the Diagnosis and Treatment of Dementia and Cognitive Impairment in China" (published by Human Health Press), and a series of 17 articles on diagnosis and treatment guidelines, which provided guidance documents for standardizing clinical practice of dementia in China. (7) Editor-in-chief of the textbooks compiled by many colleges and universities of China's medical colleges. He has been the editor-in-chief of the 6th, 7th, and 8th editions of Neurology. In addition, he edited the standardized training materials for national residents and training materials for specialists in neurology, and made special contributions to the cultivation of medical talents in China.

This study brings together more than 150 experts in 68 well-known hospitals in China who have established memory clinics in the field of AD and cognitive impairment. The purpose of this study is to condense the national AD clinical centers and establish the largest cohort of FAD in China.

The doctors participating in the study in our hospital have medical qualifications and rich clinical experience, and are competent for the safety protection and medical diagnosis and treatment of the subjects. Qualified or trained researchers are responsible for obtaining informed consent and receiving consultations on safety issues at any time.

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14. Appendix

Appendix 1 Participants information

Basic

A1 Number	**Province Number—Hospital Number—Participant Number：** _____--_____--_____	
A2 ID number		
A3 Gender	□1 male	□2 female
A4 Age	_____ years	

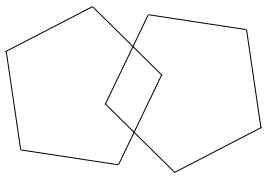
A5 Ethnicity	□1 Han，□2 Zhuang，□3 Hui，□4 Miao，□5 Tibetan，□6 Uyghur，□7 Other	
A6 Education	□ < 1 year □ 1-6 years □ > 6 years	
A7 Residence	□1 Urban □2 Rural	
A8 Home address	Urban A8.1.1 _____Province、municipality directly under the central government A8.1.2_____city A8.1.3____ district（county） A8.1.4_____Street（Road、Vallage） A8.1.5_____ Community A8.1.6_____ Building A8.1.7_____ Unit A8.1.8_____ House number A8.1.9 Post Code_____	
A9 Contact number	A9.1 Telephone（district number） _____ − _____ A9.2 Mobile_____	
A10 Handedness	□1 Dextromanulity □2 Left handedness □3 With both hands	
A11 Occupation	A11.1 Record occupational before retirement_____ □1 Agricultural, forestry, animal husbandry, fishery	□7 Soldier □8 Media, cultural and sports personnel □9 Student

	and water conservancy industry production personnel □2 Production and transportation equipment operators and related personnel □3 Business and service personnel □4 Officals □5 Administritive staff □6 Professionals	□10 No Job □11 House working □12 Religious personnel □13 Others
A12 Marrital status	□1 Married □2 Widowed □3 Divorced	□4 Separated □5 Single □6 Live together
A13 Living condition	□1 Living alone □2 Living with spouse □3 Living with children □4 Living with nanny	□5 Living with spouse and children □6 Living in nursing home or gerocormium □7Living with relatives or friends
A14 Hight（cm）	__ __ __	
A15 Body weight（kg）	__ __ __	
A16 Waistline（cm）	__ __	

A17 Blood pression （mmHg）	A17.1 Systolic__ __ __mmHg
	A17.2 Diastolic__ __ __mmHg
A18 Resting heart rate （Beat/ Minute）	__ __ __
A19 Normal vision	□0 No □1 Yes
A20 Normal hearing	□0 No □1 Yes

Appendix 2 mini-mental state examination (MMSE)[39]

Activity	Score
ORIENTATION – one point for each answer	
Ask: "What is the: (year)(season)(date)(day)(month)?"	_____
Ask: "Where are we: (state)(county)(town)(hospital)(floor)?"	_____
REGISTRATION – score 1,2,3 points according to how many are repeated	
Name three objects: Give the patient one second to say each.	
Ask the patient to: repeat all three after you have said them.	
Repeat them until the patient learns all three.	_____
ATTENTION AND CALCULATION – one point for each correct subtraction	
Ask the patient to: begin from 100 and count backwards by 7.	
Stop after 5 answers. (93, 86, 79, 72, 65)	_____
RECALL – one point for each correct answer	
Ask the patient to: name the three objects from above.	_____
LANGUAGE	
Ask the patient to: identify and name a pencil and a watch. (2 points)	_____
Ask the patient to: repeat the phrase "No ifs, ands, or buts." (1 point)	_____
Ask the patient to: "Take a paper in your right hand, fold it in half, and put it on the floor " (1 point for each task completed properly)	_____
Ask the patient to: read and obey the following: "Close your eyes." (1 point)	_____
Ask the patient to: write a sentence. (1 point)	_____
Ask the patient to: copy a complex diagram of two interlocking pentagons. (1 point)	_____

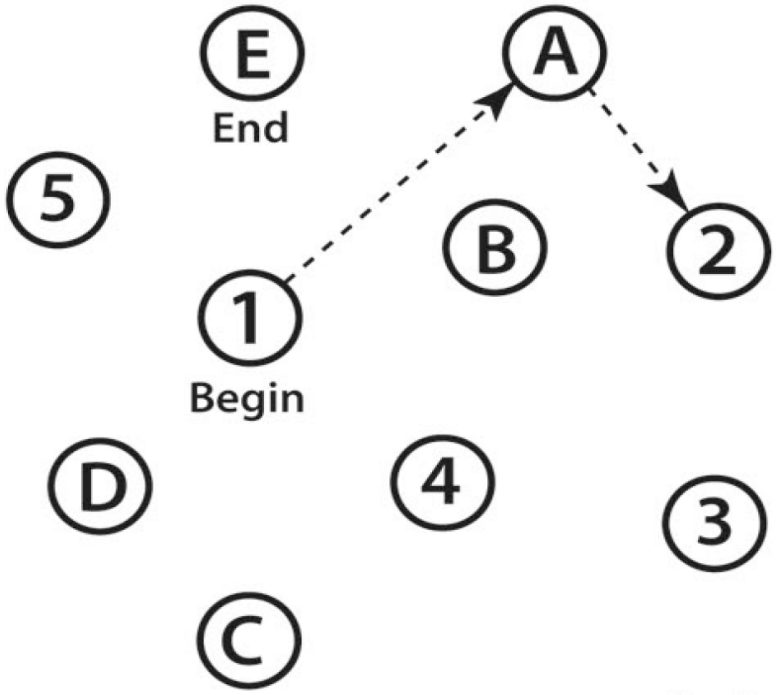


TOTAL (0-30): _____

Appendix 3 Montreal Cognitive Assessment (MoCA) [40]

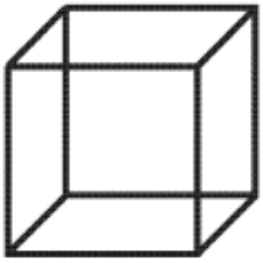
Montreal Cognitive Assessment (MoCA) was designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. Time to administer the MoCA is approximately 10 minutes. The total possible score is 30 points; a score of 26 or above is considered normal.

	contents	Administration and Scoring Instructions	score
1	Alternating Trail Making	Administration: The examiner instructs the subject: "Please draw a line, going from a number to a letter in ascending order. Begin here [point to (1)] and draw a line from 1 then to A then to 2 and so on. End here [point to (E)]." Scoring: Allocate one point if the subject successfully draws the following pattern: 1 −A- 2- B- 3- C- 4- D- 5- E, without drawing any lines that cross. Any error that is not immediately self-corrected earns a score of 0.	



1 score□

2	Visuoconstructional Skills (Cube)	Administration: The examiner gives the following instructions, pointing to the cube: “Copy this drawing as accurately as you can, in the space below”. Scoring: One point is allocated for a correctly executed drawing. • Drawing must be three-dimensional • All lines are drawn • No line is added • Lines are relatively parallel and their length is similar (rectangular prisms are accepted) A point is not assigned if any of the above-criteria are not met.	



2 score□

3 Visuoconstructional Skills (Clock)

Administration: Indicate the right third of the space and give the following instructions: “Draw a clock. Put in all the numbers and set the time to 10 past 11”.
Scoring: One point is allocated for each of the following three criteria:
• Contour (1 pt.): the clock face must be a circle with only minor distortion acceptable (e.g., slight imperfection on closing the circle);
• Numbers (1 pt.): all clock numbers must be present with no additional numbers; numbers must be in the correct order and placed in the approximate quadrants on the clock face; Roman numerals are acceptable; numbers can be placed outside the circle contour;
• Hands (1 pt.): there must be two hands jointly indicating the correct time; the hour hand must be clearly shorter than the minute hand; hands must be centered within the clock face with their junction close to the clock center.
A point is not assigned for a given element if any of the above-criteria are not met.
At the same time, the score is given by the 15-point method (see table below), not counting the total score.

	Contents	Yes	No	score
1	Shape similar to the clock	1	0	
2	Round circumference	1	0	
3	Diameter >2.5 cm (if the first painting is small, encourage it to draw larger)	1	0	
4	All the numbers are inside the circle	1	0	
5	First locate 12,6,3,9	1	0	
6	The numbers are arranged at regular intervals (symmetrically arranged on a 12-6 axis). If it is so, skip the 7th question	2	0	
7	If there is a spatial alignment error, it can be corrected (with traces of alteration)	1	0	
8	Only Arabic numerals	1	0	
9	Only the Arabic numerals 1-12 appear	1	0	
10	1-12 appears in sequence, no omission or misalignment	1	0	
11	Only 2 pointers	1	0	
12	The pointer is like an arrow	1	0	
13	The hour hand is between 11 and 12	1	0	
14	The minute hand is longer than the hour hand	1	0	
15	There is no problem under the column	1	0	
	1)The minute hand points to 10 o'clock			
	2)Write the words 11:10			
	3)Extra pointer or circle			
	4)Any letters, words or diagrams appear			
	5)Any extra lines appear below the circle			

3 score ☐——3-point method

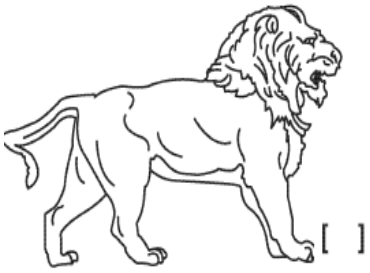
3.1 score☐☐——15-point method

4 Naming

Administration: Beginning on the left, point to each figure and say: “Tell me the name of this animal”.
Scoring: One point each is given for the following responses: (1) lion (2) giraffe

4 score☐

(3) camel or dromedary.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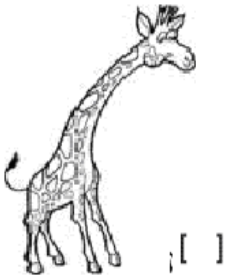
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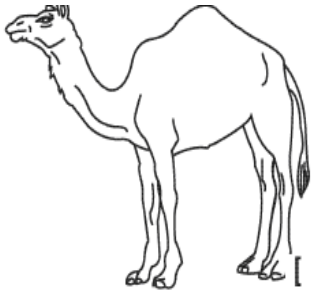

[ ]

5 Memory

Administration: The examiner reads a list of 5 words at a rate of one per second, giving the following instructions: "This is a memory test. I am going to read a list of words that you will have to remember now and later on. Listen carefully. When I am through, tell me as many words as you can remember. It doesn't matter in what order you say them". Mark a check in the allocated space for each word the subject produces on this first trial. When the subject indicates that (s) he has finished (has recalled all words), or can recall no more words, read the list a second time with the following instructions: "I am going to read the same list for a second time. Try to remember and tell me as many words as you can, including words you said the first time." Put a check in the allocated space for each word the subject recalls after the second trial.

At the end of the second trial, inform the subject that (s)he will be asked to recall these words Again by saying, "I will ask you to recall those words again at the end of the test."

Scoring: No points are given for Trials One and Two.

	Face	Velvet	School	Daisy	Red
First					
Second					

6 Forward Digit Span

Give the following instruction: "I am going to say some numbers and when I am through, repeat them to me exactly as I said them". Read the five number sequence at a rate of one digit per second.

Scoring: Allocate one point for each sequence correctly repeated.

21854 [ ]

6 score☐

7 Backward Digit Span

Administration: Give the following instruction: "Now I am going to say some more numbers, but when I am through you must repeat them to me in the backwards order." Read the three number sequence at a rate of one digit per second.

Scoring: Allocate one point for each sequence correctly repeated, (N.B.: the correct response for the backwards trial is 2-4-7).

742 [ ]

7 score☐

8 Vigilance

Administration: The examiner reads the list of numbers at a rate of one per second, after giving the following instruction: "I am going to read a sequence of numbers. Every time I say the number 1, knock the table once. If I say a different letter, do not knock the table".

Scoring: Give one point if there is zero to one errors (an error is a knock on a wrong number or a failure to knock on number 1).

52139411806215194511141905112 [ ]

8 score☐

9	Serial 7s (Refer to the MMSE result score)	Administration: The examiner gives the following instruction: “Now, I will ask you to count by subtracting seven from 100, and then, keep subtracting seven from your answer until I tell you to stop.” Give this instruction twice if necessary. Scoring: This item is scored out of 3 points. Give no (0) points for no correct subtractions, 1 point for one correction subtraction, 2 points for two-to-three correct subtractions, and 3 points if the participant successfully makes four or five correct subtractions. Count each correct subtraction of 7 beginning at 100. Each subtraction is evaluated independently; that is, if the participant responds with an incorrect number but continues to correctly subtract 7 from it, give a point for each correct subtraction. For example, a participant may respond “92 – 85 – 78 – 71 – 64” where the “92” is incorrect, but all subsequent numbers are subtracted correctly. This is one error and the item would be given a score of 3. [ ]**93** [ ]**86** [ ]**79** [ ]**72** [ ]**65**	9 score☐
10	Sentence repetition	Administration: The examiner gives the following instructions: “I am going to read you a sentence. Repeat it after me, exactly as I say it [pause]: I only know that John is the one to help today.” Following the response, say: “Now I am going to read you another sentence. Repeat it after me, exactly as I say it [pause]: The cat always hid under the couch when dogs were in the room.” Scoring: Allocate 1 point for each sentence correctly repeated. Repetition must be exact. Be alert for errors that are omissions (e.g., omitting "only", "always") and substitutions/additions (e.g., "John is the one who helped today;" substituting "hides" for "hid", altering plurals, etc.). I only know that John is the one to help today. [ ] The cat always hid under the couch when dogs were in the room. [ ]	10score☐
11	Verbal fluency	Administration: "Please tell as many animals as you can, as soon as possible. The time is 1 minute, are you ready? Start." Stop after one minute. Scoring: If the subject names ≥11 within 1 minute, score 1 point. At the same time, record the examiner's answer as much as possible. Deified animals such as dragons, phoenixes, and unicorns are also correct. please record : 2.11.1(0-15 s)_____ 2.11.2(16-30 s)_____ 2.11.3(31-45 s)_____ 2.11.4(46-60 s)_____ [ ]_____(N≥11 words)	11score☐
12	Abstraction	Administration: The examiner asks the subject to explain what each pair of words has in common, starting with the example: “Tell me how an orange and a banana are alike”. If the subject answers in a concrete manner, then say only one additional time: “Tell me another way in which those items are alike”. If the subject does not give the appropriate response (fruit), say, “Yes, and they are also both fruit.” Do not give any additional instructions or clarification. After the practice trial, say: “Now, tell me how a train and a bicycle are alike”. Following the response, administer the second trial, saying: “Now tell me how a ruler and a watch are alike”. Do not give any additional instructions or prompts. Scoring: Only the last two item pairs are scored. Give 1 point to each item pair	12score☐

correctly answered. The following responses are acceptable:

[ ] Train-bicycle： Means of transportation, means of travelling, you take trips in both;

[ ] Ruler-watch ： Measuring instruments, used to measure.

The following responses are not acceptable: Train-bicycle = they have wheels; Ruler-watch = they have numbers.

13 Delayed recall

Administration: The examiner gives the following instruction: "I read some words to you earlier, which I asked you to remember. Tell me as many of those words as you can remember." Make a check mark (√) for each of the words correctly recalled spontaneously without any cues, in the allocated space.
Scoring: Allocate 1 point for each word recalled freely without any cues.
Following the delayed free recall trial, prompt the subject with the semantic category cue provided below for any word not recalled. Make a check mark (√) in the allocated space if the subject remembered the word with the help of a category or multiple-choice cue. Prompt all non-recalled words in this manner. If the subject does not recall the word after the category cue, give him/her a multiple choice trial, using the following example instruction, "Which of the following words do you think it was, NOSE, FACE, or HAND?"
Use the following category and/or multiple-choice cues for each word, when appropriate:

	Face	Velvet	School	Daisy	Red
No prompt					
category cue	part of the body	type of fabric	type of building	type of flower	a colour
multiple choice	nose, face, hand	denim, cotton, velvet	church, school, hospital	rose, daisy, tulip	red, blue, green

Scoring: No points are allocated for words recalled with a cue. A cue is used for clinical information purposes only and can give the test interpreter additional information about the type of memory disorder. For memory deficits due to retrieval failures, performance can be improved with a cue. For memory deficits due to encoding failures, performance does not improve with a cue.

13score☐

14 Orientation

Administration: The examiner gives the following instructions: "Tell me the date today". If the subject does not give a complete answer, then prompt accordingly by saying: "Tell me the [year, month, exact date, and day of the week]." Then say: "Now, tell me the name of this place, and which city it is in."
Scoring: Give one point for each item correctly answered. The subject must tell the exact date and the exact place (name of hospital, clinic, office). No points are allocated if subject makes an error of one day for the day and date.
[ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

14score☐

15 Total score

15score☐

☐

TOTAL SCORE: Sum all sub scores listed on the right-hand side. Add one point for an individual who has 12 years or fewer of formal education, for a possible maximum of 30 points.
A final total score of 26 and above is considered normal.

Appendix 4 Neuropsychiatric Inventory (NPI-Q) [41]

Scoring levels with example definitions for the NPI-Q

Frequency of occurrence (1~4)

1. Occasionally: less than once per week
2. Often: about once per week
3. Frequently: several times per week but less than every day
4. Very frequently: once or more per day

Severity of behavior (1~3)

1. Mild: mildly distressing to the patient and not a major problem
2. Moderate: distressing to the patient but easily overcome by reassurance
3. Marked: distressing to the patient and difficult to redirect or deal with

Distress to you (0~5)

0. Not at all
1. Minimally: rarely distressing and easily tolerated
2. Mildly: occasionally distressing, but not a significant problem
3. Moderately: somewhat distressing and problematic, but usually tolerable
4. Severely: very distressing and difficult to cope with
5. Very severely or extremely: markedly distressing and extremely difficult to cope with

The score for each item is calculated by multiplying the frequency by the intensity; and the total score for the NPI is calculated by adding the scores of all symptoms.

Thus, scores may range from 0 to a maximum of 144, where the higher the score, the more severe the psychopathology.

Summary table for NPI-Q scores.

Symptom		Frequency	Severity	Total (frequency × severity)	Distress to you
1. Delirium	Does the patient have a false idea, such as thinking that someone else has stolen his/her things? Do you suspect that someone is harming him?				
2. Hallucinations	Does the patient have hallucinations such as visual hallucinations or auditory hallucinations? See or hear something or sound that doesn't exist? Talking to someone who doesn't actually exist?				
3. Agitation/aggression	Does the patient often refuse help from others? Unmanageable? Stubborn? Shouting at others? Snoring others?				
4. Depression/dysphoria	Does the patient show grief or express a depression?				
5. Anxiety	Is it uncomfortable after the patient is separated from the caregiver? Does the patient have mental stress such as shortness of breath, sigh, can't relax or feel nervous?				
6. Joy/euphoria	Is the patient too happy and feeling too good? Feeling humorous and laughing at things that others are not interesting about? Joy that doesn't match the situation?				
7. Apathy	Did the patient lose interest in activities that were previously of interest? Indifferent to other people's activities and plans?				
8. Disinhibition	Does the patient lose self-control, such as talking to strangers like an acquaintance? Or talk regardless of the feelings of others?				
9. Irritability/lability	Does the patient show impatience or crazy behavior? Can't bear the delay? Can't wait patiently for the planned activities?				
10. Abnormal motor behaviour	Does the patient repeat nonsense activities, such as turning around a house, playing with buttons, bandaging with a rope, etc.? Or other repetitive activities?				
11. Sleep disorders	Does the patient wake others up at night? Get up early in the morning? Frequent snoring during the day?				
12. Eating disorders	Does the patient's weight decrease or increase? Do you like the taste of food changes?				
13. Total score					

Appendix 5 Hamilton Anxiety scale (HAMA) [42]

	Asymptomatic 0	Minor 1	Moderate 2	Heavier 3	Serious 4
1. Anxious mood	☐0	☐1	☐2	☐3	☐4
2. nervous	☐0	☐1	☐2	☐3	☐4
3. afraid	☐0	☐1	☐2	☐3	☐4
4. insomnia	☐0	☐1	☐2	☐3	☐4
5. memory, attention disorder	☐0	☐1	☐2	☐3	☐4
6. depression mood	☐0	☐1	☐2	☐3	☐4
7. somatic anxiety: the muscular system	☐0	☐1	☐2	☐3	☐4
8. somatic anxiety: sensory system	☐0	☐1	☐2	☐3	☐4
9. cardiovascular system symptoms	☐0	☐1	☐2	☐3	☐4
10. respiratory symptoms	☐0	☐1	☐2	☐3	☐4
11. gastrointestinal symptoms	☐0	☐1	☐2	☐3	☐4
12. reproductive system symptoms	☐0	☐1	☐2	☐3	☐4
13. autonomic nervous system symptoms	☐0	☐1	☐2	☐3	☐4
14. performance at the time of the talks	☐0	☐1	☐2	☐3	☐4
15. Total points	Score				

Appendix 6 Hamilton depression scale (HAMD) [45]

Item	0	1	2	3	4	score
1. Depressed Mood	No	Only tell when asked	Spontaneously expressed in conversation	The patient express this emotion from expressions, gestures, sounds or crying without words.	The patient's speech and non-verbal expressions (expressions, movements) are almost completely expressed as such emotions.	
2. Guilt	No	Blame yourself and feel that you have been hurting others	Think that you have committed a crime, or think twice about past mistakes and mistakes	Think that the current illness is a punishment for yourself, or that there is a crime of sin	Evil wants to be accused or threatening	
3. Suicide	No	He feels that living is meaningless.	Wishes he were dead, Often think of things related to death	Negative concept(Suicidal ideas)	Serious suicidal behavior	
4. Difficulty in falling asleep	No	Sometimes it is difficult to fall asleep, and still can't sleep for half an hour after going to bed.	Difficulty in falling asleep every night	/	/	
5. Sleep is not deep	No	Shallow sleep, nightmare	Woke up in the middle of the night (before 12 o'clock) (not including the toilet)	/	/	
6. Wake up early	No	Wake up early, wake up 1 hour earlier than usual, but can fall asleep again.	Can't fall asleep after waking up early.	/	/	
7. Work and Interests	Normal	Only tell when asked	Spontaneously, directly or indirectly, lose interest in activities, work, or learning, such as being slouched, hesitant, unable to persist or force himself to work or activity	Reduced activity time or reduced effectiveness, inpatients who participate in ward labor or entertainment for less than 3 hours a day.	Stop working because of the current illness, the residents can not complete the daily affairs of the ward without participating in any activities or without the help of others.	
8. Retardation: Slowness of thought, speech, and activity Apathy, Stupor	No	Slight retardation at interview	Obvious retardation at interview	Interview difficult	Complete stupor	
9. Agitation	No	feel a little uneasy when checking	Obviously uneasy or small movements	Can't sit still, stand up in the inspection	Pick up hands, bite fingers, pull hair, bite lips	
10. Mental anxiety	No	Only tell when asked	Spontaneously expressed in conversation	Expressions and words reveal obvious worries	Fears	

11. Somatic anxiety: dry mouth, bloating, diarrhea, snoring, abdominal cramps, palpitations, headaches, excessive ventilation and sighs, as well as frequent urination and sweating.	No	Mild	Moderate, with a positive appeal symptom	Severe, the above symptoms are serious, affecting life, need to be treated	Seriously affecting life and activities	⌊___⌋
12. Gastrointestinal symptoms	Normal	Loss of appetite, but eat without the encouragement of others	Eating requires someone to urge or request , or need to apply laxatives or digestives	/	/	⌊___⌋
13. Systemic symptoms	Normal	Four limbs, heavy back and neck, back pain, headache, muscle pain, general weakness	Significant symptoms	/	/	⌊___⌋
14. Sexual symptoms: loss of libido, menstrual disorders, etc.	Normal	Mild	Severe	Not sure or not suitable for the test, not included in the total score		⌊___⌋
15. Hypochondriasis	No	Self-absorption (bodily)	Preoccupation with health	Querulous attitude	Hypochondriacal delusions	⌊___⌋
16. Loss of Weight	No	Weight loss of 1 kg or more in a week	Weight loss of more than 2 kg in a week			⌊___⌋
17. Self-awareness	Yes	I know that I am sick, but I am poor at food, environment, work, etc.	Completely deny that there is a disease			⌊___⌋
18 Total score	(Note: scores ⩽ 17 can be selected)					⌊___⌋

Appendix 7 modified Hachinski ischemic scale(m-HIS) [50]

	Feature	Yes	No
1	Abrupt onset	□2	□0
2	Stepwise deterioration	□1	□0
3	Somatic complaint	□1	□0
4	Emotional incontinence	□1	□0
5	History or presence of hypertension	□1	□0
6	History of strokes	□2	□0
7	Focal neurological symptoms	□2	□0
8	Focal neurological signs	□2	□0
9	Total (attention：score ≥4 can be chosen)	|____|.|____|	

Appendix 8 WHO-UCLA Verbal Learning Test [51]

Administration: I will give you some words of the object below. After I finish reading, please tell me the words you remember. You can say what you remember, not in order. Then I will give you a few more times. Please tell me every word you remember, including the words you have said before, and see how much you can remember at the end, ok?

The examiner clearly reads out at the speed of 1 second per word, records with Arabic numerals 1, 2, 3, etc., and records the number of insertions and the number of repetitions. Insertion of the same item twice or more is counted as one insertion, and each repetition is counted as one repetition, but the repetition of the inserted item is not counted as a repetition. Recall time every 2 minutes.

If the test has been carried out in the past 3 months, the date of the record check (year/month/day) / / 0000/00/00 = not determined

project	1.1 first pass	2.1 second pass	3.1 third pass
arm			
cat			
ax			
bed			
aircraft			
ear			
dog			
hammer			
chair			
car			
eye			
horse			
knife			
bell			
bicycle			

The correct number	1.2	2.2	3.2
Insert word record	1.3	2.3	3.3
Number of insertions	1.4	2.4	3.4
Repeat number	1.5	2.5	3.5
Memory strategy	☐1Yes ☐2No	☐1Yes ☐2No	☐1Yes ☐2No

4 the correct number	3 times correct total recall	(Abnormal reference value≤18)
5 insert number	3 times the total number of inserts	
6 repeat number	Repeated recalls in 3 times	
7 Memory Strategy (According to object classification memory)	☐1Yes	☐2No

Delayed Recall

Administration: "Remember the words I have just read to you several times? Please remember as much as possible" (2 minutes of recall time. Record with 1, 2, 3 and other Arabic numerals, and record the number of insertions And the number of repetitions. The insertion of the same item multiple times is counted as one insertion, and each repetition is counted as one repetition, but the repetition of the inserted item is not counted as a repetition.)".

7.2	Correct number of recalls (reference value ≤ 6)	Correct number of recalls (reference value ≤ 6)
7.3	Insert word record	
7.4	Number of insertions	Number of insertions

7.5	Repeat number	Repeat number
7.6	Memory strategy (memory according to object classification) ☐1 Yes ☐0 No	

Cued Recall

Administration: The 15 words you have learned before can be divided into 5 categories. I will give you some tips below. Please continue to try to recall the words you have memorized. Record the picture "√".

Item category	8.1Record		
Body class	☐ arm	☐ ears	☐ eyes
Animal	☐ cat	☐ dog	☐ horse
Tools	☐ Axe	☐ hammer	☐knife
Furniture	☐ bed	☐ chair	☐ clock
Vehicle type	☐Airplane	☐ car	☐Bicycle

8.2	Measurable number	

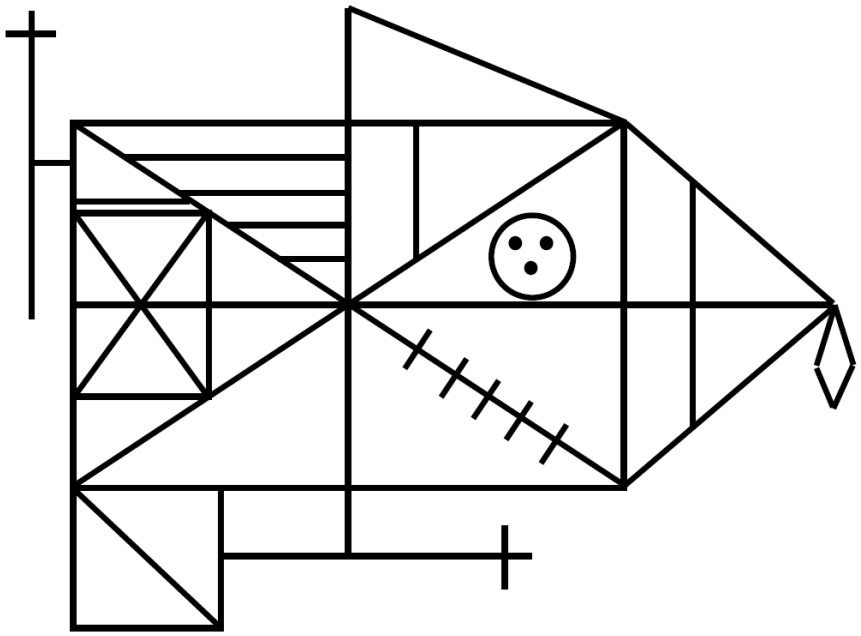
Recognition

Administration: I will read 30 words below, among which I have just let you remember, some are added, please tell me which ones I let you remember and which ones are added. (Non-shadows are remembered - target words; shadows are interference items). For all words, just answer "yes" in the cell that is "yes" at the back, as long as the answer to "no" is snoring in the cell "No".

	Yes	No		Yes	No
mirror			lips		
hammer			tree		
knife			arm		
candle			nose		
motorcycle			sun		
ax			truck		
bell			eye		
chair			fish		
aircraft			ear		
tortoise			bicycle		
horse			snake		
thigh			Bench		
dog			bus		
table			bed		
Cat			car		

9.1	The number of non-shadowed "yes"	
9.2	The number of "yes" in the shadow	
9.3	9.1 minus 9.2	

Appendix 9 Rey-Osterrieth Complex Figure Test [52]



Administration: Please draw an identical picture according to the picture.

Scoring: Consider each of the 18 units separately (the highest score is 36 points). Appraise accuracy of each unit and relative position within the whole of the design. For each unit count as follows:

Correct	Placed properly	2 points
	Placed poorly	1 points
Distorted or incomplete but recognizable	Placed properly	1 points
	Placed poorly	0.5 points
Absent or not recognizable		0 points
1.	Cross upper left corner, outside of rectangle	☐2 ☐1 ☐0.5 ☐0
2.	Large rectangle	☐2 ☐1 ☐0.5 ☐0
3.	Diagonal cross	☐2 ☐1 ☐0.5 ☐0
4.	Horizontal midline of (2)	☐2 ☐1 ☐0.5 ☐0
5.	Vertical midline	☐2 ☐1 ☐0.5 ☐0

6.	Small rectangle, within (2) to the left	☐2 ☐1 ☐0.5 ☐0
7.	Small segment above (6)	☐2 ☐1 ☐0.5 ☐0
8.	Four parallel lines within (2), upper left	☐2 ☐1 ☐0.5 ☐0
9.	Triangle above (2), upper right	☐2 ☐1 ☐0.5 ☐0
10.	Small vertical line within (2), below (9)	☐2 ☐1 ☐0.5 ☐0
11.	Circle with three dots, within (2)	☐2 ☐1 ☐0.5 ☐0
12.	Five parallel lines within (2) and crossing (3), lower right	☐2 ☐1 ☐0.5 ☐0
13.	Sides of triangle attached to (2) on right	☐2 ☐1 ☐0.5 ☐0
14.	Diamond attached to (13)	☐2 ☐1 ☐0.5 ☐0
15.	Vertical line within triangle (13), parallel to the right side of (2)	☐2 ☐1 ☐0.5 ☐0
16.	Horizontal line within (13), continuing (4) to the right	☐2 ☐1 ☐0.5 ☐0
17.	Cross attached to (5), below (2)	☐2 ☐1 ☐0.5 ☐0
18.	Square attached to (2), lower left	☐2 ☐1 ☐0.5 ☐0
19. Total Score☐ ☐		

Recall

Administration: Do you remember the picture I asked you to draw? Please remember and draw the picture as much as possible. If you can only recall some of the graphics, but can't determine their location, feel free to draw.

Correct	Placed properly	2 points
	Placed poorly	1 points
Distorted or incomplete but recognizable	Placed properly	1 points
	Placed poorly	0.5 points
Absent or not recognizable		0 points
1.	Cross upper left corner, outside of rectangle	☐2 ☐1 ☐0.5 ☐0
2.	Large rectangle	☐2 ☐1 ☐0.5 ☐0
3.	Diagonal cross	☐2 ☐1 ☐0.5 ☐0
4.	Horizontal midline of (2)	☐2 ☐1 ☐0.5 ☐0
5.	Vertical midline	☐2 ☐1 ☐0.5 ☐0
6.	Small rectangle, within (2) to the left	☐2 ☐1 ☐0.5 ☐0
7.	Small segment above (6)	☐2 ☐1 ☐0.5 ☐0
8.	Four parallel lines within (2), upper left	☐2 ☐1 ☐0.5 ☐0
9.	Triangle above (2), upper right	☐2 ☐1 ☐0.5 ☐0
10.	Small vertical line within (2), below (9)	☐2 ☐1 ☐0.5 ☐0
11.	Circle with three dots, within (2)	☐2 ☐1 ☐0.5 ☐0
12.	Five parallel lines within (2) and crossing (3), lower right	☐2 ☐1 ☐0.5 ☐0
13.	Sides of triangle attached to (2) on right	☐2 ☐1 ☐0.5 ☐0

14.	Diamond attached to (13)	☐2 ☐1 ☐0.5 ☐0
15.	Vertical line within triangle (13), parallel to the right side of (2)	☐2 ☐1 ☐0.5 ☐0
16.	Horizontal line within (13), continuing (4) to the right	☐2 ☐1 ☐0.5 ☐0
17.	Cross attached to (5), below (2)	☐2 ☐1 ☐0.5 ☐0
18.	Square attached to (2), lower left	☐2 ☐1 ☐0.5 ☐0
19. Total Score☐ ☐		

Appendix 10 Digit Span Test (DST) [55]

Digit Span Forward

Administration: "Now we have to do a simple test, please pay attention to listen to me to read some numbers, I repeat it after I finish reading." Read a string of numbers at a rate of one digit per second. Test 1 is done first, and the number string of the same serial number in test 2 is repeated.

Scoring method: When both tests of the same length fail, and the test completes the highest score, the test ends. The highest score for success before the end of the record is the length of the digital breadth (any test in both tests of the same item is correct).

	Test 1		Test 2
3	5-8-2	3	6-9-4
4	6-4-3-9	4	7-2-8-6
5	4-2-7-3-1	5	7-5-8-3-6
6	6-1-9-4-7-3	6	3-9-2-4-8-7
7	5-9-1-7-4-2-8	7	4-1-7-9-3-8-6
8	5-8-1-9-2-6-4-7	8	3-8-2-9-5-1-7-4
9	2-7-5-8-6-2-5-8-4	9	7-1-3-9-4-2-5-6-8
10	5-2-7-4-9-1-3-7-4-6	10	4-7-2-5-9-1-6-2-5-3

Digit Span Forward length_____

Digit Span Backward

Administration: "I will say some numbers below, please listen carefully. After I finish reading, you repeat it again. I said 7-1-9, what should you say?" If the answer is correct, he said: "Here. and also". So from the second 3 digits of the test 1, if the answer is wrong or do not understand the practice, tell the correct answer should be 9-1-7, and another example, said: "Remember, to back." Another example is 3-4-8. If this success, it starts with 3 digits. If it fails again, it will return to the 2-digit test 1; if the subject passes the example, but the 3 digits are tested twice If it fails, it will return to 2 digits and the test will end.

Scoring method: When both tests fail, and the test completes the highest score, the test ends. The highest score for success is the length of the inverse digital breadth (any test in both tests of the same item can be successful).

	Test 1		Test 2
2	2-4	2	5-8
3	6-2-9	3	4-1-5
4	3-2-7-9	4	4-9-6-8
5	1-5-2-8-6	5	6-1-8-4-3
6	5-3-9-4-1-8	6	7-2-4-8-5-6
7	8-1-2-9-3-6-5	7	4-7-3-9-1-2-8
8	9-4-3-7-6-2-5-8	8	7-2-8-1-9-6-5-3

Digit Span Backward length_____

Appendix 11 Verbal Fluency Test (VFT) [57]

Animal Fluency

Administration: "Please tell as many animals as you know as soon as possible. The time is 1 minute. Are you ready? Start." Stop after one minute.

Scoring method: Record the name of the animal that was spoken within one minute of the subject. Mythical animals such as dragons, phoenixes, and unicorns are also correct. The names spoken by the subjects can be grouped by animal species (reference: mammals, birds, fish, insects, etc.), in tandem to the names of the animals listed in each subcategory, from the second of each subcategory. The animals start counting until they switch to another sub-category, and the sub-categories of each sub-category add the total number of series in this test. The number of conversions, that is, the number of times to switch between groups. Please note that duplicate, incorrect names, nicknames are not counted in the correct number, but are counted in the concatenation and conversion numbers.

1. animal_____
2. the correct number _ _
3. the number of repetitions _ _
4. the number of errors _ _

Vegetable fluency

Administration: "Please tell as many fruits as you can, as soon as possible. The time is 1 minute. Are you ready? Start." Stop after one minute.

Grading method: Record the name of the fruit spoken by the subject within one minute. The recording method is the same as above.

1. goods_____
2. the correct number _ _
3. the number of repetitions _ _
4. the number of errors _ _

Category Switching

Administration: "Please tell as many animals and fruits as you can as fast as possible. The time is 1 minute. Are you ready? Start." Stop after one minute.

Grading method: Record the number of item names spoken by the subject within one minute. The recording method is the same as above. Tandem is the name of the item listed consecutively in each sub-category, counting from the second item in each sub-category until it is converted to another sub-category, and the sub-series of each sub-category is added to obtain the total number of series in this test. . The number of conversions, that is, the number of times to switch between groups. Please note that duplicate, incorrect names, nicknames are not counted in the correct number, but are counted in the concatenation and conversion numbers.

1. article_____
2. the correct number _ _
3. the number of repetitions _ _
4. the number of errors _ _
5. the total number of series _ _
6. the number of conversions _ _

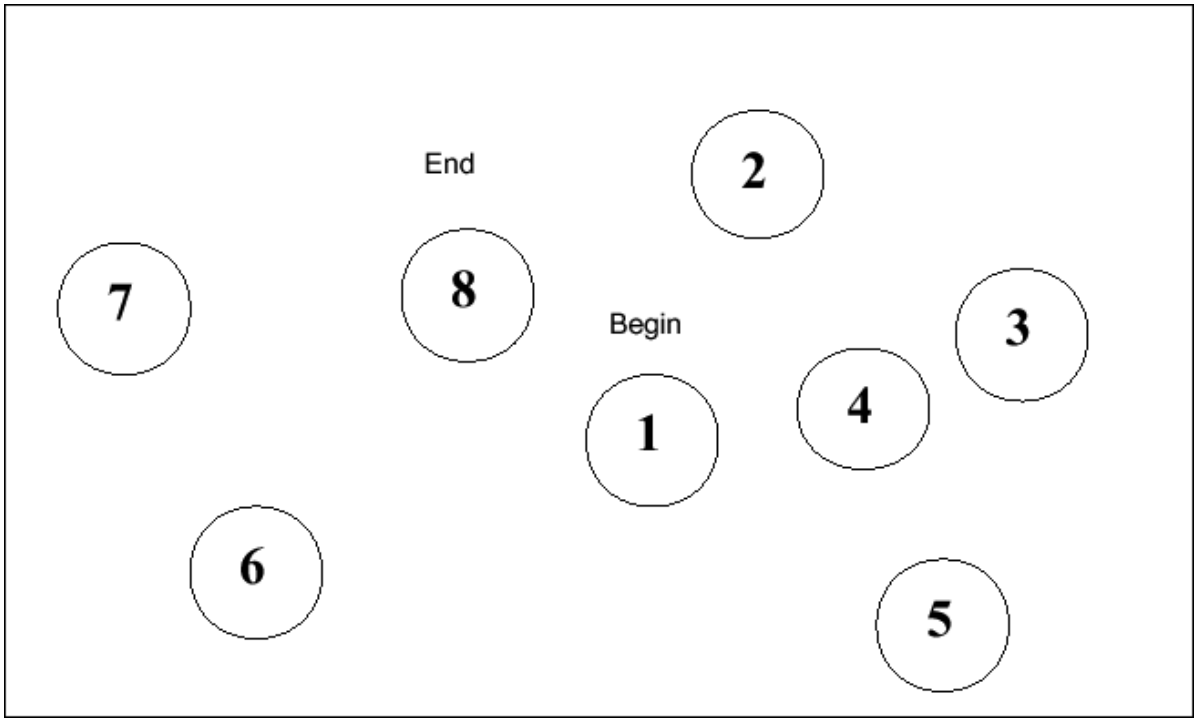
Appendix 12 Trail Making Test (TMT) [58]

Part A

Practice test

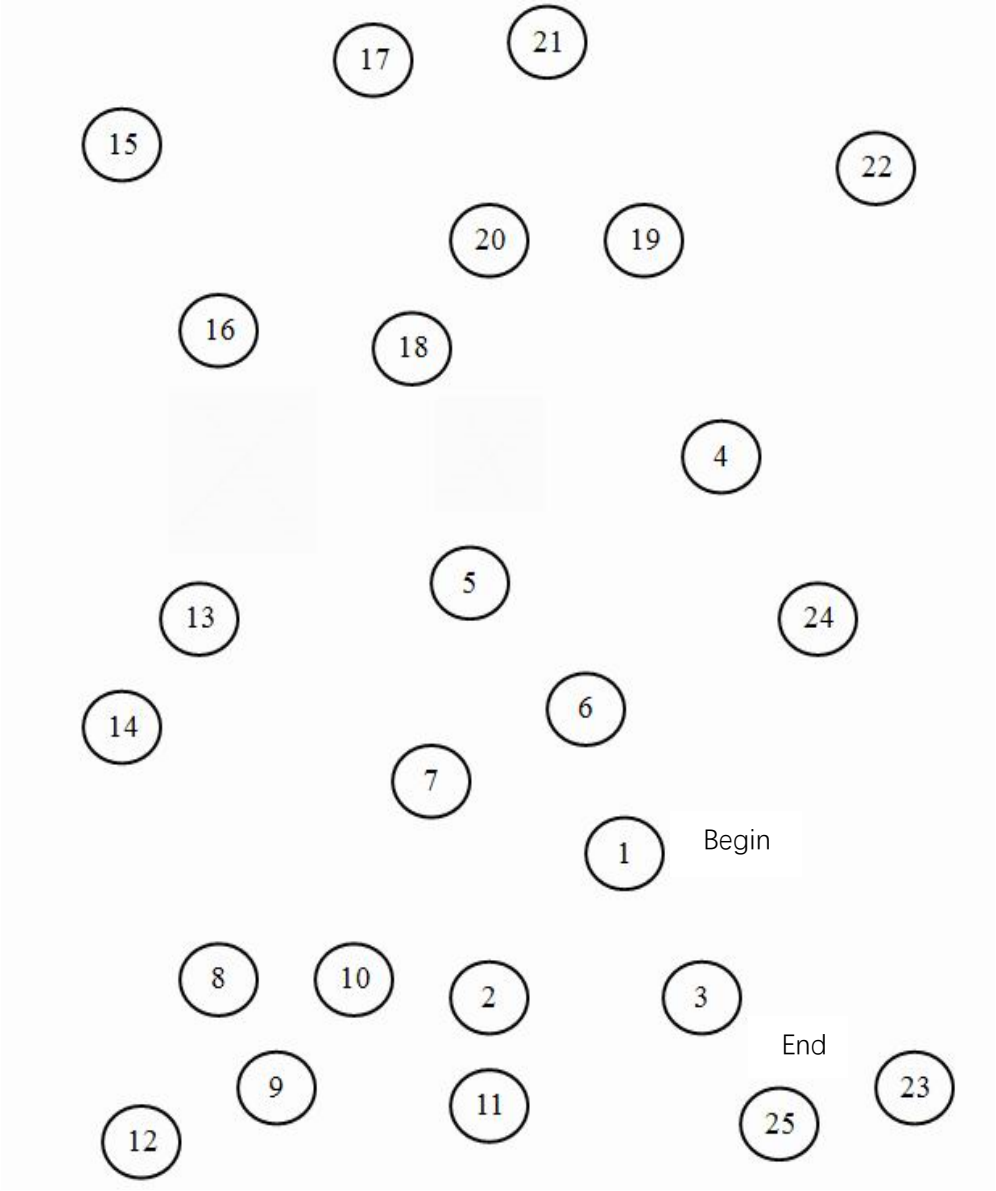
Administration: The following are some circles with numbers in the circle. I would like to ask you to connect in the order of 1-2-3... with a pen. Note: Do not leave the pen tip away from the paper and the line drawn must pass through the graphic. If I am wrong, I will point out that you need to go back and paint again. Let us give it a try now. The sooner you paint, the better. Are you ready? Start!

Note: The following is a practice test, not scored. Correct every time you make a mistake, and tell the correct connection method, while actively encouraging.



Actual test

Administration: There is a similar circle diagram here, please connect it again as above. Please note that the faster you draw, the better. Do not leave the pen tip and the lines drawn must pass through the graphic. Start here (pointing to the starting point) until here (pointing to the end). Ready? Start. (time)

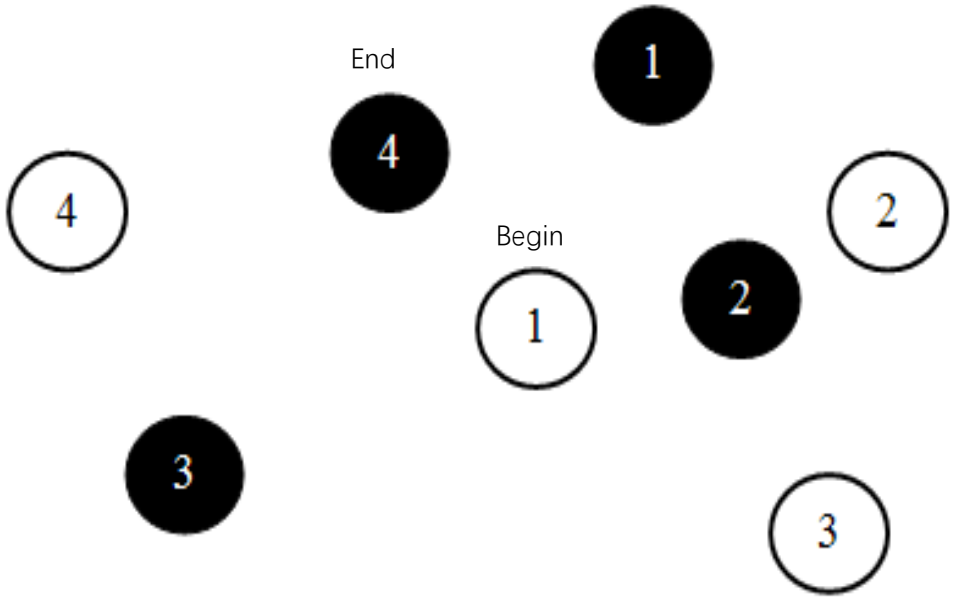


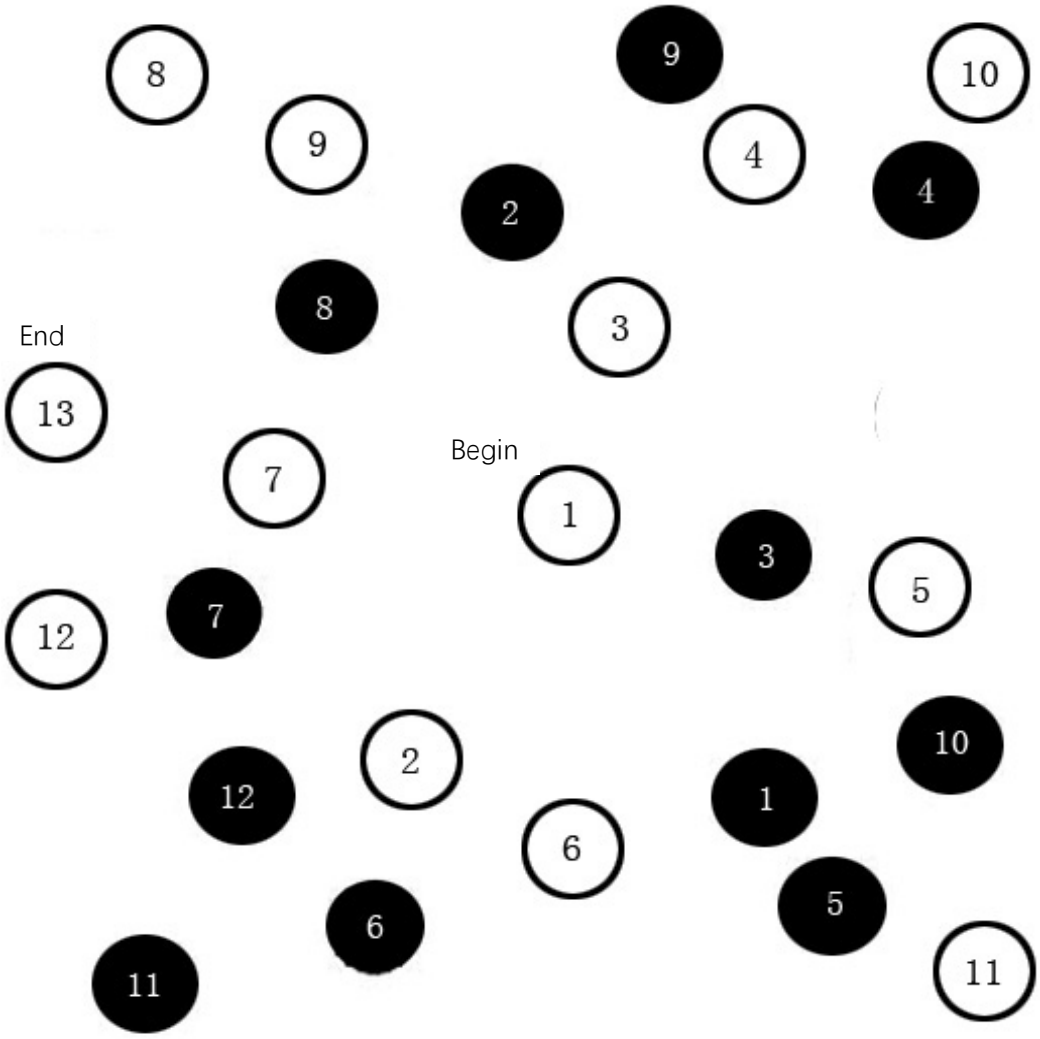
1.1 All digital completion time_____(0-150) seconds (if 150 seconds is not completed, record 150)
1.2 Number of errors_____(0-25; 88=Not applicable)
1.3 Correct number_____(0-25; 88=Not applicable)

Part B

Administration: The following are 1-13 white circle numbers and 1-12 black circle numbers. They are also scattered. Please connect them in order, i.e. white circle 1 - black circle 1 - white circle 2 - black circle 2... Note: Do not leave the pen tip away from the paper and the line drawn must pass through the graphic. If I am wrong, I will point out that you need to go back and paint again. Let us give it a try now. The sooner you paint, the better. Are you ready? Start!

Practice test





2.1 All digital completion time_____(0-300) seconds (if 300 seconds is not completed, record 300)
2.2 Number Sequence Error Number_____(0-25; 88=Not applicable)
2.3 Correct number of lines_____(0-24; 88=Not applicable)

Appendix 13 Boston Naming Test (BNT) [59]

Administration: "I will show you some pictures now, please tell me what these pictures are."

If the tester fails to answer the answer, he is prompted: For example, "This is (plant), is there another name?"

If you still can't answer, then three answers are given in order to let the tester choose. Remember to record every answer of the testier, whether correct or not (only calculate the score of spontaneous naming: correct = 1, answer = 0).

	Picture	Answer (yes = 1; wrong = 0)	If wrong, specific record	If the answer is wrong, prompt	Answer (yes = 1; wrong = 0)	If wrong, specific record	If the prompt still answers the error, please select (circle the specific choice)		
1	2. Tree			plant			Peach blossom	Tree	Fireworks
2	3. Pen			Used for writing			straw	Original ball pen	pen
3	6. Scissors			tool			Scissors	clamp	Spoon
4	8. Flowers			plant			Vegetables	straw hat	flower
5	9. Saw			Woodworking equipment			Machine gun	Saw	Knife
6	12. Broom			Used for cleaning			Mop	Writing brush	Broom
7	*14. Mushroom			food			Mushroom	Cauliflower	umbrella
8	15. Hangers			Inside the wardrobe			roof	hanger	hook
9	16. Wheelchair			Patient use			Executive chair	trolley	wheelchair
10	17. Camel			animal			camel	mountain	Cattle
11	21 racquet			Sporting goods			Ping pong paddle	racquet	mirror
12	22. Snail			animal			snails	Squid	scallop
13	24. Seahorse			Marine life			hook	abalone	Seahorse
14	25. Darts			Used to throw			Target	Darts	rocket
15	30. Harmonica			Musical instrument			Harmonica	Air conditioner	box
16	31. Rhinoceros			animal			Canned knife	hippo	rhinoceros
17	33. Igloo			Used to live			Ice house	Grass house	grave
18	36. Cactus			plant			Iron tree	cactus	cross
19	37 escalator			Use up and down			Sliced bread	Slide	escalator
20	38. Harp			Musical instrument			harp	floor lamp	piano
21	42. Stethoscope			Doctor			Earphone	Stethoscope	sphygmomanometer
22	43. Pyramid			In Egypt			Zongzi	Sphinx	pyramid
23	46. Funnel			Pour water			Funnel	Ice cream cones	Pump
24	47. Accordion			Musical instrument			Harmonica	accordion	Blinds
25	50. Compass			painting			Cotton pliers	ruler	Compasses
26	52. Tripod			For photography			tripod	Mechanical arm	easel
27	54. tongs			Appliance			Spatula	tongs	canoe
28	57. Flower shed/ trellis			Park has			Flower shed/ trellis	trash can	network
29	59. Protractor			stationery			Pound scale	Triangle ruler	Protractor
30	60. Abacus			Counting			Calculator	Abacus	Curtain
Total	31			32			33		

Appendix 14 Activities of daily living (ADL) [60]

Ask the insider to rate the following functions of the patient based on the information provided (assessed according to the level of intelligence).

Rating	Instrumental Daily Living Ability Scale (LADH) Scoring Project
1	Ability to use the phone
	☐1、take the initiative to operate the phone - check the number, dial, etc.
	☐2、can dial a few familiar numbers
	☐3、can answer the phone but not dial the phone
	☐4、cannot use the telephone
2	Shopping
	☐1、independently handle all shopping needs
	☐2、a small amount of shopping independently
	☐3、need to accompany on any purchase
	☐4、completely unable to shopping
3	Cooking
	☐1、independently plan, prepare and make the right amount of meal
	☐2、if the raw materials can be prepared to prepare enough meal
	☐3、heating, serving or cooking, or cooking but not keeping the right amount
	☐4、Need to prepare the meal and do it well
4	Hosting housework
	☐1、independently host housework or occasionally need help (such as heavy work needs family help)
	☐2、do daily light physical housework such as washing dishes, making beds
	☐3、do daily light physical housework but cannot maintain an acceptable level of cleanliness
	☐4、all housework needs help
	☐5、do not participate in any housework
5	Washing
	☐1、can complete a personal bath
	☐2、wash small pieces of clothing - wash socks, etc.

	□3、all washing must be done by others
6	Transportation
	□1、independent public transport or driving a car
	□2、travel by taxi, but no bus
	□3、take the bus when accompanied by others
	□4、can only travel by taxi or car with the help of others
	□5、no travel at all
7	Responsibility for the medical care
	□1、can take medicine seriously according to the correct time and dose
	□2、if you prepare for each dose, you can take medicine
	□3、you can't prepare your own medicine
8	Financial ability
	□1、independently handle finance (budget, write checks, pay rent, bills, go to the bank), collect and maintain income channels
	□2、manage daily shopping, but need help in banking affairs and big shopping, etc.
	□3、can't handle finance

9 IADL total score

Rating	Somatic Self-care Ability Scale (PSUS) Scoring Project
10	Bowel and bladder function at the toilet,
	□1、in the bathroom can be completely self-care, no incontinence
	□2、need to be reminded of self-cleaning, or need help, or a small number of accidents (at least once a week)
	□3、dirty or wet during sleep, more than once a week
	□4、dirty or wet when awake, more than once a week
	□5、incontinence
11	Eating
	□1、no need to help when eating
	□2、eating a small amount of time to eat and/or need to prepare special food, or need help after cleaning after meals
	□3、need moderate help for meal, and it is not neat

	☐4、all meals need a lot of help
	☐5、can't eat at all, and resist feeding by others
12	Dressing
	☐1、wear clothes, undress, and choose clothes from your wardrobe
	☐2、wearing clothes and undressing, need a small amount of help
	☐3、need moderate help in dressing and choosing clothes
	☐4、need more help in wearing clothes, but with the help of others
	☐5、can't wear clothes at all, and it is against the help of others
13	Carding (tidy, hair, nails, hands, face, clothes)
	☐1、always dressed neatly, properly decorated, no need to help
	☐2、can properly decorate themselves, occasionally need a small amount of help, such as repairing beard
	☐3、need moderate or reasonable help or guidance in combing
	☐4、all combing matters need help, but can be kept neat after others help
	☐5、actively oppose the efforts of others to help sort out
14	Walking
	☐1、walk to the venue or urban area
	☐2、Walk in the residential area or walk near a street
	☐3、walking needs help (choose 1) a() cane, b() walker, c() wheelchair
	☐1-In and out without help
	☐2-In and out need help
	☐4、don’t need to sit in a chair or a wheelchair, but you can’t push it yourself without help
	☐5、more than half of the time bedridden
15	Bathing
	☐1、take a bath (bath, shower, bath), no need to help
	☐2、need help when getting in and out of the tub
	☐3、only wash your face and hands, cannot wash other parts of the body

	□4、cannot take a bath, but with others to bathe him
	□5、cannot take a bath, and resist efforts to keep him clean

16 BADL total score_____

17 ADL total score (IADL+BADL)_____

The total score below 16 is completely normal, and greater than 16 points have different degrees of functional decline, up to 64 points. The single item is divided into 1 normal, 2 to 4 is divided into functional decline, where there are 2 or more than ≥ 3 points, or the total score is ≥ 22 points, there are obvious obstacles to function.

Appendix 15 Clinical Dementia Rating (CDR) [61]

This is a semi-structured interview. Please ask all of these questions. Ask any additional questions necessary to determine the subject's CDR. Please note information from the additional questions.

Memory Questions for Informant:

1. Does he/she have a problem with his/her memory or thinking?

☐Yes ☐No

1a. If yes, is this a consistent problem (as opposed to inconsistent)?

☐Yes ☐No

2. Can he/she recall recent events?

☐Yes ☐No

3. Can he/she remember a short list of items (shopping)?

☐Usually ☐Sometimes ☐Rarely

4. Has there been some decline in memory during the past year?

☐Usually ☐Sometimes ☐Rarely

5. Is his/her memory impaired to such a degree that it would have interfered with his/her activities of daily life a few years ago (or pre-retirement activities)? (collateral sources opinion)

☐Yes ☐No

6. Does he/she completely forget a major event (e.g., trip, party, family wedding) within a few weeks of the event?

☐Usually ☐Sometimes ☐Rarely

7. Does he/she forget pertinent details of the major event?

☐Usuallv ☐Sometimes ☐Rarelv

8. Does he/she completely forget important information of the distant past (e.g., birthdate, wedding date, and place of employment)?

☐Usually ☐Sometimes ☐Rarely

9. Tell me about some recent event in his/her life that he/she should remember. (For later testing, obtain details such as location of the event, time of day, participants, how long the event was, when it ended and how the subject or other participants got there).

Within 1 week: _____

_____

_____

Within 1 month: _____

_____

_____

10. When was he/she born? _____

11. Where was he/she born? _____

12. What was the last school he/she attended?

Name _____

Place _____

Grade _____

13. What was his/her main occupation/job (or spouse's job if subject was not employed)? _____

_____

14. What was his/her last major job (or spouse's job if subject was not employed)?

15. When did he/she (or spouse) retire and why? _____

Orientation Questions for Informant:

How often does he/she know of the exact:

1. Date of the Month?

☐Usually ☐Sometimes ☐Rarely ☐Don't know

2. Month?

☐Usually ☐Sometimes ☐Rarely ☐Don't know

3. Year?

☐Usually ☐Sometimes ☐Rarely ☐Don't know

4. Day of the Week?

☐Usually ☐Sometimes ☐Rarely ☐Don't know

5. Does he/she have difficulty with time relationships (when events happened in relation to each other)?

☐Usually ☐Sometimes ☐Rarely ☐Don't know

6. Can he/she find his/her way about familiar streets?

☐Usually ☐Sometimes ☐Rarely ☐Don't know

7. How often does he/she know how to get from one place to another outside his/her neighborhood?

☐Usually ☐Sometimes ☐Rarely ☐Don't know

8. How often can he/she find his/her way about indoors?

☐Usually ☐Sometimes ☐Rarely ☐Don't know

Judgment and Problem Solving Questions for Informant:

1. In general, if you had to rate his/her abilities to solve problems at the present time, would you consider them: As good as they have ever been

☐Good, but not as good as before

☐Fair

☐Poor

☐No ability at all

2. Rate his/her ability to cope with small sums of money (e.g., make change, leave a small tip):

☐No loss

☐Some loss

☐Severe loss

3. Rate his/her ability to handle complicated financial or business transactions (e.g., balance check-book, pay bills):

☐No loss

☐Some loss

☐Severe loss

4. Can he/she handle a household emergency (e.g., plumbing leak, small fire)?

☐As well as before

☐Worse than before because of trouble thinking

☐Worse than before, another reason (why) _____

_____

_____

5. Can he/she understand situations or explanations?

☐Usually ☐Sometimes ☐Rarely ☐ Don't know

6. Does he/she behave* appropriately [i.e., in his/her usual (premorbid) manner] in social situations and interactions with other people?

☐Usually ☐Sometimes ☐Rarely ☐Don't know

*This item rates behavior, not appearance.

Community Affairs Questions for Informant: Occupational

1. Is the subject still working? ☐Yes ☐No ☐N/A

If not applicable, proceed to item 4
If yes, proceed to item 3
If no, proceed to item 2

2. Did memory or thinking problems contribute to the subject's decision to retire? (Question 4 is next) ☐Yes ☐No ☐D/K

3. Does the subject have significant difficulty in his/her job because of problems with memory or thinking?

☐Rarely or Never ☐Sometimes ☐Usually ☐Don't know

Social

4. Did he/she ever drive a car? ☐Yes ☐No

5. Does the subject drive a car now? ☐Yes ☐No

6. If no, is this because of memory or thinking problems? ☐Yes ☐No

7. If he/she is still driving, are there problems or risks because of poor thinking? ☐Yes ☐No

8. Is he/she able to independently shop for needs?

☐Rarely or Never	☐Sometimes	☐Usually	☐Don't know
(Needs to be accompanied on any shopping trip)	(Shops for limited number of items; buys duplicate items or forgets needed items)		

9. Is he/she able to independently carry out activities outside the home?

☐Rarely or Never	☐Sometimes	☐Usually	☐Don't know
(Generally unable to perform activities without help)	(Limited and/or routine, e.g., superficial participation in church or meetings; trips to beauty parlor)	(Meaningful participation in activities, e.g., voting)	

10.Is he/she taken to social functions outside a family home? ☐Yes ☐No

If no, why not? _____

11. Would a casual observer of the subject's behavior think the subject was ill? ☐Yes ☐No

12. If in nursing home, does he/she participate well in social functions (thinking)? ☐Yes ☐No

IMPORTANT:

Is there enough information available to rate the subject's level of impairment in community affairs?

☐Yes ☐No

If not, please probe further.

Community Affairs: Such as going to church, visiting with friends or family, political activities, professional organizations such as bar association, other professional groups, social clubs, service organizations, educational programs.

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*Please add notes if needed to clarify subject's level of functioning in this area.

Home and Hobbies Questions for Informant:

1a. What changes have occurred in his/her abilities to perform household chores? _____

_____

1b. What can he/she still do well? _____

_____

2a. What changes have occurred in his/her abilities to perform hobbies? _____

_____

2b. What can he/she still do well? _____

_____

3. If in nursing home, what can he/she no longer do well (H and H)? _____

_____

4. Everyday Activities (Blessed):

	☐No loss	☐severe	☐loss
• Ability to perform household tasks	0	0.5	1

Please describe: _____

_____

5 Is he/she able to perform household chores at the level of: (Pick one Informant does not need to be asked directly).

☐No meaningful function.

(Performs simple activities, such as making a bed, only with much supervision)

☐Functions in limited activities only.

(With some supervision, washes dishes with acceptable cleanliness; sets table)

☐Functions independently in some activities.

(Operates appliances, such as a vacuum cleaner; prepares simple meals)

☐Functions in usual activities but not at usual level.

☐Normal function in usual activities.

IMPORTANT:

Is there enough information available to rate the subject's level of impairment in HOME & HOBBIES?

☐Yes ☐No

If not, please probe further.

Homemaking Tasks: Such as cooking, laundry, cleaning, grocery shopping, taking out garbage, yard work, simple care maintenance, and basic home repair.

Hobbies: Sewing, painting, handicrafts, reading, entertaining, photography, gardening, going to theater or symphony, woodworking, participation in sports.

Personal Care Questions for Informant:

*What is your estimate of his/her mental ability in the following areas?

*A box-score of 1 can be considered if the subject's personal care is impaired from a previous level, even if they do not receive prompting.

A. Dressing (Blessed)	Unaided	Occasionally misplaced buttons, etc.	Wrong sequence commonly forgotten items	Unable to dress
	0	1	2	3
B. Washing, grooming	Unaided	Needs Prompting	Sometimes needs help	Always or nearly always needs
	0	1	2	3
C. Eating habits	Cleanly proper utensils	Messily; spoon	Simple solids	Has to be fed completely
	0	1	2	3
D. Sphincter control (Blessed)	Normal complete control	Occasionally wets bed	Frequently wets bed	Doubly incontinent
	0	1	2	3

Memory Questions for Subject:

1. Do you have problems with memory or thinking? ☐ Yes ☐ No

2. A few moments ago your (spouse, etc.) told me a few recent experiences you had. Will you tell me something about those? (Prompt for details, if needed such as location of the event, time of day, participants, how long the event was, when it ended and how the subject or other participants got there).

Within 1 week

_____ 1.0 – Largely correct
_____ 0.5 – Partly correct
_____ 0.0 – Largely incorrect

Within 1 month

_____ 1.0 – Largely correct
_____ 0.5 – Partly correct
_____ 0.0 – Largely incorrect

3. I will give you a name and address to remember for a few minutes. Repeat this name and address after me: (Repeat until the phrase is correctly repeated or to a maximum of three trials).

Elements	1	2	3	4	5
	John	Brown,	42	Market Street,	Chicago
	John	Brown,	42	Market Street,	Chicago
	John	Brown,	42	Market Street,	Chicago

(Underline elements repeated correctly in each trial).

4. When were you born?

_____

5. Where were you born?

_____

6. What was the last school you attended?

Name _____

Place _____

Grade _____

7. What was your main occupation job (or spouse if not employed)? _____

8. What was your last major job (or spouse if not employed)?

_____

9. When did you (or spouse) retire and why?

_____

10. Repeat the name and address I asked you to remember:

Elements	1	2	3	4	5
	John	Brown,	42	Market Street,	Chicago

(Underline elements repeated correctly).

Orientation Questions for Subject:

Record the subject's answer verbatim for each question

1. What is the date today? ☐Correct ☐Incorrect

_____

2. What day of the week is it? ☐Correct ☐Incorrect

_____

3. What is the month? ☐Correct ☐Incorrect

_____

4. What is the year? ☐Correct ☐Incorrect

_____

5. What is the name of this place? ☐Correct ☐Incorrect

_____

6. What town or city are we in? ☐Correct ☐Incorrect

_____

7. What time is it? ☐Correct ☐Incorrect

_____

8. Does the subject know who the informant is (in your judgment)? ☐Correct ☐Incorrect

_____

Judgment and Problem-Solving Questions for Subject:

Instructions: If initial response by subject does not merit a grade 0, press the matter to identify the subject's best understanding of the problem. Circle nearest response.

Similarities:

Example: "How are a pencil and pen alike? (Writing instruments) How are these things alike?" Subject's Response

1. turnip……cauliflower_____

(0 = vegetables)
(1 = edible foods, living things, can be cooked, etc.)
(2 = answers not pertinent; differences; buy them)

2. desk……bookcase

(0 = furniture, office furniture; both hold books)
(1 = wooden, legs)
(2 = not pertinent, differences)

Differences:

Example: "What is the difference between sugar and vinegar? (sweet vs sour) What is the difference between these things?"

3. lie……mistake_____

(0 = one deliberate, one unintentional)
(1 = one bad the other good – or explains only one)
(2 = anything else, similarities)

4. river……canal_____

(0 = natural - artificial)
(2 = anything else)

Calculations:

5. How many nickels in a dollar? ☐Correct ☐Incorrect

6. How many quarters in $6.75? ☐Correct ☐Incorrect

7. Subtract 3 from 20 and keep subtracting 3 from each new number all the way down.

☐Correct ☐Incorrect

Judgment:

8. Upon arriving in a strange city, how would you locate a friend that you wished to see?

(0 = try the telephone book, go to the courthouse for a directory; call a mutual friend)
(1 = call the police, call operator (usually will not give address)
(2 = no clear response)

9. Subject's assessment of disability and station in life and understanding of why he/she is present at the examination (may have covered, but rate here):

☐Good Insight ☐Partial Insight ☐Little Insight

Global CDR

Use all the information you get to make the most appropriate judgment. Evaluate the six functional domains separately, fill in the scores in the corresponding "_", and note that the scores are scored only when the decline in ability is caused by cognitive impairment. If the severity of the dysfunction is between two levels, it is assessed in principle at a severe level. Finally, the scores of the six functional domains are combined and the CDR overall score (global CDR) is summarized according to the following principles:

1. Memory (M) is the main item and the other 5 items are secondary items;

2. When M=0.5, CDR≠0, can only be =0.5 or 1;

3. CDR=M (memory score):

1) When at least 3 secondary items are the same as the memory score;

2) When there are only 1 or 2 minor item scores = M, no more than 2 minor item scores are on either side of M;

3) When the 3 minor items score on one side of the memory score and the other 2 minor item scores on the other side of the memory score;

4) When at least 3 minor items are 0, if M=0.5, then CDR=0.5;

5) When only one minor item is ≥ 0.5, if M = 0, CDR = 0.

4. CDR≠M (memory score)

1) When 3 or more secondary item scores are greater or less than M, CDR = most secondary item scores;

2) When M=0.5, at least 3 minor items are ≥1, CDR=1;

3) When M=0, 2 or more minor items ≥0.5, CDR=0.5;

4) When M ≥ 1, CDR ≠ 0, at this time if most other minor items = 0, CDR = 0.5.

5. The principle of proximity: when the above principles are not met, the score of the closest secondary item of CDR=M (eg: M and the score of a secondary item = 3, the score of 2 secondary items = 2, 2 The score of the secondary item is 1, CDR = 2).

CDR domain total score (sum of boxes)

Refers to the arithmetic sum of the scores of all six functional domains.

CDR supplement

According to the test situation, it is not included in the total score.

	Impairment				
	None **0**	Questionable **0.5**	Mild **1**	Moderate **2**	Severe **3**
Memory	No memory loss or inconsistent forgetfulness	Consistent slight forgetfulness; partial recollection of events; "benign" forgetfulness	Moderate memory loss; more marked for recent events; defect interferes with everyday activities	Severe memory loss; only highly learned material retained; new material rapidly lost	Severe memory only fragments remain
Orientation	Fully oriented	Fully oriented except for slight difficulty with time relationships	Moderate difficulty with time relationships; oriented for place at examination; may have geographic disorientation elsewhere	Severe difficulty with time relationships; usually disoriented to time, often to place	Oriented to person only

Judgment &Problem Solving	Solves everyday problems & handles business & financial affairs well; judgment good in relation to past performance	Slight impairment in solving problems, similarities, and differences	Moderate difficulty in handling problems, similarities, and differences; social judgment usually maintained	Severely impaired in handling problems, similarities, and differences; social judgment usually impaired	Unable to make judgments or solve problems
Community Affairs	Independent function at usual level in job, shopping, volunteer and social groups	Slight impairment in these activities	Unable to function independently at these activities although may still be engaged in some; appears normal to casual inspection	No pretense of independent function outside home Appears well enough to be taken to functions outside a family home	Appears too ill to be taken to functions outside a family home
Home and Hobbies	Life at home, hobbies, and intellectual interests well maintained	Life at home, hobbies, and intellectual interests slightly impaired	Mild but definite impairment of function at home; more difficult chores abandoned; more complicated hobbies and interests abandoned	Only simple chores preserved; very restricted interests, poorly maintained	No significant function in home
Personal Care	Fully capable of self-care		Needs prompting	Requires assistance in dressing, hygiene, keeping of personal effects	Requires much help with personal care; frequent incontinence
_____CDR sum of boxes					
_____global CDR					

Score only as decline from previous usual level due to cognitive loss, not impairment due to other factors.

Appendix 16 Study glossary

Abbreviations	Abbreviated Term
ADL	Activity of daily living
AA	Alzheimer's association
AD	Alzheimer's disease
APP	Amyloid precursor protein gene
APOE	Apolipoprotein E
ADAD	Autosomal dominant Alzheimer’s disease
AAO	Average age at onset
BOLD	Blood oxygen level dependent
BNT	Boston naming test
CRF	Case report form
CSF	Cerebrospinal fluid
CDR	Clinical dementia rating
CCDS	Consensus coding sequence
CFAN	The chinese familial alzheimer’s disease network
COAST	The cognition and aging study
DLB	Dementia with lewy bodies
DSM-IV-R	Diagnostic and Statistical Manual of Mental Disorders-IV-R
DWI	Diffusion weighted imaging
DSB	Digit span backward
DSF	Digit span forward
DST	Digit span test
DIAN	Dominantly inherited alzheimer network
EOAD	Early onset of Alzheimer's disease
EDV	End diastolic velocity
EYO	Estimated years to symptom onset
FAD	Familial Alzheimer's disease
FDG	[18F]Fluorodeoxy glucose
FLAIR	Fluid attenuated inversion recovery
FTD	Frontotemporal dementia
GWAS	Genome-wide association studies
HAMA	Hamilton anxiety scale
HAMD	Hamilton depression scale
IHHT	Intermittent hypoxic-hyperoxic training
IMT	Intima-media thickness
LMMs	Linear mixed models
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MCMC	Markov chain Monte Carlo
MTA	Medial temporal lobe atrophy
MeDi	Mediterranean diet
mRNA	Messenger ribonucleic acid
MCI	Mild cognitive impairment
MMSE	Mini-mental state examination

M-HIS	Modified hachinski ischemic scale
MoCA	Montreal cognitive assessment
NINCDS-ADRDA	National institute of neurological and communicative disorders and stroke-AD and related disorders association criteria
NIA	National institute on aging
NIA-AA	National institute on aging-Alzheimer’s association
NPI-Q	Neuropsychiatric inventory questionaire
NPT	Non-pharmacological treatment
PDD	Parkinson's disease dementia
PSV	Peak systolic velocity
PIB	Pittsburgh compound B
PCR	Polymerase chain reaction
PET	Positron emission tomography
PSEN1	Presenilin 1
PSEN2	Presenilin 2 gene
ROCFT	Rey-Osterrieth complex figure test
SNP	Single nucleotide polymorphism
SAD	Sporadic Alzheimer's disease
SUVR	Standardized uptake value ratio
T1WI	T1weighted image
T2WI	T2weighted image
TMT	Trail making test
WHO-UCLA AVLT	The world health organization university of California-Los Angeles auditory verbal learning test
VaD	Vascular dementia
VFT	Verbal fluency test

Appendix 17 Participating unit and participant number

A total of 8 digits: the number of each unit (3) + the number of the participant (5), with a horizontal bar in the middle. For example, the number of eighth participant in Xuanwu Hospital is 001-00008. The number of each participating unit is shown in the table below.

NO.	Unit name	NO.	Unit name
001	Xuanwu Hospital, Capital Medical University	035	Jiangxi Provincial People's Hospital
002	Beijing Tian Tan Hospital, Capital Medical University	036	Anshanshi Changda the Hospital
003	Beijing Chao-Yang Hospital, Capital Medical University	037	Affiliated Zhongshan Hospital of Dalian University
004	Fu Xing Hospital, Capital Medical University	038	The First Hospital of China medical University
005	Peking Union Medical College Hospital	039	Baotou Central Hospital
006	Peking University First Hospital	040	General Hospital of Ningxia Medical University
007	Peking University Third Hospital	041	People's Hospital of Ningxia Hui Autonomous Region
008	Chinese PLA General Hospital	042	The Affiliated Hospital of Qingdao University
009	China-Japan Friendship Hospital	043	78th Hospital of the People's Liberation Army
010	Beijing Geriatric Hospital	044	Qilu Hospital of Shandong University
011	Dalian Municipal Hospital Affiliated of Dalian Medical University	045	Qilu Hospital of Shandong University (Qingdao)
012	Fujian Medical University Union Hospital	046	Shangdong Provincial Hospital
013	Guangzhou Brain Hospital	047	QingDao Municipal Hospital
014	Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University	048	First Hospital of Shanxi Medical University
015	The first Affiliated Hospital of Guangxi Medical University	049	Tangdu Hospital, Fourth Military Medical University
016	The Affiliated Hospital of Guizhou Medical University	050	The first Affiliated Hospital of Xi' an Jiao Tong University
017	HanDan Central Hospital	051	Rui Jin Hospital Shanghai Jiao Tong University School of Medicine
018	Heibei General Hospital	052	Renji Hospital Shanghai Jiao Tong University School of Medicine
019	Shijiazhuang First Hospital	053	Shanghai Changzheng Hospital
020	Tangshan Workers Hospital	054	Affiliated Hospital of North Sichuan Medical University
021	Hennan Provincial People's Hospital	055	Tianjin Huanhu Hospital
022	KaiFeng Central Hospital	056	General Hospita of Tianjin Medical University
023	People's Hospital of Zhengzhou	057	Xinjiang Autonomous Region Chinese Medicine Hospital
024	The First Affiliated Hospital of Harbin Medical University	058	Ningbo City Medical Treatment Center Lihuili Hospital
025	Tongji Hospital, Huazhong University of Science and Technology	059	The First Affiliated Hospital of Wenzhou Medical University
026	People's Hospital Affiliated Hubei University of Medicine	060	The First Affiliated Hospital, Zhejiang University
027	Zhongnan Hospital of Wuhan University	061	Shao Yifu Hospital, Zhejiang University of Medicine

028	The Third Xiangya Hospital of Central South University	062	Zhejiang Provincial People's Hospital
029	Xiangya Hospital, Central South University	063	Daping Hospital of the Third Military Medical University
030	The First Hospital of Jilin University	064	The Second Affiliated Hospital of Chongqing Medical University
031	China-Japan Friendship Hospital of Jilin University	065	The First Hospital Affiliated Anhui Medical University
032	Subei People's Hospital	066	Chongqing General Hospital
033	Affiliated Hospital of Nantong University	067	Dongfang Hospital, Beijing University of Chinese Medicine
034	Xuzhou Mine General Hospital	068	Zigong First People's Hospital