Stroke incidence and cognitive outcomes of intracranial atherosclerotic stenosis: study protocol for a multicenter, prospective, observational cohort study

Qianqian Si1#, Yuming Teng1, Caiyuan Liu1, Wei Zhuzhuang Yuan1, Xiaoyuan Fan2, Xiaofan Zhang2, Zongmuyu Zhang2, Xingshi Li3, Qing Liu4, Peng Wang4, Zhongrui Yan5, Bo Wu6, Qiang Liu6, Hangjuan Li7, Yan Ji8, Yuncai Ran9, Bo Song8, Shiguang Zhu10, Hongyan Li11, Jingxia Guan12, Manli Zhao13, Yonggang Hao14, Pengfei Wang15, Hong Bian16, Ningwen Wang17, Yulin Wang18, Yuning Pan19, Hongwei An20, Rong Guo21, Cong Han22, Junshi Zhang23, Hebo Wang24, Yong You25, Hongquan Jiang26, Zifan Liu27, Jinghi Liu28, Dingbo Tao29, Xiangyu Piao30, Jianguang Zhang31, Pei Wang32, Shen Yang33, Zhou Liu34, Xiue Wei35, Kai Han36, Zhimin Shi37, Aihua Liu38, Zuwon Zhang39, Chunye Ma40, Baichen Wang41, Gejuan Zhang42, Chengguang Song43, Guilian Zhang44, Xiao Yang45, Bing Chen46, Baoquan Lu47, Beilei Chen48, Meng Zuo49, Kun Han50, Xiaodan Zhang50, Wenfeng Cao51, Lingfeng Wu51, Qi Li52, Xiaokun Geng53, Junshan Zhou54, Mengfei Zhong55, Minghua Wang56, Yangmei Chen57, Jiachun Liu58, Tingrui Wang59, Youqing Deng60, Weihai Xu1

1Department of Neurology, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China; 2Department of Radiology, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China; 3Center for Rehabilitation Medicine, Department of Neurology, Zhejiang Provincial People's Hospital (Affiliated People's Hospital, Hangzhou Medical College), Hangzhou, China; 4Department of Neurology, Jining No. 1 People's Hospital, Jining, China; 5Department of Neurology, West China Hospital, Sichuan University, Chengdu, China; 6Department of Neurology, Tianjin Neurological Institute, Tianjin Medical University General Hospital, Tianjin, China; 7Department of Neurology, 920 Hospital of Joint Service Support Force of the People's Liberation Army, Kunming, China; 8Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; 9Department of Magnetic resonance, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; 10Department of Neurology, Affiliated Hospital of Xuzhou Medical University, Xuzhou, China; 11Department of Neurology, People's Hospital of Xinjiang Uygur Autonomous Region, Xinjiang Clinical Research Center for Stroke and Neurological Rare Disease, Urumqi, China; 12Department of Neurology, Renmin Hospital of Wuhan University, Wuhan, China; 13Department of Encephalopathy, Weifang Traditional Chinese Medicine Hospital, Weifang, China; 14Department of Neurology, Dushi Lake Hospital Affiliated to Soochow University, Suzhou, China; 15Department of Neurology, Weihai Municipal Hospital, Cheeloo College of Medicine, Shandong University, Weihai, China; 16Department of Neurology, Central Hospital Affiliated to Shandong First Medical University, Jining, China; 17Department of Neurology, Binzhou People's Hospital, Binzhou, China; 18Department of Neurology, First Hospital of Qinhua Medical College, Jinan, China; 19Department of Radiology, Ningbo First Hospital, Ningbo Hospital of Zhejiang University, Ningbo, China; 20Department of Neurology, Lanzhou Traditional Chinese Medical Hospital, Lanzhou, China; 21Function Department of Neurology, The People's Hospital of China Medical University, The People's Hospital of Liaoning Province, Shenyang, China; 22Department of Neurosurgery, The Fifth Medical Center, Chinese PLA General Hospital, Beijing, China; 23Department of Neurology, Huaihe Hospital of Henan University, Kaifeng, China; 24Department of Neurology, Hebei General Hospital, Shijiazhuang, China; 25Department of Neurology, The Second Affiliated Hospital of Hainan Medical University, Haikou, China; 26Department of Neurology, The First Affiliated Hospital of Harbin Medical University, Harbin, China; 27Department of Neurology, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China; 28Department of Neurology, The First Affiliated Hospital of Guangxi Medical University, Nanning, China; 29Department of Neurology, The First Affiliated Hospital of Dalian Medical University, Dalian, China; 30Department of Neurology, Shengli Hospital, Dalian University, Dalian, China; 31Department of Neurology, Chengde Central Hospital, Chengde, China; 32The Third Department of Neurology, Baoding First Central Hospital, Baoding, China; 33Department of Neurology, Wuhu No.1 People's Hospital, Wuhu, China; 34Department of Neurology, Affiliated Hospital of Guangdong Medical University, Zhanjiang, China; 35Department of Neurology, The Second Affiliated Hospital of Zhejiang Medical University, Xuzhou, China; 36Department of Neurology, Jiaozuo People's Hospital, Jiaozuo, China; 37Department of Neurology, People's Hospital of Ningxia Hui Autonomous Region, Yinchuan, China; 38Department of Interventional Neuroradiology, Beijing Tiantan Hospital, Capital Medical University, Beijing, China; 39Department of Neurology, Chongqing University Jianguang Hospital, Chongqing, China; 40Department of Neurology, The Second Affiliated Hospital of Dalian Medical University, Dalian, China; 41Department of Neurology, Tongde Hospital of
Zhejiang Province, Hangzhou, China; 11Department of Neurology, The Affiliated Hospital of Northwest University, Xi’an No. 3 Hospital, Xi’an, China; 12Department of Neurology, Liaoning province Benxi Central Hospital, Benxi, China; 13Department of Neurology, The Second Affiliated Hospital of Xi’an Jiaotong University, Xi’an, China; 14Department of Neurology, General Hospital of Ningsia Medical University, Yinchuan, China; 15Department of Radiology, General Hospital of Ningsia Medical University, Yinchuan, China; 16Department of neurology, Tangshan Gongren Hospital, Tangshan, China; 17Department of Neurology, Northern Jiangsu People's Hospital, Jiangsu, China; 18Department of Neurology, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China; 19Department of Neurology, Hwa Mei Hospital, University of Chinese Academy of Sciences, Ningbo Institute of Life and Health Industry, University of Chinese Academy of Sciences, Ningbo, China; 20Department of neurology, People's Hospital of Jiangxi province and The First Affiliated Hospital of Nanchang Medical College, Nanchang, China; 21Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China; 22Department of Neurology, Beijing Luhe Hospital, Capital Medical University, Beijing, China; 23Department of Neurology, Nanjing First Hospital, Nanjing Medical University, Nanjing, China; 24Department of MRI, Shengli Oilfield Central Hospital, Shandong, China; 25Department of Neurology, Daqing People's Hospital, Daqing, China; 26Department of Neurology, Second Affiliated Hospital of Chongqing Medical University, Chongqing, China; 27Department of Neurointervention, Beijing Sanbo Brain Hospital, Capital Medical University, Beijing, China; 28Department of Neurology, Binzhou Central Hospital, Binzhou, China; 29Department of Neurology, the First Hospital of Nanchang, The Third Affiliated Hospital of Nanchang University, Nanchang, China.

Contributions: (I) Conception and design: W Xu, C Liu; (II) Administrative support: W Xu; (III) Provision of study materials or patients: W Xu; (IV) Collection and assembly of data: Q Si, Y Teng, C Liu, W Yuan, X Fan, X Zhang, Z Zhang, M Li, Q Liu, P Wang, Z Yan, B Wu, Q Liu, H Li, Y Ji, Y Ran, S Zhu, H Li, J Guan, M Zhao, Y Hao, P Wang, H Bian, N Wang, Y Wang, Y Pan, H An, R Guo, C Han, J Zhang, H Wang, Y You, H Jiang, Z Liu, J Liu, D Tao, X Piao, J Zhang, P Wang, S Yang, Z Liu, X Wei, K Han, Z Shi, A Liu, Z Zhang, C Ma, B Wang, B Song, G Zhang, C Song, G Zhang, X Yang, B Chen, B Lu, B Chen, M Zuo, K Han, X Zhang, W Cao, L Wu, Q Li, X Geng, J Zhou, M Zhong, M Wang, Y Chen, J Liu, T Wang, Y Deng; (V) Data analysis and interpretation: Q Si, Y Teng, C Liu, W Xu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*These authors contributed equally to this work as co-first authors.

Correspondence to: Weihai Xu, MD, Department of Neurology, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Shuaidyuan1, Dongcheng District, Beijing 100730, China. Email: xuwh@pumch.cn.

**Background:** Intracranial atherosclerotic stenosis (ICAS) is one of the leading causes of stroke worldwide. Current diagnostic evaluations and treatments remain insufficient to assess the vulnerability of intracranial plaques and reduce the recurrence of stroke in symptomatic ICAS. On the other hand, asymptomatic ICAS is associated with an increased risk of cognitive impairment. The pathogenesis of ICAS related cognitive decline is largely unknown. The aim of SICO-ICAS study (stroke incidence and cognitive outcomes of ICAS) is to elucidate the pathophysiology of stroke and cognitive impairment in ICAS population, comprehensively evaluating the complex interactions among life-course exposure, genomic variation, vascular risk factors, cerebrovascular burden and coexisting neurodegeneration.

**Methods:** SICO-ICAS is a multicenter, prospective, observational cohort study. We aim to recruit 3,000 patients with symptomatic or asymptomatic ICAS (>50% or occlusion) who will be followed up for ≥12 months. All participants will undergo pre-designed magnetic resonance imaging packages, blood biomarkers testing, as well as detailed cognitive domains assessment. All participants will undergo clinical visits every 6 months and telephone interviews every 3 months. The primary outcome measurement is ischemic stroke or cognitive impairment within 12 months after enrollment.

**Discussion:** This study will establish a large prospective ICAS cohort, hopefully discover new biomarkers associated with vulnerable intracranial plaques, identify subjects at high risk for incident ischemic stroke or cognitive impairment, and eventually propose a precise diagnostic and treatment strategy for ICAS population.

**Trial Registration:** Chinese Clinical Trials Register ChiCTR2200061938.

**Keywords:** Intracranial atherosclerosis stenosis (ICAS); stroke; cognitive impairment
Introduction

Intracranial atherosclerotic stenosis (ICAS) is a major cause of stroke and associated with substantial morbidity and mortality (1,2). Currently, the rate of recurrent stroke is high in symptomatic ICAS and few biomarkers have been found to identify the vulnerable plaques (3). On the other hand, asymptomatic ICAS accounts for 7% in Chinese patients aged ≥40 years (4) and is related to an increased risk of cognitive impairment (5,6). Early recognition of cognitive impairment in ICAS patients has raised concerns, as these individuals may greatly benefit from early intervention of vascular risk factors. However, the pathogenesis of ICAS related cognitive decline is largely unknown.

Beyond the degree of luminal stenosis, intraplaque hemorrhage and positive artery remodeling have been found valuable for identifying vulnerable plaques by high-resolution intracranial vessel wall imaging (IVWI) (7-11). However, relying solely on the information from IVWI to predict future stroke is far from sufficient. Multimodal magnetic resonance imaging (MRI) examinations should be performed to quantitatively evaluate the effects of ICAS on cerebral blood flow (CBF), brain structure and function. In addition to blood lipid and glucose, attention should be paid to blood pressure variability, a potential indicator associated with stroke occurrence (12). The impact of the environment on ICAS should not be ignored. Epidemiological studies have shown that ischemic stroke is closely related to air pollution (13).

According to the Atherosclerosis Risk in Communities (ARIC) study, the overall prevalence of cognitive impairment is 39.3% in the elderly and 47.1% in patients with ICAS (14). Clinical studies have observed that cognitive decline in patients with ICAS occurs several years before a stroke event (15,16). It is uncertain which intracranial artery stenosis results in a higher risk of cognitive impairment (14,17,18). The interactions among ICAS, small vessel disease (SVD) and Alzheimer’s disease (AD) pathology need to be clarified. It is expected to build a comprehensive research framework to disclose the impact of ICAS on brain damage and cognition in vivo, and establish a score system for predicting cognitive impairment in ICAS population.

For the treatment of high-risk symptomatic ICAS patients, several studies have shown that the intensive medical treatment is superior to intravascular therapy (19,20). Recently, proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors, which are novel lipid-lowering medications, have attracted increasing attention. In patients with coronary atherosclerosis, PCSK9 inhibitors significantly reduced the risk of combined cardiovascular events by 20% (21), and effectively induced plaque regression (22). There has been no evidence regarding their effectiveness on intracranial plaque reversal.

To address all above challenges, we launch a large prospective ICAS cohort study. Multimodal MRI examinations, blood sample testing, and longitudinal neuropsychological assessment will be performed, with an emphasis on systematically investigating the influence of ICAS on stroke occurrence and cognitive impairment.

Methods

Design

This is a multicenter, prospective, observational cohort study, which plans to recruit ICAS patients who will be followed up for a minimum of 1 year. Patients will be divided into symptomatic and asymptomatic ICAS based on whether they had previously suffered an ischemic stroke or transient ischemic attack (TIA) in the territory of ICAS. The disease effects of ICAS will be assessed comprehensively through multimodal MRI examinations, neuropsychological assessment battery, blood sample testing, and routine examinations for vascular-related risk factors. Patients will undergo longitudinal cognitive and functional assessments during clinical visits every 6 months and telephone interviews every 3 months. An overview and schedule of assessments of this study is displayed in Figure 1 and Table 1. This study has obtained approval from the institutional review board at Peking Union Medical College Hospital (PUMCH), the Chinese Academy of Medical Sciences (JS-3479D, April 14, 2022), and the ethics boards of local participating centers. All participants will be required to provide written informed consent and the study will be performed according to the Declaration of Helsinki (as revised in 2013).
Number of centers

The study is being conducted from August 2022 to August 2024. Approximately 100 tertiary centers in China will participate in this study. So far, more than 60 centers have participated in the first batch. Sites that do not recruit subjects within 3 months since site initiation may be closed.

Participants

The inclusion criteria are as follows: (I) patients aged

© Annals of Translational Medicine. All rights reserved.
<table>
<thead>
<tr>
<th>General procedures</th>
<th>Screening</th>
<th>Baseline</th>
<th>Month 3*</th>
<th>Month 6*</th>
<th>Month 9*</th>
<th>Month 12*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed consent</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical and family history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mRS score&lt;sup&gt;a&lt;/sup&gt;</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>NIHSS score&lt;sup&gt;b&lt;/sup&gt;</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Routine laboratory tests (blood sample)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testing for exposome (blood sample)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional cranial MRIs&lt;sup&gt;d&lt;/sup&gt;</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3D time-of-flight MRA or CTAs&lt;sup&gt;e&lt;/sup&gt;</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVWI&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>pCASL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>3D structural T1WI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>DTI&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Resting state fMRIs&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Carotid artery ultrasound or other choice&lt;sup&gt;i&lt;/sup&gt;</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>24 h ambulatory blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>12 lead EEG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Neuropsychological assessment battery&lt;sup&gt;j&lt;/sup&gt;</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>ICAS-TICA&lt;sup&gt;k&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Concomitant therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Adverse events</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

<sup>a</sup>, this score will be collected in symptomatic subjects with stroke history; <sup>b</sup>, this score will be collected in symptomatic subjects with acute stroke event; <sup>c</sup>, the complete blood count, hepatic and renal function, blood lipids [not limited to total cholesterol, triglyceride, LDL-C, HDL-C, lipoprotein(a)], HbA1c, HCY and CRP are necessary; <sup>d,e,i</sup>, if images from local hospitals are used in screening, the examinations should be retaken in centers at baseline; <sup>f</sup>, patients included in the sub-study should retake this imaging after 6 months of therapy; <sup>g,h,j</sup>, symptomatic patients with a history of stroke or TIA <3 months will undergo these assessments at 3 months from the onset of stroke or TIA; <sup>h</sup>, if the score is below to 32 and the patient judged to be cognitively normal at the last visit, this patient will be considered at high risk of developing cognitive impairment, and the subsequent clinical evaluation is suggested; <sup>i</sup>, the time-points of follow-up will keep up with the time of initial neuropsychological assessment battery, i.e., asymptomatic ICAS and symptomatic ICAS patients (with a history of stroke or TIA ≥3 months) will undergo telephone interviews at 3 and 9 months and clinical visits at 6 and 12 months after enrollment, while symptomatic ICAS patients with a history of stroke or TIA <3 months will undergo telephone interviews at 6 and 12 months and clinical visits at 9 and 15 months from the onset of stroke or TIA. SICO-ICAS, stroke incidence and cognitive outcomes of intracranial atherosclerotic stenosis; mRS, modified Rankin score; NIHSS, National Institutes of Health Stroke Scale; MRA, magnetic resonance angiography; CTA, computed tomography angiography; IVWI, intracranial vessel wall imaging; pCASL, pseudo-continuous arterial spin labeling; DTI, diffusion tensor imaging; fMRI, resting state functional MRI; EEG, electrocardiogram; ICAS-TICA, ICAS version of telephone interview for cognition assessment; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, glycated hemoglobin; HCY, homocysteine; CRP, C-reactive protein; TIA, transient ischemic attack.
between 18 and 85 years; (II) patients with ICAS [defined as intracranial artery stenosis (>50% or occlusion) at Willis circle branch [intracranial internal carotid artery (ICA), middle cerebral artery (MCA), anterior cerebral artery (ACA), posterior cerebral artery (PCA), basilar artery (BA) or intracranial segment of the vertebral artery (VA)]] confirmed by magnetic resonance angiography (MRA) or computed tomography angiography (CTA); (III) patients with a modified Rankin score (mRS) ≤ 2, which indicates no extra help is needed for daily life (note: a score of 0 represents no symptoms, while a score of 6 signifies death); and (IV) patients included in the sub-study about PCSK9 inhibitors requiring an acute ischemic stroke within 7 days of symptom onset and in the same territory of the symptomatic ICAS [a total National Institutes of Health Stroke Scale (NIHSS) score of 1–15].

The exclusion criteria are as follows: (I) patients with >50% extracranial artery stenosis; (II) patients with relative or absolute contraindications to MRI (e.g., metals in the body, claustrophobia, etc.); (III) those with intracranial stenosis due to non-atherosclerotic etiologies, such as vasculitis, dissection, moyamoya disease, embolus, immune system diseases, or other causes; (IV) patients with other known etiological types of stroke, such as cerebral hemorrhage or subarachnoid hemorrhage, and cardiac cerebral embolism; (V) patients diagnosed with neurodegenerative diseases such as AD, Lewy body dementia, frontotemporal dementia or Parkinson’s disease; (VI) patients with a history of traumatic brain injury, multiple sclerosis, encephalitis, tumors, poisoning, syphilis, and serious heart, lung, liver, kidney, or endocrine system diseases; and (VII) patients who refuse to sign the informed consent.

We also will enroll healthy controls who have no stenoses of intracranial or extracranial vessels, and no history of stroke or TIA, cognitive impairment, traumatic brain injury, brain tumors, etc. The recruitment of these individuals will primarily come from communities and health examinations.

**Clinical data collection**

(I) Demographic characteristics: age, sex, education years, occupation, marital status, residence status, height, weight, waist circumference, physical activity, sleep duration and questionnaire about exposome; (II) previous medical history: stroke or TIA, hypertension, diabetes, hyperlipidemia, atrial fibrillation, myocardial infarction, heart failure, smoking, alcohol consumption, vitamin B12 deficiency, hyperthyroidism or hypothyroidism, immune system diseases, tumors, sleep disorders, anxiety and depression, brain trauma, poisoning, substance abuse, and other diagnosed neurological and psychiatric disorders; (III) family history: cardiovascular and cerebrovascular diseases, dementia, etc.; (IV) medications: stroke prevention drugs and concomitant medications; (V) routine laboratory tests [complete blood count, hepatic and renal function, blood lipids, glycated hemoglobin (HbA1c), homocysteine, C-reactive protein (CRP), other indicators related to vascular or cognitive impairment, and apolipoprotein E (APOE) genotyping (when available)]; and (VI) general examinations [carotid artery ultrasound, 12 lead electrocardiogram, Holter electrocardiogram, echocardiography, and 24 h ambulatory blood pressure (if available)].

**Blood sample biomarkers testing**

To test for additional biomarkers, a 12-mL venous blood sample will be collected and centrifuged at 2,000 rpm for 10 minutes at room temperature, after which blood cells, plasma, or serum will be separated as appropriate and stored at −80°C for further study. Specifically, we will test biomarkers for exposomes such as heavy metals exposure. Furthermore, neurodegenerative biomarkers (amyloid-β, P-Tau, etc.), axon injury biomarkers (nerve filament light chain protein, etc.), and other multi-omics analyses (proteomics, transcriptomics, metabolomics, and genomics) will be performed as an optional choice.

**Neuropsychological assessment battery**

(I) Overall cognition: mini-mental state examination (MMSE), Montreal cognitive assessment (MoCA); (II) daily living ability: activities of daily living scale (ADL), consisting of 14 items (23); (III) mental behavior: Hamilton anxiety and depression scale (24); (IV) a neuropsychological battery: (i) memory: auditory verbal learning test (AVLT, Chinese version), paired associate word learning, Rey complex figure recall test, episodic memory, working memory; (ii) visual-spatial function: Rey complex figure copy test, copy cube; (iii) attention: trail making test A (TMT-A), digit span backward and forward; (iv) executive function: TMT-A and TMT-B, digit symbol substitution, clock drawing test; (v) language: Boston naming test, word fluency, picture talk; and (V) evaluation of clinical dementia: using the clinical dementia rating (CDR) scale (25); a score of 0 signifies no dementia and a score of 3 denotes severe dementia. The
overall assessment will take about 60 to 90 minutes.

We will first construct a Z-score for each test using the means and standard deviations from the Chinese norm. If more than half of the tests within the same cognitive domain are less than −1.5 Z, the cognitive domain will be considered impaired. If a patient has at least one impaired domain, their daily life ability and CDR should be evaluated by a neurologist who is specialized in cognitive decline. Patients whose daily life ability is relatively preserved (ADL ≤16 points) and CDR scale is ≥0.5 will be classified into the mild cognitive impairment group. Meanwhile, patients whose daily life ability is impaired (ADL >16 points) and CDR scale is ≥1 will be classified into the dementia group. Finally, the etiological classification for cognitive impairment can be acquired, and Hachinski Ischemic Scale (HIS) (26) will be used to facilitate the differentiation diagnosis of vascular dementia and AD (27-29) (see below for the follow-up process).

**Multimodal MRI examinations**

Every participant will undergo pre-designed imaging packages, containing conventional cranial MRI [T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), T2-weighted fluid attenuation inversion recovery (FLAIR), diffusion-weighted imaging (DWI), and T2*-WI or susceptibility-weighted imaging (SWI)], three-dimensional time-of-flight MRA, IVWI, three-dimensional pseudo-continuous arterial spin labeling (3D-pCASL), and 3D structural T1WI. Diffusion tensor imaging (DTI) and resting-state functional MRI (fMRI) are possible alternatives (if available). The above multimodal MRI examinations evaluate several aspects of ICAS, including the degree of stenosis, plaques information, CBF, brain structure, brain function, and SVD markers. The overall examination will take about 40 to 60 minutes.

During the MRI scanning, all participants will be asked to close their eyes, keep their heads still, and avoid sleep or cognitive activity. For the scanning process and parameters requirements refer to our previous study (30) and the expert consensus of the Chinese Brain Imaging Alliance on standardized acquisition and analysis of AD brain MRI.

**Follow-up**

Generally, all patients will be followed up for at least 1 year and will retake the neuropsychological assessment battery every 6 months during clinical visits and undergo a simple cognitive assessment every 3 months by telephone interview. The specific follow-up process of patients with symptomatic and asymptomatic ICAS is shown below.

Patients with asymptomatic ICAS will be followed up by telephone interviews at 3 and 9 months after enrollment, including end-point events assessment (see below), mRS score, daily living ability, control of vascular risk factors, medications status, as well as conducting a simple cognitive test by ICAS version of telephone interview for cognition assessment (ICAS-TICA). At 6 and 12 months after enrollment, the patients will be followed up face-to-face and will retake the neuropsychological assessment battery, along with repeated measurement of routine laboratory tests.

Symptomatic ICAS patients with a history of stroke or TIA ≥3 months will undergo the same follow-up process as those with asymptomatic ICAS. However, symptomatic ICAS patients with a history of stroke or TIA <3 months will undergo the initial neuropsychological assessment battery at 3 months from the onset of stroke or TIA. Based on the patients’ cooperation, the optional advanced MRI examinations (DTI, resting-state fMRI) will also be performed 3 months from the onset of ischemic cerebrovascular event. The patients will subsequently be followed up by telephone interviews at 6 and 12 months and then face-to-face follow-up at 9 and 15 months from the event onset. The contents of the follow-up with symptomatic ICAS will be consistent with asymptomatic ICAS.

**Outcome**

The primary outcome measurement of this study is ischemic stroke or cognitive impairment within 12 months after enrollment. Ischemic stroke recurrence is defined by a focal neurological defect (confirmed by new ischemic lesions on DWI) and the diagnosis of cognitive impairment is described above. The secondary outcome measurements are the mRS score, daily living ability, other acute vascular events (new cerebral hemorrhage/subarachnoid hemorrhage, myocardial infarction, visceral hemorrhage, heart failure, pulmonary embolism, arrhythmia, etc.), and vascular death events (death from ischemic/hemorrhagic stroke, myocardial infarction, visceral hemorrhage, heart failure, pulmonary embolism, sudden death, arrhythmia, etc.).

**Treatments**

We will select 20 centers to join in the sub-study to observe the real-world effectiveness of PCSK9 inhibitors
(evolocumab, subcutaneously at a dose of 140 mg every 2 weeks) in patients with symptomatic ICAS who had suffered an ischemic stroke within 7 days. Baseline and repeated IVWI examinations after 6 months of therapy will be performed to compare intracranial plaque burden changes between patients receiving PCSK9 inhibitors plus high-intensity statins and those receiving high-intensity statins alone. The primary efficacy endpoint is the nominal change in percent atheroma volume (PAV) of the lesion side from baseline to 6 months. The PAV will be calculated in line with a previous study (22). Exploratory endpoints include the changes in lipid parameters and CRP level, the percentage of patients with PAV regression, imaging changes of other plaque features (stenosis degree, plaque length, maximum thickness, etc.), ischemic stroke recurrence, cognitive impairment, other acute vascular events, and vascular death events. Individual treatments applied in the remaining centers will comply with the clinical guidelines (31).

**Adverse events**

All exploratory clinical events and laboratory adverse events that occur during the study will be reported and recorded.

**Data management and interpretation**

All participating centers will collect clinical data and complete the pre-designed electronic case report form (eCRF) on a commercial Internet database (brainscience.cc).

All blood samples will be delivered to and stored in the core labs (PUMCH) for unified management and analysis. The images will be interpreted independently by a radiologist and a neurologist who do not know the patients’ clinical information. The diagnosis of mild cognitive impairment and dementia, and the etiological classification will be independently interpreted by two neurologists who are specialized in cognitive disorders, and differences between the two observers will be resolved by a third expert to reach a consensus.

**Sample size and statistical analysis**

The ARIC study (14) showed that the prevalence rates of cognitive impairment with ICA, MCA, ACA, BA, VA, and PCA plaques are 38.7%, 40.4%, 53.6%, 44.3%, 44.9%, and 49.0%, respectively. Meanwhile, based on the preliminary results of our unpublished data, the prevalence of cognitive impairment in the ICA, MCA, ACA, BA, VA, and PCA stenosis groups were 50.0%, 32.7%, 33.3%, 37.5%, 53.3%, and 58.3%, separately. Considering an attrition rate of 15%, the 95% confidence interval of the estimated total sample size is 2,527–2,699. Therefore, we plan to enroll approximately 3,000 cases. Regarding the sub-study for the evaluation of comparative effectiveness of statins with and without PCSK9 inhibitors, 220 patients in each treatment arm will be needed to achieve a 90% power at a 2-sided significance level of 0.05 to detect a nominal treatment difference of 0.9% (22), assuming a 2.9% standard deviation. Considering the potential attrition rate of 20%, 275 patients in each arm will be recruited. Further, we also plan to enroll about 2000 healthy controls on the basis of feasibility.

Descriptive statistical analysis will be used to present the demographic and participant characteristics. Continuous data will be described by the mean (standard deviation) or median (inter-quartile range), and categorical data will be compared using the percentile. Continuous variables conforming to normal distribution will be compared using the two-sample t-test or one-way analysis of variance (ANOVA). Meanwhile, continuous variables that do not conform to a normal distribution will be compared using the Mann-Whitney U test or Kruskal-Wallis test. The Chi-square test or Fisher’s exact test will be used for categorical variables. The relationships between baseline features and specific risk factors and prognostic events will be analyzed by multivariable logistic regression or Cox proportional hazards regression models, as appropriate. Propensity score matching method will be used in the comparative effectiveness study to generate a 1:1 matched cohort for patients treated with statins plus PCSK9 inhibitors versus solely statins. Cross-sectional analyses will also be conducted to explore the risk factors distinguishing symptomatic and asymptomatic ICAS.

**Discussion**

There is growing concern about the link among ICAS, stroke, and dementia (5,32). It is pressingly needed to establish a large prospective ICAS cohort to comprehensively assess the vulnerability of intracranial plaques, reveal the underlying mechanisms of ICAS related cognitive decline, and ultimately improve current prevention and intervention strategies for ICAS population.

The main objectives of the SICO-ICAS study are as follows. Firstly, this study will provide a panoramic assessment of the vulnerability of intracranial plaques.
by comparing the differences in multimodal imaging biomarkers between patients with symptomatic and asymptomatic ICAS, including IVWI (morphology of vessel wall, intracranial plaques features, neovascularization), pCASL (CBF), 3D structural T1WI (grey matter structure), DTI (white matter tracts), and resting-state fMRI (functional connectivity, brain network). Blood samples contain clues that allow us to match biological changes back to life-course exposure (33). Traditional risk factors like low-density lipoprotein cholesterol (LDL-C), HbA1c, and blood pressure variability will also be analyzed. Secondly, this study will calculate the prevalence of mild cognitive impairment and dementia in ICAS patients and analyze the relevant risk factors. The complete biological research framework examined by multimodal imaging biomarkers including brain vessels, network, and reserve will be integrated to comprehensively investigate the pathologies of ICAS on brain damage and cognition. The testing of blood biomarkers for neurodegeneration and axon injury-related factors and the APOE ε4 allele will be acquired if needed. The mechanisms that link ICAS with SVD and AD pathology in the development of dementia will be explored.

Thirdly, the disease effects of ICAS will be further clarified by comparing ICAS patients to healthy controls. Fourthly, this study will assess the real-world effectiveness of PCSK9 inhibitors in symptomatic patients with acute ischemic stroke.

There are several limitations in this study. Firstly, this is an observational cohort study, so there may be some residual confounding bias even after multivariable adjustment. Secondly, participants in this study will be followed up for at least 1 year; however, the incidence of stroke with asymptomatic ICAS patients and the incidence of cognitive impairment may be relatively low at the 1-year stage. Lastly, the histological evidence to validate the imaging results, such as the plaque components, is lacking; although IVWI findings have been confirmed by postmortem pathological results (34-36).

In conclusion, this study has been designed to provide in-depth insights into the pathophysiology of ICAS and its disease effects on stroke and cognitive impairment. It is anticipated to eventually establish a precise diagnostic and treatment strategy for ICAS population.

Acknowledgments

Funding: This work is supported by the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (project No. 2020-RC320-001) and the National Science Fund for Distinguished Young Scholars (grant No. 82025013).

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3570/coif). WX serves as an unpaid editorial board member of Annals of Translational Medicine from Jun 2019 to May 2024. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the institutional review board at Peking Union Medical College Hospital, the Chinese Academy of Medical Sciences (JS-3479D, April 14, 2022), and the ethics boards of local participating centers. All participants will be required to provide written informed consent and the study will be performed according to the Declaration of Helsinki (as revised in 2013).

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

31. Turan TN, Zaidat OO, Gronseth GS, et al. Stroke Prevention in Symptomatic Large Artery Intracranial...


(English Language Editor: A. Kassem)