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Complete List of Authors:	Wang, Xiang ; Shandong Provincial Hospital, Department of Neurology Zhao, Yuanyuan; Shandong Provincial Hospital, Department of Neurology Ji, Xiaokang; Shandong University School of Public Health, Department of biostatistics Sang, Shaowei; Shandong University Qilu Hospital, Department of clinical epidemiology Shao, Sai; Shandong Medical Imaging Research Institute, Department of Radiology Yan, Peng; Shandong Provincial Hospital, Department of Neurology Li, Shan; Shandong Provincial Hospital, Department of Neurology Li, JiFeng; Shandong Provincial Hospital, Department of Neurology Wang, Guangbin; Shandong Medical Imaging Research Institute, Department of Radiology Lu, Ming; Shandong University, Department of clinical epidemiology Du, YiFeng; Shandong Provincial Hospital, Department of Neurology Xue, Fuzhong; Shandong University School of Public Health, Department of biostatistics Qiu, Chengxuan; Shandong Provincial Hospital, Department of Neurology; Karolinska Institutet-Stockholm University, Department of Neurobiology, Care Sciences and Society Sun, QinJian; Shandong Provincial Hospital, Department of Neurology
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Xiang Wang[a], Yuanyuan Zhao[a], Xiaokang Ji[b], Shaowei Sang[c], Sai Shao[d], Peng Yan[a], Shan Li[a], Jifeng Li[a], Guangbin Wang[d], Ming Lv[c], Yifeng Du[a], Fuzhong Xue[b], Chengxuan Qiu[a,e], Qinjian Sun[a]*

[a]Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, China

[b]Department of Biostatistics, School of Public Health, Shandong University, Jinan, Shandong, China

[c]Department of Clinical Epidemiology, Qilu Hospital affiliated to Shandong University, Jinan, Shandong, China

[d]Department of Radiology, Shandong Medical Imaging Research Institute Affiliated to Shandong University, Jinan, Shandong, China

[e]Aging Research Center, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet-Stockholm University, Stockholm, Sweden

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*Corresponding author:

Qinjian Sun, Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong University, #324 Jingwu Weiqi Road, Jinan, Shandong 250021, P. R. China.

Email: sqj1210@163.com

ABSTRACT

Purpose: The population-based Kongcun Town Asymptomatic Intracranial Artery Stenosis (KT-aICAS) study aims to investigate the prevalence and major cardiovascular risk factors (CRFs) or biomarkers related to the development and prognosis of aICAS.

Participants: The KT-aICAS study included 2311 rural residents who were aged ≥40 years and living in Kongcun Town, Shandong Province, China. Baseline examination was conducted from October 2017 to October 2018, during which information on demographics, socioeconomics, personal and family medical history, and lifestyle factors was collected through face-to-face interviews, physical examination, and blood tests. Asymptomatic ICAS was initially screened using transcranial Doppler examination and then diagnosed using magnetic resonance angiography. Atherosclerosis in carotid arteries was diagnosed via carotid ultrasonography. High-resolution magnetic resonance imaging was further used to evaluate the vessel wall of aICAS. Neuropsychological assessments were performed in the subjects diagnosed with aICAS.

Findings to date: Of the 2311 participants, 2027 completed the diagnostic procedure and aICAS was detected in 154 persons, resulting in an overall prevalence of 7.6%. The prevalence of aICAS increased with advancing age from 5.1% in participants aged 40-49 years to 12.7% in those aged ≥70 years (*P*<0.001). Asymptomatic ICAS was detected in 305 intracranial arteries, including 221 (72.5%) in the anterior circulation and 84 (27.5%) in the posterior circulation (*P*<0.001). In addition, major CRFs were highly prevalent among middle-aged and elderly rural dwellers who were free of stroke.

Future plans: Follow-up examinations will be performed every 3 years. This study will increase our knowledge about the natural history of aICAS and facilitate studies of aICAS-associated disorders among rural-dwelling Chinese adults, such as ischaemic stroke and vascular cognitive impairment.

Strengths and limitations of this study

- This population-based study integrated TCD and MRA to detect aICAS among residents living in rural communities, which is a feasible approach to obtain a relatively accurate prevalence of aICAS in rural residents.
- High-resolution MRI scans were used to further identify characteristics of aICAS.
- Neuropsychological evaluation in this study covers global cognitive function and multiple cognitive domains.
- This study used TCD as a screening tool for aICAS, which might have missed some cases and underestimated the prevalence of aICAS.
- The study sample was selected from only one rural area, and thus, cautiousness is needed when generalizing the study findings to other rural populations in China.

INTRODUCTION

Intracranial artery stenosis (ICAS) due to atherosclerosis is a leading cause of ischaemic stroke worldwide, especially in Asian populations[1]. In addition, intracranial atherosclerosis has been associated with cognitive impairment [2]. Atherosclerotic lesions may develop and progress silently over years, subsequently impair cognitive function, or even suddenly cause clinical stroke. Therefore, the detection of ICAS in the asymptomatic phase may facilitate early preventive and therapeutic interventions and thus delay the progression to symptomatic stroke and cognitive impairment[3, 4]. Several population-based studies that used non-invasive tools, such as transcranial ultrasound or magnetic resonance angiography (MRA), to investigate the prevalence of asymptomatic ICAS (aICAS) suggested that the prevalence of aICAS was around 9% in Caucasian populations[5, 6] and 12% in African-Americans [6], but as high as 24.5% in Asian populations[7-10]. However, data remain limited with regard to the prevalence of aICAS among rural residents in China, where cardiovascular risk factors (CRFs) are highly prevalent and poorly controlled[11].

Furthermore, the natural history and prognosis of aICAS remains poorly understood. The Warfarin versus Aspirin Symptomatic Intracranial Disease trial showed that the risk of stroke from aICAS was relatively low among patients with co-existing symptomatic ICAS and aICAS[12]. In addition, asymptomatic atherosclerotic stenosis of the middle cerebral artery (MCA) is apparently not a malignant disease[13, 14]. These studies suggest that aICAS may have a benign long-term prognosis. It is worth noting that these previous studies have either focused on selective patient groups (co-existence of asymptomatic and symptomatic stenosis) or targeted special vessels (e.g., MCA), and the general population-based studies that aimed at investigating the prognosis of aICAS remain scarce. Indeed, a population-based prospective study of 2807 healthy volunteers (mean age, 62.0 years) in Japan found that aICAS was a risk factor for clinical stroke[15]. However, the predictors for the conversion and progression of aICAS to the symptomatic stage remain largely unclear.

Symptomatic ICAS is an independent risk factor for cognitive decline. However, whether aICAS is associated with subsequent cognitive decline remains to be explored. Recently, a prospective cohort study found that asymptomatic severe MCA stenosis (MCAS) with poor collateral circulation was associated with declines in multiple cognitive domains characterized by vascular cognitive impairment[16]. The association between cognitive function and aICAS in other intracranial arteries remains to be clarified.

Therefore, we initiate the Kongcun Town aICAS (KT-aICAS) study that aims: (1) to investigate occurrence (e.g., prevalence and incidence) and distribution of aICAS among middle-aged and older adults living in rural communities; (2) to explore CRFs of aICAS and clinical biomarkers that may predict the progression from aICAS to ischaemic stroke; (3) to assess the association between aICAS and cognitive impairment; and (4) to build a comprehensive database that facilitates longer-term multidisciplinary research regarding the biomedical, clinical, neuroimaging, and epidemiological aspects of aICAS.

Cohort description

Study Design

At baseline, a population-based cross-sectional study was conducted to detect the prevalence and distribution of aICAS. A subsequent prospective cohort study will be performed to determine the incidence rates of aICAS, examine the association between aICAS and ischaemic stroke or cognitive impairment, and explore a variety of CRFs, clinical features, imaging markers or biomarkers for the conversion from aICAS to symptomatic ICAS and the potential cognitive consequences.

Study Communities and Population

The KT-aICAS Study targeted all registered residents who were aged ≥40 years, free of a history of stroke, and living in the Kongcun Town of Pingyin county in Shandong Province in October 2017. Kongcun Town is a rural township that is located ~76 km west of Jinan, the capital of Shandong Province, China. Kongcun Town includes ~30000 residents in an area of ~5000 hectares. The majority of people in the town are farmers. Since the 2000s, the School of Public Health at Shandong University has been working closely with the town government and local health professionals to promote the health of local residents. This has involved regular collection of data on demographics, lifestyle factors, health history, and diet. Following the baseline assessment in 2017-2018, all participants will be invited to undertake a follow-up assessment every 3 years with the aim of detecting patients with aICAS and identifying incident clinical stroke and cognitive impairment. Figure 1 shows the basic design of the KT-aICAS Study and the flowchart of the study population and assessments.

Ethics Approval and Consent to Participate

The protocol of the KT-aICAS Study was reviewed and approved by the ethical standards committees on human experimentation at Shandong Provincial Hospital Affiliated to Shandong University. Written informed consent was obtained from all of the participants. The study was conducted in accordance with the principles for medical research involving human subjects expressed in the Declaration of Helsinki. The face-to-face interviews were scheduled at the most convenient times for the participants. Respondents were free to refuse participation or withdraw from the study at any time. Any case of serious illness that had not been detected previously was urgently referred to the hospital.

Baseline Assessments

Baseline assessments were performed in October 2017-October 2018, which included a two-phase design as briefly described below.

Phase 1

From October to November 2017, we performed a phase 1 assessment, a screening phase that aimed to collect baseline data and identify people at high risk of aICAS. During this phase, all participants underwent structured questionnaire survey, transcranial Doppler (TCD) examination, and colour Doppler ultrasound examination (neck).

Face-to-face interviews. A standardized questionnaire was used to collect data on demographic features, health and family history (e.g., chronic diseases), CRFs (e.g., hypertension, diabetes, and obesity), lifestyle factors (e.g., diet habits, smoking, alcohol consumption, tea consumption, and physical activity), menstrual and reproductive history, quality of life, and current use of medication. Typically, the interview took approximately 30 minutes. Then, all participants underwent physical and clinical examinations, which included BP and anthropometric measurements, carotid artery B-mode ultrasonography, and TCD examination.

BP and anthropometric measurements. All measurements were performed by trained nurses. After a 5-min rest, arterial blood pressure was measured twice at a 5-min interval using an automated sphygmomanometer with the subject in the seated position. The mean value of the two measurements was used in the analysis. Hypertension was defined as blood pressure ≥140/90 mmHg or use of antihypertensive drugs. Height was measured without shoes using a standard right-angle device and a fixed measurement tape (to the nearest 0.5 cm). Body weight without heavy clothing was measured. Body mass index (BMI) was calculated as height (meters) divided by weight (kilograms) squared. We defined obesity as a BMI ≥28 kg/m^2 and overweight as a BMI of 24-27.9 kg/m^2.

TCD ultrasound examination. Participants were examined with a portable machine (VIASYS Companion III, Nicolet, Washington, United States) by two experienced physicians with expertise in sonography after a 5-min rest in a quiet and comfortable room. The bilateral MCA, anterior cerebral artery (ACA), posterior cerebral artery (PCA), vertebral artery (VA), and basilar artery (BA) were examined with a 2 MHz probe through a temporal window, occipital window, and eye window, respectively. The depth, peak systolic velocity (PSV), mean flow velocity (MFV), end diastolic velocity, and pulsatility index were measured for each artery. The following MFV cut-offs were used for the diagnosis of ≥50% intracranial stenosis of various arteries according to the criteria described in the Stroke Outcomes and Neuroimaging of Intracranial Atherosclerosis (SONIA) trial[17]: MFV ≥100 cm/s for MCA stenosis, MFV ≥80 cm/s for VA stenosis, and MFV ≥80 cm/s for BA stenosis. Because the ACA and PCA were not evaluated in the SONIA trial, we followed previously validated criteria to define ≥50% stenosis in these vessels[18]: MFV ≥80 cm/s and ≥30% different when compared to the contralateral ACA segment for ACA stenosis and MFV ≥80 cm/s for PCA stenosis. The criteria for ≥70% stenosis were as follows[19]: MCA: MFV ≥120 cm/s or a stenotic/prestenotic ratio ≥3 or low velocity; VA and BA: MFV ≥110 cm/s or a stenotic/prestenotic ratio ≥3. In addition, the presence of turbulence or musical sounds and an abnormal segmental velocity were considered for the diagnosis of stenosis.

Carotid ultrasonography examination. Carotid ultrasound examination was performed in the head-straight flat supine position by two experienced physicians to measure intimal medial thickness (IMT), PSV, and EDV of the common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) with a 7-MHz linear transducer (Siemens ACUSON P500). First, the structure of the vascular wall of the full-length of the CCA and the bifurcation and the initial segment of the ICA as well as atherosclerotic plaques and intravascular echo were measured in a cross-section. Then, the IMT of the distal segment of the CCA (1 to 1.5 cm below the bifurcation level), the internal diameter of the ICA, and the distal segment of the CCA were measured in longitudinal sections. The diameter was defined as the vertical distance between the upper edge of the posterior wall of the vessel and the inferior border of the anterior wall. When vessel stenosis occurred, the residual diameter and original diameter were measured. The thickness of the IMT was defined as the vertical distance between the upper edge of the posterior wall of the vessel and the upper edge of the outer membrane. Location, morphology, and integrity of the fibrous cap and the acoustic characteristics of the plaque were observed, and the size of the plaque was measured. Intimal thickening was defined as intimal-middle film thickness ≥1.0 mm, and plaque was defined as focal intimal-middle film thickness ≥1.5 mm[20].

Laboratory tests. After overnight fasting, peripheral venous blood samples were taken in the morning. The blood was then allowed to clot in a 37 °C water bath for 30 minutes before it was centrifuged at 3,000 rpm for 15 minutes. Then, the serum supernatant was collected and separated into 5 aliquots (200 μL per aliquot) and immediately stored in liquid nitrogen. Blood tests were conducted at the certified clinical laboratory of the Shandong Provincial Hospital Affiliated to Shandong University. We measured a complete blood count, fasting blood glucose (FBG), liver function, renal function, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglyceride, apolipoprotein genotyping, homocysteine, supersensitivity C-reactive protein, and insulin. The remaining serum samples were stored at -80 °C until further analyses. Diabetes was defined as FBG ≥7.0 mmol/l or current use of blood glucose-lowering agents or insulin injection. High cholesterol was defined as total cholesterol ≥6.20 mmol/l or current use of hypolipidaemic drugs, and dyslipidaemia was defined as total cholesterol ≥6.20 mmol/l, triglycerides ≥1.8 mmol/l, HDL-C <1.11 mmol/l or LDL-C ≥3.36 mmol/l or current use of hypolipidaemic drugs.

Phase 2

From April to October 2018, participants who were screened positive and their age- and sex-matched controls screened negative for ICAS by TCD in Phase 1 underwent Phase 2 assessment, which included neuropsychological assessments, structural brain MRI scans, and MRA.

MRI protocol and acquisition. All invited participants underwent brain MRI scans on a 3.0T scanner (Achieve; Philips Medical Systems, Best, The Netherlands) with an 8-channel head coil. The core MRI protocol included MRA, dynamic susceptibility contrast-enhanced perfusion-weighted imaging (DSC-PWI), diffusion tensor imaging (DTI), T1-weighted imaging with 3D variable refocusing flip angle volume isotropic turbo spin-echo acquisition (3D T1VISTA), 3D T2VISTA, fluid-attenuated inversion recovery images, and contrast-enhanced T1VISTA. The post-enhanced T1VISTA parameters were the same as those used for pre-T1VISTA. A gadolinium contrast medium (Gd-DTPA, Magnevist, Beijing BeiLu Pharmaceutical Co., Ltd., Beijing, China) was intravenously injected at the third acquisition (0.2 mL/kg or 4.5 mL/s followed immediately by a 30 mL physiological saline flush). The extent of stenosis in the five evaluated arteries (bilateral MCA, bilateral intracranial ICA, and BA) was classified into five grades by consensus as normal, mild (signal reduction <50%), moderate (signal reduction ≥50% and <70%), severe (signal reduction ≥70%), or occlusion (focal signal loss with the presence of distal signal). The diagnosis and grading of stenosis was performed by a neurologist specializing in stroke and a clinical neuroradiologist.

Neuropsychological evaluation. Neuropsychological assessments were performed by experienced neuropsychologists trained specifically for this project and included tests of global cognitive function and specific cognitive domains, such as memory, executive function, attention, visuospatial function, and affection. The Chinese versions of the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) were performed to assess global cognitive function[21]. Specific cognitive domains (e.g., episodic memory, verbal fluency, attention, and executive function) were assessed using the following neuropsychological tests: (1) the Rey Auditory Verbal Learning Test Learning (AVLT: direct recall, delayed recall, and word list recognition) for episodic memory[22], the Fuld-object Memory Evaluation (FOM) for object memory[23], (2) the Category Verbal Fluency test (CVF) for verbal performance and executive function[24], (3) the Trail Making Test (TMT

A&B) for executive function[25], (4) the Wechsler Memory Scale-III Forward Digit Span (FDS) and Backward Digit Span(BDS) for attention and executive function[26], and (5) the Wechsler block design test and Clock Drawing Test (CDT) for visuospatial and motor skills[27]. In addition, depressive symptoms were assessed using the Hamilton depression Scale (HAMD) [28]. The Barthel index was used to assess basic activities of daily living (ADL) [29].

Quality control procedure. Before the beginning of the study, all staff were centrally trained and required to demonstrate competency to perform different assessments, including interviews, biospecimen acquisition, cerebrovascular assessments, and cognitive testing. Psychologists, neuroradiologists, and neurologists attended a standardization meeting to ensure the consistency of application of the evaluation criteria. The collection and processing of the data were checked and verified by two or even three people, and the entry of the collected data was completed via integral double entry.

Statistical Analysis

The data are presented as frequencies (%) for categorical variables and as the mean (SD) or median (interquartile range, IQR) for continuous variables. Characteristics of the study participants by sex were compared using the general linear regression model for continuous variables and chi-square test for categorical variables. We describe the distribution of major CRFs and aICAS. We used the statistical packages R (http://www.r-project.org; version 3.4.3) and EmpowerStats (www.empowerstats.com; X&Y Solutions Inc.) for all analyses.

Follow-up assessments

All survivors of the baseline participants will be invited to undertake a follow-up evaluation in the third year. The same questionnaire used for the baseline survey will be used during the follow-up examination for collecting epidemiological, neuropsychological, and clinical data. Analysis of biomarkers as well as TCD test, carotid ultrasonography examination, and brain MRI scan will also be performed.

Patient and public involvement

No patients or public were involved in the development of research questions and the design of the project. However, patients or participants would benefit from participating this project because health care advices were provided during the examination. Furthermore, in the future patients may benefit from the knowledge generated from this project once it becomes publicly available. All participant-related information was de-identified from the database to preserve privacy.

Findings to date

In this ongoing prospective cohort study of middle-aged and older adults who were free of clinical stroke, we demonstrate the feasibility of a two-phase procedure to study the epidemiology and nature history of aICAS in rural communities by integrating epidemiological approaches with clinical, neuroimaging, and neuropsychological techniques.

Table 1 summarises the characteristics of baseline participants. The mean age of the 2311 participants was 57.6 years, and 53.4% were women. Compared to men, women were older, less educated, and less to be single, and had higher levels of total cholesterol, triglycerides, LDL-C, and BMI, but lower levels of diastolic pressure and HDL-C. There was no significant sex difference in levels of FBG or systolic pressure and prevalence of hypertension. Men were much more likely to smoke and consume alcohol than women, whereas women were more likely to be obese. Women had a higher prevalence of diabetes and higher dyslipidemia levels than men. These results suggested that major CRFs were highly prevalent in rural-dwelling middle-aged and elderly people in China and that there were substantial sex differences in certain lifestyle factors, such as smoking and alcohol consumption, which were highly prevalent only in men (figure 2). Given the well-established aetiological relationship of CRFs with atherosclerosis and clinical stroke, our study suggests that active interventions to target these modifiable risk factors are urgently needed to counteract the huge burden of stroke and dementia in rural areas of China.

Of the 2311 participants, 2027 completed the diagnostic procedure for aICAS, and aICAS was diagnosed in 154 persons (92 women and 62 men), which corresponded to the overall prevalence of 7.6% (8.7% in women vs. 6.4% in men, P=0.055). The prevalence of aICAS increased with advancing age, from 5.1% in individuals aged 40-49 years to 12.7% in those aged ≥70 years (figure 3). A total of 305 intracranial arteries were diagnosed with aICAS; 221 (72.5%) were involved in ACA, MCA or ICA, and 84 (27.5%) were involved in PCA, VA or BA (table 2). The distributions of aICAS by regional arteries were as follows: ACA, 55 (18.0%) arteries; MCA, 95 (31.1%) arteries; ICA, 71 (23.3%) arteries; PCA, 38 (12.5%) arteries; VA, 25 (8.2%) arteries; BA, 21 (6.9%) arteries. The arteries in the anterior circulation were more frequently involved than arteries in the posterior circulation.

Most previous studies have used either TCD or MRA alone to diagnose aICAS, and different detection approaches and procedures may contribute to the substantial variations in the reported prevalence of aICAS. For example, the prevalence of aICAS was reported to be 13.2% among coal mine workers aged ≥40 years in a study that used only TCD to detect aICAS, whereas another study of people aged ≥40 years (mean age, 58.1 years) from Korea reported that the prevalence of aICAS was 24.5% in all intracranial vessels[7]. We used a two-phase approach that included a screening phase with TCD examination followed by a diagnostic phase with MRA, and we found that the overall prevalence of aICAS was 7.6% in this rural-dwelling middle-aged and elderly population. TCD examination, when used as a tool for detecting arterial stenosis, has the advantage of convenience and non-invasiveness but also has a high negative predictive value (~90%); thus, TCD is suitable for screening arterial stenosis in a large-scale population study. However, the positive predictive value of TCD examination is only ~50%. In contrast, MRA provides a good positive predictive value for ICAS but may not be suitable for large-scale community-based studies due to its high costs and inconvenience[17]. In this study, we combined TCD with MRA to detect aICAS, which is feasible especially among people who are living in the remote rural communities. This approach will allow us to obtain a more accurate estimate of aICAS prevalence than that was achieved in previous studies used either TCD or MRA alone.

We also found that the distribution of aICAS was more common in the anterior circulation than in the posterior circulation, in accordance with a study from Japan[30]. The variations in the regional arterial distribution of aICAS are likely due to differences in the anatomical and haemodynamic characteristics between the anterior and posterior circulation.

For example, in the anterior circulation, the higher number of vascular branches may increase the risk of atherosclerosis[31]. In addition, TCD has relatively low sensitivity in detecting posterior circulation stenosis[32], and this may have contributed, in part, to the differences observed in the regional arterial distribution of aICAS.

In summary, in the community-based KT-aICAS study, aICAS affected nearly one-tenth of the rural-dwelling middle-aged and elderly residents, and its prevalence increased with advanced age. Anterior circulation arteries were most vulnerable to atherosclerotic lesions. In addition, major CRFs were highly prevalent among middle-aged and elderly rural dwellers who were free of stroke. These findings may have important implications for the primary prevention of aICAS, clinical stroke, and cognitive impairment. Future follow-up data from this cohort may contribute to the understanding of the epidemiology and natural history as well as cognitive consequences of aICAS among rural residents in China.

Strengths and Limitations

This cohort study targeted residents living in rural communities in China, to whom the research community has so far paid little attention. Second, we integrated TCD and MRA into a two-phase procedure to detect aICAS in a large-scale population study, and this approach is likely to allow us to obtain a relatively accurate estimate of the prevalence of aICAS. Furthermore, high-resolution MRI scans could help further evaluate the characteristics of arterial stenosis and avoid the contamination of ICAS cases with cases involving other non-atherosclerotic diseases[33]. In addition, high-resolution MRI can be used to detect intraplaque haemorrhage, lipid cores, and artery fibrous caps, thus providing further insight into the long-term prognosis of aICAS. To the best of our knowledge, this is the first community-based study of aICAS among an Asian population that used high-resolution MRI as well as PWI images and DTI images. Finally, this community-based study includes comprehensive neuropsychological assessments that covers global cognitive function (MMSE and MoCA) and multiple cognitive domains (e.g., memory, verbal fluency, attention, and executive function), and thus, will facilitate future exploration of specific cognitive profiles associated with aICAS and its progression.

Our study also has limitations. First, we used TCD as a screening tool for arterial stenosis, which might have missed some cases of aICAS, and thus, have underestimated the overall prevalence of aICAS. Furthermore, in case of a poor or incomplete echo window for TCD, people without any stenosis in the VA, BA, and ACA were considered to be non-stenotic. This might also have resulted in the underestimation of the true prevalence of aICAS in this population. Finally, our study sample was selected from only one rural area, and this should be kept in mind when generalizing our findings to other rural populations.

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Author Contributions QS, FX, and YD conceived the research question and designed the study. XW and FX conducted the statistical analysis, interpreted the results, and wrote the first and subsequent drafts of the manuscript. XJ, SwS, SS, YZ, PY, SL, FJ, GW, and ML contributed to the epidemiological investigation. All authors contributed to data interpretation and critical revisions of the manuscript, and approved the final version of the submitted manuscript.

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Competing interests None declared.

Participant consent Obtained.

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Table 1. Characteristics of the study participants by gender

Characteristics [b]	Total (n=2311)	Men (n=1076)	Women (n=1235)	P[a]
Age (years), mean (SD)	57.6(10.5)	56.3(10.3)	58.8(10.5)	<0.001
Age group (years), n(%)				<0.001
40-49	613(26.6)	353(32.9)	260(21.1)	
50-59	733(31.8)	334(31.1)	399(32.4)	
60-69	607(26.3)	240(22.3)	367(29.8)	
⩾70	352(15.3)	147(13.7)	205(16.7)	
Marriage states, n (%)				<0.001
Married	252(10.9)	72(6.7)	180(14.6)	
Single status	2058(89.1)	1003(93.3)	1055(85.4)	
Educational level, n (%)				<0.001
Illiterate	354(15.3)	36(3.3)	318(25.8)	
Elementary	679(29.4)	247(23.0)	432(35.0)	
Middle school	976(42.3)	582(54.1)	394(31.9)	
High school and above	301(13.0)	211(19.6)	90(7.3)	
Body mass index (kg/m^2), mean (SD)	25.2(3.8)	24.8(3.2)	25.5(4.1)	<0.001
Systolic pressure (mm Hg), mean (SD)	145.0(22.4)	144.5(21.3)	145.4(23.4)	0.365
Diastolic pressure (mm Hg), mean (SD)	88.3(12.7)	89.9(13.0)	86.9(12.2)	<0.001
Fasting blood glucose (mmol/l), mean (SD)	6.1(1.8)	6.0(1.8)	6.1(1.9)	0.084
LDL-C (mmol/l), mean (SD)	3.0(0.7)	2.9(0.7)	3.1(0.7)	<0.001
HDL-C (mmol/l), mean (SD)	1.6(0.4)	1.6(0.4)	1.6(0.3)	0.031
Triglycerides (mmol/l), mean (SD)	1.4(0.9)	1.3(0.9)	1.5(0.9)	<0.001
Total cholesterol (mmol/l), mean (SD)	5.4(1.0)	5.2(0.9)	5.5(1.0)	<0.001
Smoking, n (%)	504(21.9)	489(45.6)	15(1.2)	<0.001
Alcohol consumption ,n (%)	727(31.5)	672(62.5)	55(4.5)	<0.001
Obesity, n (%)	437(19.2)	165(15.5)	272(22.5)	<0.001
Dyslipidemia, n (%)	922(41.7)	378(36.2)	544(46.7)	<0.001
Hypertension, n (%)	1294(56.6)	592(55.3)	702(57.8)	0.220
Diabetes , n (%)	725(32.9)	319(30.5)	406(34.9)	0.028

aICAS, n (%)	154(7.6)	62(6.4)	92(8.6)	0.055

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SD: standard deviation; aICAS: asymptomatic intracranial artery stenosis.

[a]P values were for the test of differences between male and female. [b]The numbers of participants with missing value were 1 for marriage states and educational level, 38 for body mass index, 29 for blood pressure, 175 for fasting blood glucose, 106 for LDL-C, HDL-C, triglycerides and total cholesterol, 8 for smoking, 2 for alcohol consumption, and 38 for obese status, 102 for dyslipidemia, 26 for hypertension, 104 for diabetic status, and 284 for aICAS (paticipants completed the diagnostic procedure for aICAS was 2027) .

Table 2. Arterial distribution of asymptomatic intracranial atherosclerotic stenosis (aICAS) according to severity of stenosis (Total number of arteries with aICAS = 305)

Arteries	Severity of intracranial arterial stenosis, n (%)				
	Mild	Moderate	Severe	Occlusion	Total
ICA	28 (9.2)	20 (6.6)	17 (5.6)	6 (2.0)	71(23.3)
MCA	45 (14.8)	21 (6.9)	23 (7.6)	6 (2.0)	95(31.2)
ACA	22 (7.2)	17 (5.6)	16 (5.3)	0 (0.0)	55(18.0)
PCA	10 (3.3)	9 (3.0)	18 (5.9)	1 (0.3)	38(12.5)
VA	4 (1.3)	2 (0.7)	8 (2.6)	11 (3.3)	25(8.2)
BA	13 (4.3)	3 (1.0)	5 (1.6)	0 (0.0)	21(6.9)

MCA, middle cerebral artery; ACA, anterior cerebral artery; ICA, intracranial internal carotid artery; PCA, posterior cerebral artery; VA, vertebral artery; BA, basilar artery.

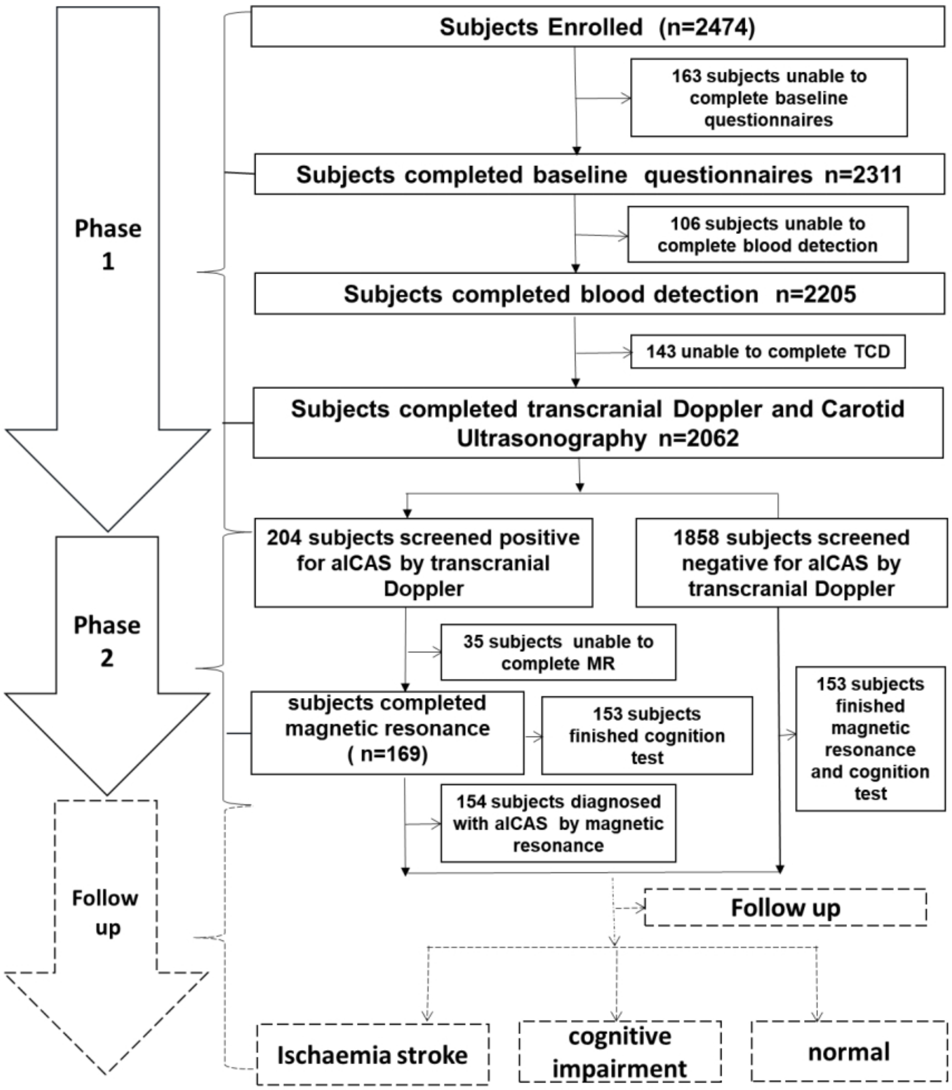


Figure 1 Flowchart of the study

316x353mm (72 x 72 DPI)

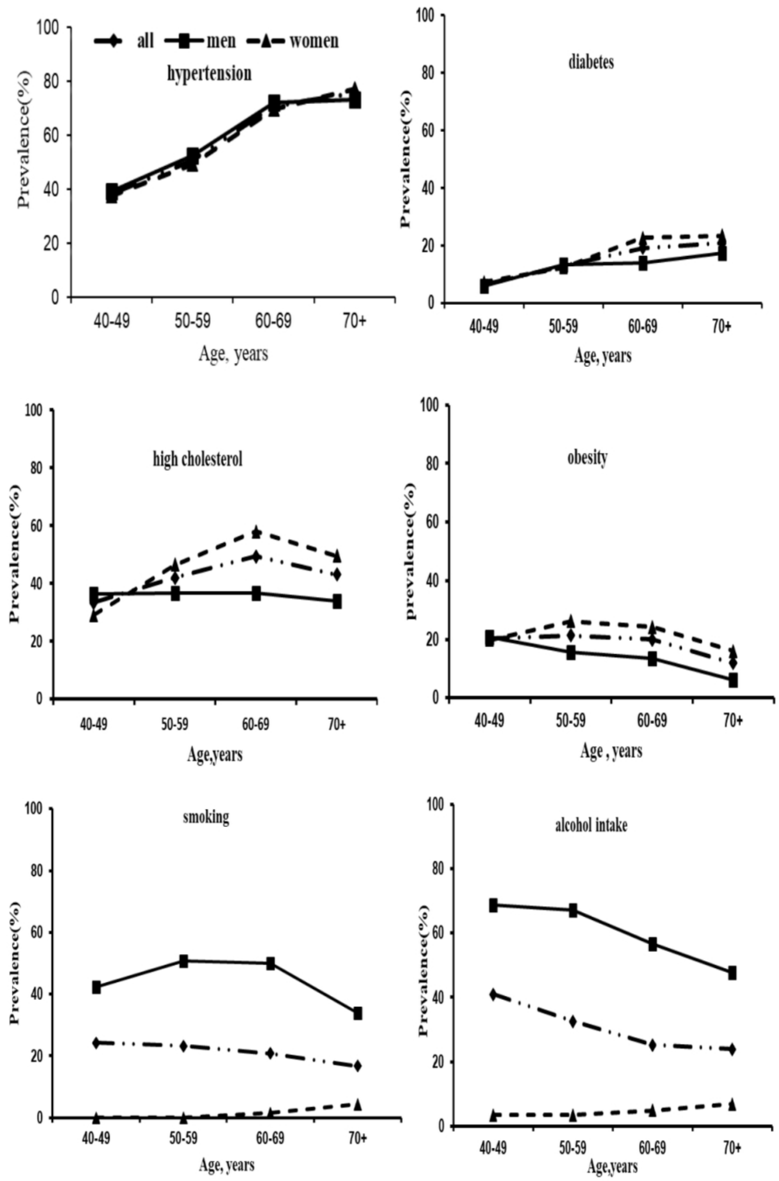
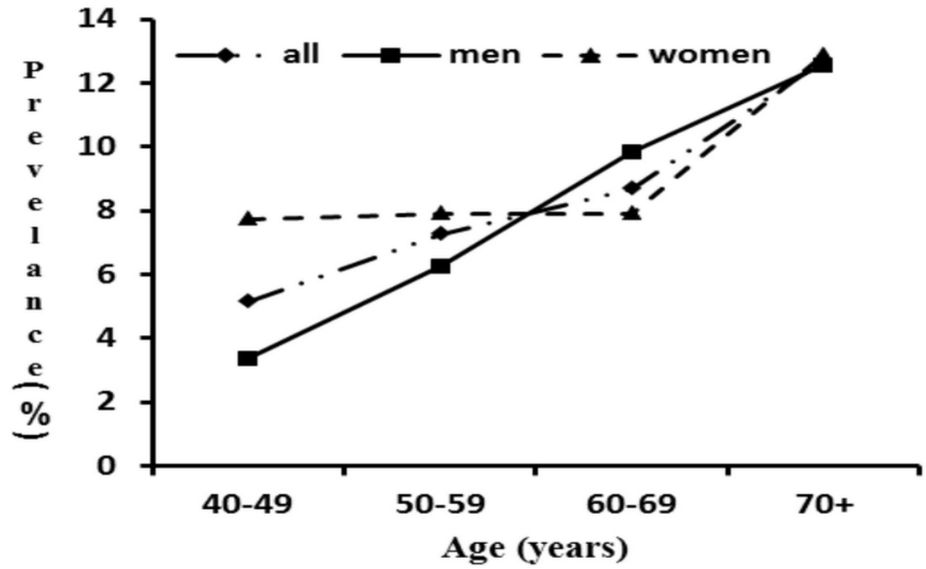


Figure 2 Age- and gender-specific prevalence (per 100 population) of cardiovascular risk factors

101x145mm (300 x 300 DPI)



all: P for trend<0.001; Men: P for trend<0.001; Women: P for trend =0.140

Figure 3 Age- and gender-specific prevalence (per 100 population) of aICAS

78x56mm (300 x 300 DPI)

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Cohort profile: the Kongcun Town Asymptomatic Intracranial Artery Stenosis Study in Shandong, China

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Cohort profile: the Kongcun Town Asymptomatic Intracranial Artery Stenosis Study in Shandong, China

Xiang Wang[a], Yuanyuan Zhao[a], Xiaokang Ji[b], Shaowei Sang[c], Sai Shao[d], Peng Yan[a], Shan Li[a], Jifeng Li[a], Guangbin Wang[d], Ming Lv[c], Yifeng Du[a], Fuzhong Xue[b], Chengxuan Qiu[a,e], Qinjian Sun[a]*

[a]Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, China

[b]Department of Biostatistics, School of Public Health, Shandong University, Jinan, Shandong, China

[c]Department of Clinical Epidemiology, Qilu Hospital Affiliated to Shandong University, Jinan, Shandong, China

[d]Department of Radiology, Shandong Medical Imaging Research Institute Affiliated to Shandong University, Jinan, Shandong, China

[e]Aging Research Center, Department of Neurobiology, Care Science and Society, Karolinska Institutet-Stockholm University, Stockholm, Sweden

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*Corresponding author:

Qinjian Sun, Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong University, #324 Jingwu Weiqi Road, Jinan, Shandong 250021, P. R. China.

Email: sqj1210@163.com

ABSTRACT

Purpose: The population-based Kongcun Town Asymptomatic Intracranial Artery Stenosis (KT-aICAS) study aims to investigate the prevalence and major cardiovascular risk factors (CRFs) or biomarkers related to the development and prognosis of aICAS.

Participants: The KT-aICAS study included 2311 rural residents who were aged ≥40 years and living in Kongcun Town, Shandong Province, China. Baseline examination was conducted from October 2017 to October 2018, during which information on demographics, socioeconomics, personal and family medical history, and lifestyle factors was collected through face-to-face interviews, physical examination, and blood tests. Asymptomatic ICAS was initially screened using transcranial Doppler examination and then diagnosed using magnetic resonance angiography. Atherosclerosis in carotid arteries was diagnosed via carotid ultrasonography. High-resolution magnetic resonance imaging was further used to evaluate the vessel wall of aICAS. Neuropsychological assessments were performed in the participants diagnosed with aICAS.

Findings to date: Of the 2311 participants, 2027 (87.7%) completed the diagnostic procedure and aICAS was detected in 154 persons, resulting in an overall prevalence of 7.6%. The prevalence of aICAS increased with advancing age from 5.1% in participants aged 40-49 years to 12.7% in those aged ≥70 years (P<0.001). Asymptomatic ICAS was detected in 305 intracranial arteries, including 221 (72.5%) in the anterior circulation and 84 (27.5%) in the posterior circulation (P<0.001). In addition, major CRFs were highly prevalent among middle-aged and elderly rural dwellers who were free of clinical stroke.

Future plans: Follow-up examinations will be performed every 3 years following the baseline examination. This study will increase our knowledge about the natural history of aICAS and facilitate studies of aICAS-associated disorders among rural-dwelling Chinese adults, such as ischaemic stroke and vascular cognitive impairment.

Strengths and limitations of this study

- This population-based study integrated TCD and MRA to detect aICAS among residents living in rural communities, which is a feasible approach to obtain a relatively accurate prevalence of aICAS in rural residents.
- High-resolution MRI scans were used to further identify characteristics of aICAS.
- Neuropsychological evaluation in this study covers global cognitive function and multiple cognitive domains.
- This study used TCD as a screening tool for aICAS, which might have missed some cases and underestimated the prevalence of aICAS.
- The study sample was selected from only one rural area, and thus, cautiousness is needed when generalizing the study findings to other rural populations in China.

INTRODUCTION

Intracranial artery stenosis (ICAS) due to atherosclerosis is a leading cause of ischaemic stroke worldwide, especially in Asian populations[1]. In addition, intracranial atherosclerosis has been associated with cognitive impairment [2]. Atherosclerotic lesions may develop and progress silently over years, subsequently impair cognitive function, or even suddenly cause clinical stroke. Therefore, the detection of ICAS in the asymptomatic phase may facilitate early preventive and therapeutic interventions, and thus delay the progression to symptomatic stroke and cognitive impairment[3, 4]. Several population-based studies that investigated the prevalence of asymptomatic ICAS (aICAS, ie, ICAS without a history of stroke) by using non-invasive tools, such as transcranial ultrasound or magnetic resonance angiography (MRA), suggested that the prevalence of aICAS ranged from around 9% in Caucasian populations[5, 6] and 12% in African-Americans [6] to as high as 24.5% in Asian populations[7-10]. The substantial racial differences in the prevalence and distribution of ICAS could be due partly to different genetic and environmental factors [11]. Therefore, this study about Asian populations who are more vulnerable to ICAS may shed light on the effects of genetic backgrounds, circumstances or social customs, and habits difference on the development of ICAS. Moreover, data remain limited with regard to the prevalence of aICAS among rural residents in China, where cardiovascular risk factors (CRFs) are highly prevalent and poorly controlled[12].

Furthermore, the natural history and prognosis of aICAS remains poorly understood. The Warfarin versus Aspirin Symptomatic Intracranial Disease trial showed that the risk of stroke from aICAS was relatively low among patients with co-existing symptomatic ICAS and aICAS[13]. In addition, asymptomatic atherosclerotic stenosis of the middle cerebral artery (MCA) is apparently not a malignant disease[14, 15]. These studies suggest that aICAS may have a benign long-term prognosis. It is worth noting that these previous studies have either focused on selective patient groups (co-existence of asymptomatic and symptomatic stenosis) or targeted special vessels (e.g., MCA), and the general population-based studies that aimed at investigating the prognosis of aICAS remain scarce. Indeed, a population-based prospective study of 2807 healthy volunteers (mean age, 62.0 years) in Japan found that aICAS was a risk factor for clinical stroke[16]. However, the predictors for the conversion and progression of aICAS to the symptomatic stage remain largely unclear.

Symptomatic ICAS (i.e., ICAS with a history of stroke) is an independent risk factor for cognitive decline. However, whether aICAS is associated with subsequent cognitive decline remains to be explored. Recently, a clinical-based study suggested that in participants with asymptomatic severe MCA stenosis, poor collateral circulation was associated with declines in multiple cognitive domains characterized by vascular cognitive impairment[17]. The association between cognitive function and aICAS in other intracranial arteries remains to be clarified.

Therefore, we initiated the Kongcun Town aICAS (KT-aICAS) study in Shandong Province, China. This cohort profile aimed to describe the establishment of the study cohort, baseline assessments, and some of the findings from this cohort.

Cohort description

Study Design and Purposes

KT-aICAS is designed as a population-based prospective cohort study. The purposes of the KT-aICAS were: (1) to investigate occurrence (e.g., prevalence and incidence) and distribution of aICAS among middle-aged and older adults living in rural communities; (2) to explore CRFs of aICAS and clinical biomarkers that may predict the progression from aICAS to ischaemic stroke; (3) to assess the association between aICAS and cognitive impairment; and (4) to build a comprehensive database that facilitates longer-term multidisciplinary research regarding the biomedical, clinical, neuroimaging, and epidemiological aspects of aICAS. At baseline, a cross-sectional study was conducted to detect the prevalence and distribution of aICAS. A subsequent follow-up assessment will be performed to determine the incidence rates of aICAS, examine the association between aICAS and ischaemic stroke or cognitive impairment, and explore a variety of CRFs, clinical features, imaging markers or biomarkers for the conversion from aICAS to symptomatic ICAS, clinical stroke, and the potential cognitive consequences.

Study Communities and Population

The KT-aICAS Study targeted all registered residents who were aged ≥40 years, free of a history of stroke, and living in the Kongcun Town of Pingyin county in Shandong Province in October 2017. Kongcun Town is a rural township that is located ~76 km west of Jinan, the capital of Shandong Province, China. Kongcun Town includes ~30000 residents in an area of ~5000 hectares. The majority of people in the town are farmers. Since the 2000s, the School of Public Health at Shandong University has been working closely with the town government and local health professionals to promote the health of local residents. This helped improve the cooperation from local residents (e.g., high participation rate at baseline and possibly high adherence to future follow-ups) for implementing this project. Following the baseline assessment in 2017-2018, all participants will be invited to undertake a follow-up assessment every 3 years with the aim of detecting patients with aICAS and identifying incident clinical stroke and cognitive impairment. Figure 1 shows the basic design of the KT-aICAS Study and the flowchart of the study population and assessments.

Ethics Approval and Consent to Participate

The protocol of the KT-aICAS Study was reviewed and approved by the ethical standards committees on human experimentation at Shandong Provincial Hospital Affiliated to Shandong University. Written informed consent was obtained from all of the participants. The study was conducted in accordance with the principles for medical research involving human subjects expressed in the Declaration of Helsinki. The face-to-face interviews were scheduled at the most convenient times for the participants. Respondents were free to refuse participation or withdraw from the study at any time. Any case of serious illness that had not been detected previously, was urgently referred to the local hospital for further examinations. All participant-related information was de-identified from the database to preserve privacy.

Baseline Assessments

Baseline assessments were performed in October 2017-October 2018, which included a two-phase design as described below.

Phase 1

From October to November 2017, we performed a phase 1 assessment, a screening phase that aimed to collect baseline data and identify people at high risk of aICAS. During this phase, all participants underwent a structured questionnaire survey, a transcranial Doppler (TCD) examination, and a colour Doppler ultrasound examination (neck) on the same day.

Face-to-face interviews. A standardized questionnaire was used to collect data on demographic features, health and family history (e.g., chronic diseases), CRFs (e.g., hypertension, diabetes, and obesity), lifestyle factors (e.g., diet habits, smoking, alcohol consumption, tea consumption, and physical activity), menstrual and reproductive history, quality of life, and current use of medication [18, 19]. Typically, the interview took approximately 30 minutes. Then, all participants underwent physical and clinical examinations, which included BP and anthropometric measurements, carotid artery B-mode ultrasonography, and TCD examination.

BP and anthropometric measurements. All measurements were performed by trained nurses. After a 5-min rest, arterial blood pressure was measured twice at a 5-min interval using an automated sphygmomanometer with the participant in the seated position. The mean value of the two measurements was used in the analysis. Hypertension was defined as blood pressure ≥140/90 mmHg or use of antihypertensive drugs. Height was measured without shoes using a standard right-angle device and a fixed measurement tape (to the nearest 0.5 cm). Body weight without heavy clothing was measured. We defined obesity as a body mass index (BMI) ≥28 kg/m^2 and overweight as a BMI of 24-27.9 kg/m^2.

TCD ultrasound examination. Participants were examined with a portable machine (VIASYS Companion III, Nicolet, Washington, United States) by two experienced physicians with expertise in sonography after a 5-min rest in a quiet and comfortable room. The bilateral MCA, anterior cerebral artery (ACA), posterior cerebral artery (PCA), vertebral artery (VA), and basilar artery (BA) were examined with a 2 MHz probe through a temporal window, occipital window, and eye window, respectively. The depth, peak systolic velocity (PSV), mean flow velocity (MFV), end diastolic velocity, and pulsatility index were measured for each artery. The following MFV cut-offs were used for the diagnosis of ≥50% intracranial stenosis of various arteries according to the criteria described in the Stroke Outcomes and Neuroimaging of Intracranial Atherosclerosis (SONIA) trial[20]: MFV ≥100 cm/s for MCA stenosis, MFV ≥80 cm/s for VA stenosis, and MFV ≥80 cm/s for BA stenosis. Because the ACA and PCA were not evaluated in the SONIA trial, we followed previously validated criteria to define ≥50% stenosis in these vessels[21]: MFV ≥80 cm/s and ≥30% different when compared to the contralateral ACA segment for ACA stenosis and MFV ≥80 cm/s for PCA stenosis. The criteria for ≥70% stenosis were as follows[22]: MCA: MFV ≥120 cm/s or a stenotic/prestenotic ratio ≥3 or low velocity; VA and BA: MFV ≥110 cm/s or a stenotic/prestenotic ratio ≥3. In addition, the presence of turbulence or musical sounds and an abnormal segmental velocity were considered for the diagnosis of stenosis.

Carotid ultrasonography examination. Carotid ultrasound examination was performed in the head-straight flat supine position by two experienced physicians to measure intimal medial thickness (IMT), PSV, and EDV of the common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) with a 7-MHz linear transducer (Siemens ACUSON P500). First, the structure of the vascular wall of the full-length of the CCA and the bifurcation and the initial segment of the ICA as well as atherosclerotic plaques and intravascular echo were measured in a cross-section. Then, the IMT of the distal segment of the CCA (1 to 1.5 cm below the bifurcation level), the internal diameter of the ICA, and the distal segment of the CCA were measured in longitudinal sections. The diameter was defined as the vertical distance between the upper edge of the posterior wall of the vessel and the inferior border of the anterior wall. When vessel stenosis occurred, the residual diameter and original diameter were measured. The thickness of the IMT was defined as the vertical distance between the upper edge of the posterior wall of the vessel and the upper edge of the outer membrane. Location, morphology, and integrity of the fibrous cap and the acoustic characteristics of the plaque were observed, and the size of the plaque was measured. Intimal thickening was defined as intimal-middle film thickness ≥1.0 mm, and plaque was defined as focal intimal-middle film thickness ≥1.5 mm[23].

Laboratory tests. After overnight fasting, participants came to the local clinic, and peripheral venous blood samples were taken in the morning. The blood was then allowed to clot in a 37 °C water bath for 30 minutes before it was centrifuged at 3,000 rpm for 15 minutes. Then, the serum supernatant was collected and separated into five aliquots (200 μL per aliquot) and immediately stored in liquid nitrogen. Blood tests were conducted at the certified clinical laboratory of the Shandong Provincial Hospital Affiliated to Shandong University. We measured a complete blood count in the whole blood as well as serum fasting blood glucose (FBG), liver function, renal function, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglyceride, apolipoprotein genotyping, homocysteine, supersensitivity C-reactive protein, and insulin. The remaining serum samples were stored at -80 °C until further analyses. Diabetes was defined as FBG ≥7.0 mmol/l or current use of blood glucose-lowering agents or insulin injection. High cholesterol was defined as total cholesterol ≥6.20 mmol/l or current use of hypolipidaemic drugs, and dyslipidaemia was defined as total cholesterol ≥6.20 mmol/l, triglycerides ≥1.8 mmol/l, HDL-C <1.11 mmol/l or LDL-C ≥3.36 mmol/l or current use of hypolipidaemic drugs.

Phase 2

From April to October 2018, participants who were screened positive, and a group of age- and sex-matched controls (1:1) who were screened negative for ICAS by TCD in Phase 1, underwent Phase 2 assessment the day in our hospital, which included neuropsychological assessments, structural brain MRI scans, and MRA.

MRI protocol and acquisition. All invited participants underwent brain MRI scans on a 3.0T scanner (Achieve; Philips Medical Systems, Best, The Netherlands) with an 8-channel head coil. The core MRI protocol included MRA, dynamic susceptibility contrast-enhanced perfusion-weighted imaging (DSC-PWI), diffusion tensor imaging (DTI), T1-weighted imaging with 3D variable refocusing flip angle volume isotropic turbo spin-echo acquisition (3D T1VISTA), 3D T2VISTA, fluid-attenuated inversion recovery images, and contrast-enhanced T1VISTA. The post-enhanced T1VISTA parameters were the same as those used for pre-T1VISTA. A gadolinium contrast medium (Gd-DTPA, Magnevist, Beijing BeiLu Pharmaceutical Co., Ltd., Beijing, China) was intravenously injected at the third acquisition (0.2 mL/kg or 4.5 mL/s followed immediately by a 30 mL physiological saline flush). The extent of stenosis in the five evaluated arteries (bilateral MCA, bilateral intracranial ICA, and BA) was classified into five grades by consensus as normal, mild (signal reduction <50%), moderate (signal reduction ≥50% and <70%), severe (signal reduction ≥70%), or occlusion (focal signal loss with the presence of distal signal). The diagnosis and grading of stenosis was performed by a neurologist specializing in stroke and a clinical neuroradiologist.

Neuropsychological evaluation. Neuropsychological assessments were performed by experienced neuropsychologists trained specifically for this project and included tests of global cognitive function and specific cognitive domains, such as memory, executive function, attention, visuospatial function, and mood. The Chinese versions of the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) were performed to assess global cognitive function[24]. Specific cognitive domains (e.g., episodic memory, verbal fluency, attention, and executive function) were assessed using the following neuropsychological tests: (1) the Rey Auditory Verbal Learning Test Learning (RAVLT: direct recall, delayed recall, and word list recognition) for episodic memory[25], the Fuld-object Memory Evaluation (FOM) for object memory[26], (2) the Category Verbal Fluency test (CVF) for verbal performance and executive function[27], (3) the Trail Making Test (TMT A&B) for executive function[28], (4) the Wechsler Memory Scale-III Forward Digit Span (FDS) and Backward Digit Span(BDS) for attention and executive function[29], and (5) the Wechsler block design test and Clock Drawing Test (CDT) for visuospatial and motor skills[30]. In addition, depressive symptoms were assessed using the Hamilton depression Scale (HAMD) [31]. The Barthel index was used to assess basic activities of daily living (ADL) [32].

Quality control procedure. Before the beginning of the study, all staff were centrally trained and required to demonstrate competency to perform different assessments, including interviews, biospecimen acquisition, cerebrovascular assessments, and cognitive testing. Psychologists, neuroradiologists, and neurologists attended a standardization meeting to ensure the consistency of application of the evaluation criteria. The collection and processing of the data were checked and verified by two or even three people, and the entry of the collected data was completed via integral double entry.

Statistical Analysis

The data are presented as frequencies (%) for categorical variables and as the mean (SD) or median (interquartile range, IQR) for continuous variables. Characteristics of the study participants by sex were compared using the general linear regression model for continuous variables and chi-square test for categorical variables. We describe the distribution of major CRFs and aICAS. We used the statistical packages R (http://www.r-project.org; version 3.4.3) and EmpowerStats (www.empowerstats.com; X&Y Solutions Inc.) for all analyses.

Follow-up assessments

All survivors of the baseline participants will be invited to undertake a follow-up evaluation in the third year. The same questionnaire used for the baseline survey will be used during the follow-up examination for collecting epidemiological, neuropsychological, and clinical data. Analysis of biomarkers as well as TCD test, carotid ultrasonography examination, and brain MRI scan will also be performed.

Patient and public involvement

No patients or public were involved in the development of research questions and the design of the project. Cardiovascular risk factors and stenosed arteries would be screened out during the project. Thus, primary and secondary prevention of stroke will surely benefit from this project. Furthermore, in the future patients may benefit from the knowledge generated from this project once it becomes publicly available.

Findings to date

In this ongoing prospective cohort study of middle-aged and older adults who were free of clinical stroke, we have demonstrated the feasibility of a two-phase procedure to study the epidemiology and nature history of aICAS in rural communities by integrating epidemiological approaches with clinical, neuroimaging, and neuropsychological techniques.

The characteristics of baseline participants in KT-aICAS are reported in table 1. The mean age of the 2311 participants was 57.6 years, and 53.4% were women. Compared with men, women were older, less educated, and less likely to be single, and had higher levels of total cholesterol, triglycerides, LDL-C, and BMI, but lower levels of diastolic pressure and HDL-C. There was no significant sex difference in levels of FBG or systolic pressure and prevalence of hypertension. Men were much more likely to smoke and consume alcohol than women across all age groups, whereas women had a higher prevalence of diabetes, obesity and a higher cholesterol level than men mainly in groups over 60 years of age (figure 2). The sociocultural tradition in China, especially in rural regions, could explain the sex differences in behavioural factors, whereas the differences in health conditions might be partially contributable to sex differences in factors such as educational achievement, socioeconomic position, lifestyles, and dietary habits. Taken together, our data showed that major CRFs were highly prevalent in rural-dwelling middle-aged and elderly people in China and that there were substantial sex differences in certain lifestyle factors. We have reported that these CRFs, especially when coexisting, are strongly correlated with aICAS [33]. The potential cognitive consequence of CRFs and aICAS including the characteristics of vessel plaque image evaluated by 3T MRI will be analysed in a future study. Therefore, given the well-established aetiological relationship of CRFs with atherosclerosis and clinical stroke, our study suggests that active interventions to target these modifiable risk factors are urgently needed to counteract the huge burden of stroke and dementia in rural areas of China.

Of the 2311 participants, 2027 (87.7%) completed the diagnostic procedure for aICAS. Out of the 2027 participants, 437 (21.5%) participants have poor temporal window, and 154 persons (92 women and 62 men) were diagnosed with aICAS, which corresponded to the overall prevalence of 7.6% (8.7% in women vs. 6.4% in men, P=0.055). The overall prevalence of aICAS increased with advancing age, from 5.1% in individuals aged 40-49 years to 12.7% in those aged ≥70 years (figure 3). There was a sex difference in the patterns of age-specific prevalence of aICAS, such that the prevalence increased steadily with age in men, but in women the prevalence was relatively stable from 40 to 60 years of age, and then substantially increase. The reasons for the sex differences is not fully understood, but the fast increase in the prevalence of aICAS with age after 60 years of age might be partially due to a decrease in estrogen levels among postmenopausal women [34].

A total of 305 intracranial arteries were diagnosed with aICAS; 221 (72.5%) were involved in ACA, MCA or ICA, and 84 (27.5%) were involved in PCA, VA or BA (table 2). The distributions of aICAS by regional arteries were as follows: ACA, 55 (18.0%) arteries; MCA, 95 (31.1%) arteries; ICA, 71 (23.3%) arteries; PCA, 38 (12.5%) arteries; VA, 25 (8.2%) arteries; BA, 21 (6.9%) arteries. The arteries in the anterior circulation were more frequently involved than arteries in the posterior circulation.

Most previous studies have used either TCD or MRA alone to diagnose aICAS, and different detection approaches and procedures may contribute to the substantial variations in the reported prevalence of aICAS. For example, the prevalence of aICAS was reported to be 13.2% among coal mine workers aged ≥40 years in a study that used only TCD to detect aICAS, whereas another study of people aged ≥40 years (mean age, 58.1 years) from Korea reported that the prevalence of aICAS was 24.5% in all intracranial vessels[7]. We used a two-phase approach that included a screening phase with TCD examination followed by a diagnostic phase with MRA, and we found that the overall prevalence of aICAS was 7.6% in this rural-dwelling middle-aged and elderly population. TCD examination, when used as a tool for detecting arterial stenosis, has the advantage of convenience and non-invasiveness but also has a high negative predictive value (~90%); thus, TCD is suitable for screening arterial stenosis in a large-scale population study. However, the positive predictive value of TCD examination is only ~50%. In contrast, MRA provides a good positive predictive value for ICAS but may not be suitable for large-scale community-based studies due to its high costs and inconvenience[20]. In this study, we combined TCD with MRA to detect aICAS, which is feasible especially among people who are living in the remote rural communities. This approach will allow us to obtain a more accurate estimate of aICAS prevalence than that was achieved in previous studies used either TCD or MRA alone.

We also found that the distribution of aICAS was more common in the anterior circulation than in the posterior circulation, in accordance with a study from Japan[35]. The variations in the regional arterial distribution of aICAS are likely due to differences in the anatomical and haemodynamic characteristics between the anterior and posterior circulation.

For example, in the anterior circulation, the higher number of vascular branches may increase the risk of atherosclerosis[36]. In addition, TCD has relatively low sensitivity in detecting posterior circulation stenosis[37], and this may have contributed, in part, to the differences observed in the regional arterial distribution of aICAS.

In summary, in the community-based KT-aICAS study, aICAS affected nearly one-tenth of the rural-dwelling middle-aged and elderly residents, and its prevalence increased with advanced age. Anterior circulation arteries were most vulnerable to atherosclerotic lesions. In addition, major CRFs were highly prevalent among middle-aged and elderly rural dwellers who were free of stroke. These findings may have important implications for the primary prevention of aICAS, clinical stroke, and cognitive impairment. Future follow-up data from this cohort may contribute to the understanding of the epidemiology and natural history as well as cognitive consequences of aICAS among rural residents in China.

Strengths and Limitations

This cohort study targeted residents living in rural communities in China, to whom the research community has so far paid little attention. Second, we integrated TCD and MRA into a two-phase procedure to detect aICAS in a large-scale population study, and this approach is likely to allow us to obtain a relatively accurate estimate of the prevalence of aICAS. Furthermore, high-resolution MRI scans could help further evaluate the characteristics of arterial stenosis and avoid the contamination of ICAS cases with cases involving other non-atherosclerotic diseases[38]. In addition, high-resolution MRI can be used to detect intraplaque haemorrhage, lipid cores, and artery fibrous caps, thus providing further insight into the long-term prognosis of aICAS. To the best of our knowledge, this is the first community-based study of aICAS among an Asian population that used high-resolution MRI as well as PWI images and DTI images. Finally, this community-based study includes comprehensive neuropsychological assessments that covers global cognitive function (MMSE and MoCA) and multiple cognitive domains (e.g., memory, verbal fluency, attention, and executive function), and thus, will facilitate future exploration of specific cognitive profiles associated with aICAS and its progression.

Our study also has limitations. First, we used TCD as a screening tool for arterial stenosis, which might have missed some cases of aICAS, and thus, have underestimated the overall prevalence of aICAS. Furthermore, in case of a poor or incomplete echo window for TCD, people without any stenosis in the VA, BA, and ACA were considered to be non-stenotic. This might also have resulted in the underestimation of the true prevalence of aICAS in this population. Finally, our study sample was selected from only one rural area, and this should be kept in mind when generalizing our findings to other rural populations.

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Author Contributions QS, FX, and YD conceived the research question and designed the study. XW and FX conducted the statistical analysis. XW, CQ, and QS wrote the first and subsequent drafts of the manuscript. XJ, SwS, SS, YZ, PY, SL, JL, GW, and ML contributed to the epidemiological investigation. All authors contributed to data interpretation and critical revisions of the manuscript, and approved the final version of the submitted manuscript.

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Competing interests None declared.

Participant consent Obtained.

Ethics approval Shandong Provincial Hospital Affiliated to Shandong University in Jinan, Shandong, China (2017566).

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Table 1. Characteristics of the study participants by gender

Characteristics[b]	Total (n=2311)	Men (n=1076)	Women (n=1235)	P Value[a]
Age (years), mean (SD)	57.6(10.5)	56.3(10.3)	58.8(10.5)	<0.001
Age group (years), n(%)				<0.001
40-49	613(26.6)	353(32.9)	260(21.1)	
50-59	733(31.8)	334(31.1)	399(32.4)	
60-69	607(26.3)	240(22.3)	367(29.8)	
≥70	352(15.3)	147(13.7)	205(16.7)	
Marital status[b], n (%)				<0.001
Single	252(10.9)	72(6.7)	180(14.6)	
Married	2058(89.1)	1003(93.3)	1055(85.4)	
Educational level[b], n (%)				<0.001
Illiterate	354(15.3)	36(3.3)	318(25.8)	
Elementary	679(29.4)	247(23.0)	432(35.0)	
Middle school	976(42.3)	582(54.1)	394(31.9)	
High school and above	301(13.0)	211(19.6)	90(7.3)	
Body mass index (kg/m^2)[b], mean (SD)	25.2(3.8)	24.8(3.2)	25.5(4.1)	<0.001
Systolic pressure (mm Hg)[b], mean (SD)	145.0(22.4)	144.5(21.3)	145.4(23.4)	0.365
Diastolic pressure (mm Hg)[b], mean (SD)	88.3(12.7)	89.9(13.0)	86.9(12.2)	<0.001
Fasting blood glucose (mmol/l)[b], mean (SD)	6.1(1.8)	6.0(1.8)	6.1(1.9)	0.084
LDL-C (mmol/l)[b], mean (SD)	3.0(0.7)	2.9(0.7)	3.1(0.7)	<0.001
HDL-C (mmol/l)[b], mean (SD)	1.6(0.4)	1.6(0.4)	1.6(0.3)	0.031
Triglycerides (mmol/l)[b], mean (SD)	1.4(0.9)	1.3(0.9)	1.5(0.9)	<0.001
Total cholesterol (mmol/l)[b], mean (SD)	5.4(1.0)	5.2(0.9)	5.5(1.0)	<0.001
Dyslipidemia[b], n (%)	922(41.7)	378(36.2)	544(46.7)	<0.001
Smoking[b], n (%)	504(21.9)	489(45.6)	15(1.2)	<0.001
Alcohol consumption[b], n (%)	727(31.5)	672(62.5)	55(4.5)	<0.001
Obesity[b], n (%)	437(19.2)	165(15.5)	272(22.5)	<0.001
Hypertension[b], n (%)	1294(56.6)	592(55.3)	702(57.8)	0.220

Diabetes[b], n (%)	725(32.9)	319(30.5)	406(34.9)	0.028
Carotid stenosis[b], n (%)	59(2.9)	33(3.4)	26(2.5)	0.202
aICAS[b], n (%)	154(7.6)	62(6.4)	92(8.6)	0.055
MMSE score[c], mean (SD)	23.4(5.4)	24.7(4.4)	22.5(5.9)	0.001
MoCA score[c], mean (SD)	17.9(5.3)	18.6(4.8)	17.5(5.6)	0.088

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; aICAS: asymptomatic intracranial artery stenosis; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment.

[a]P values are for the test of differences between male and female.

[b]The number of participants with missing value were one for marital status and educational level, 38 for body mass index and obese status, 29 for blood pressure, 175 for fasting blood glucose, 106 for lipid measurements (LDL-C, HDL-C, triglycerides, and total cholesterol), eight for smoking, two for alcohol consumption, 102 for dyslipidemia, 26 for hypertension, 104 for diabetes, 284 for carotid stenosis, and 284 for aICAS (of the 2311 participants, 2027 completed the diagnostic procedure for aICAS).

[c]MMSE and MoCA tests were performed in 308 participants, including 154 with aICAS and 153 age- and sex-matched controls.

Table 2. Arterial distribution of asymptomatic intracranial atherosclerotic stenosis (aICAS) according to severity of stenosis (Total number of arteries with aICAS = 305)

Arteries	Severity of intracranial arterial stenosis, n (%)				
	Mild	Moderate	Severe	Occlusion	Total
ICA	28 (9.2)	20 (6.6)	17 (5.6)	6 (2.0)	71(23.3)
MCA	45 (14.8)	21 (6.9)	23 (7.6)	6 (2.0)	95(31.2)
ACA	22 (7.2)	17 (5.6)	16 (5.3)	0 (0.0)	55(18.0)
PCA	10 (3.3)	9 (3.0)	18 (5.9)	1 (0.3)	38(12.5)
VA	4 (1.3)	2 (0.7)	8 (2.6)	11 (3.3)	25(8.2)
BA	13 (4.3)	3 (1.0)	5 (1.6)	0 (0.0)	21(6.9)

MCA, middle cerebral artery; ACA, anterior cerebral artery; ICA, intracranial internal carotid artery; PCA, posterior cerebral artery; VA, vertebral artery; BA, basilar artery.

Figure Legends

Figure 1 Flowchart of the study

Figure 2 Age- and gender-specific prevalence (per 100 population) of cardiovascular risk factors

Figure 3 Age- and gender-specific prevalence (per 100 population) of aICAS

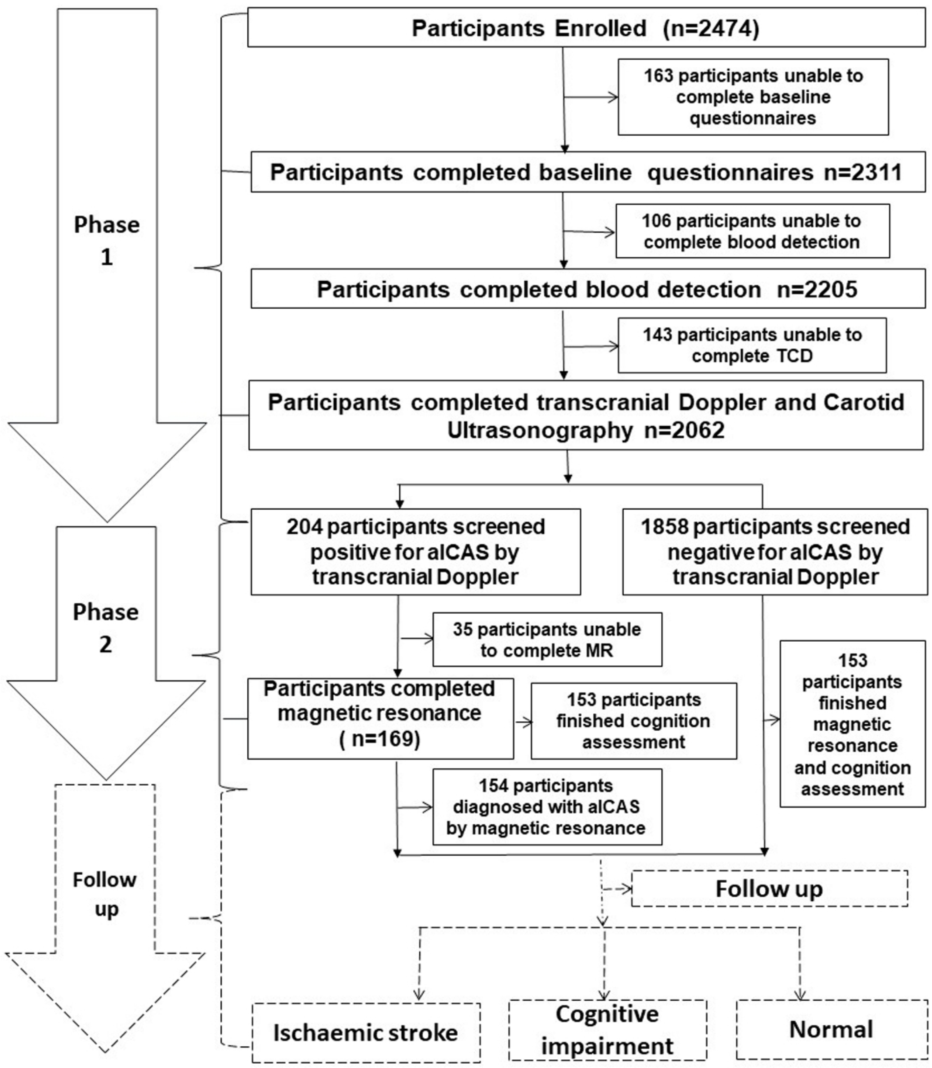


Figure 1 Flowchart of the study

236x263mm (300 x 300 DPI)

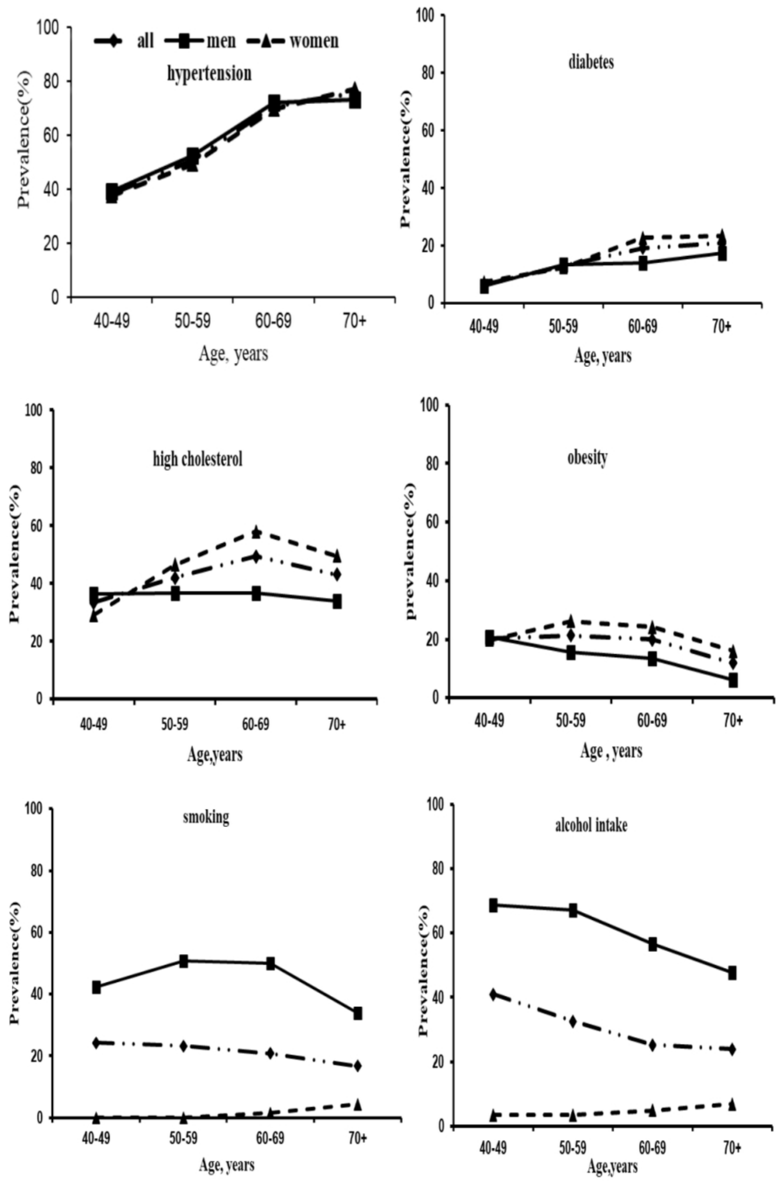
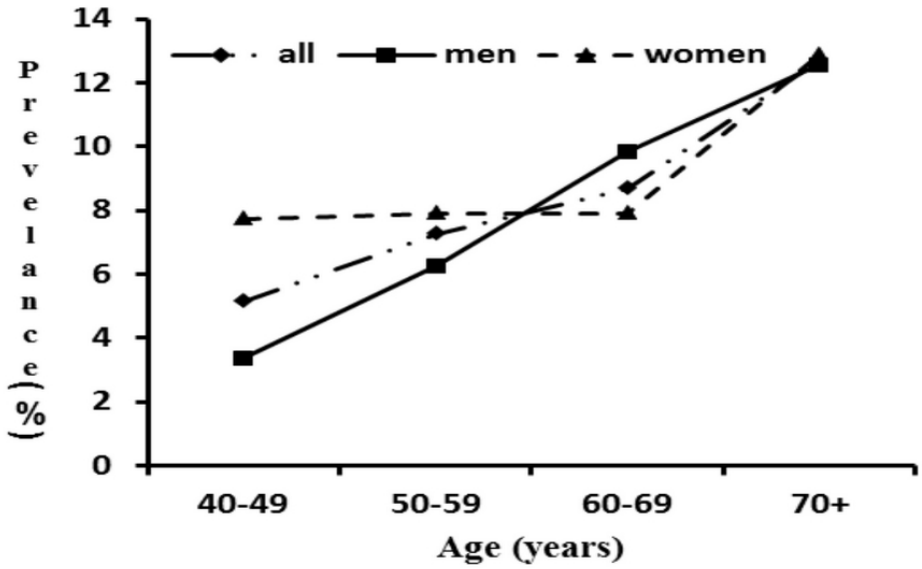


Figure 2 Age- and gender-specific prevalence (per 100 population) of cardiovascular risk factors

101x145mm (300 x 300 DPI)



all: P for trend<0.001; Men: P for trend<0.001; Women: P for trend =0.140

Figure 3 Age- and gender-specific prevalence (per 100 population) of aICAS

78x56mm (300 x 300 DPI)

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Cohort profile: the Kongcun Town Asymptomatic Intracranial Artery Stenosis Study in Shandong, China

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Complete List of Authors:	Wang, Xiang ; Shandong Provincial Hospital, Department of Neurology Zhao, Yuanyuan; Shandong Provincial Hospital, Department of Neurology Ji, Xiaokang; Shandong University School of Public Health, Department of biostatistics Sang, Shaowei; Shandong University Qilu Hospital, Department of clinical epidemiology Shao, Sai; Shandong Medical Imaging Research Institute, Department of Radiology Yan, Peng; Shandong Provincial Hospital, Department of Neurology Li, Shan; Shandong Provincial Hospital, Department of Neurology Li, JiFeng; Shandong Provincial Hospital, Department of Neurology Wang, Guangbin; Shandong Medical Imaging Research Institute, Department of Radiology Lu, Ming; Shandong University, Department of clinical epidemiology Du, YiFeng; Shandong Provincial Hospital, Department of Neurology Xue, Fuzhong; Shandong University School of Public Health, Department of biostatistics Qiu, Chengxuan; Shandong Provincial Hospital, Department of Neurology; Karolinska Universitetssjukhuset, Department of Neurobiology, Care Sciences and Society Sun, QinJian; Shandong Provincial Hospital, Department of Neurology
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Xiang Wang[a,b], Yuanyuan Zhao[a,b], Xiaokang Ji[c], Shaowei Sang[d], Sai Shao[e], Peng Yan[a,b], Shan Li[a,b], Jifeng Li[a,b], Guangbin Wang[e], Ming Lu[d], Yifeng Du[a,b], Fuzhong Xue[c], Chengxuan Qiu[a,f], Qinjian Sun[a,b]*

[a]Department of Neurology, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong, China

[b]Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, China

[c]Department of Biostatistics, School of Public Health, Shandong University, Jinan, Shandong, China

[d]Department of Clinical Epidemiology, Qilu Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong, China

[e]Department of Radiology, Shandong Medical Imaging Research Institute Affiliated to Shandong University, Jinan, Shandong, China

[f]Aging Research Center, Department of Neurobiology, Care Science and Society, Karolinska Institutet-Stockholm University, Stockholm, Sweden

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＊Corresponding author:

Qinjian Sun

Department of Neurology, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University

Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong First Medical University

＃324 Jingwu Weiqi Road, Jinan, Shandong 250021, P. R. China.

Email: sqj1210@163.com

ABSTRACT

Purpose: The population-based Kongcun Town Asymptomatic Intracranial Artery Stenosis (KT-aICAS) study aims to investigate the prevalence of aICAS and major cardiovascular risk factors (CRFs) or biomarkers related to the development and prognosis of aICAS.

Participants: The KT-aICAS study included 2311 rural residents who were aged ≥40 years and living in Kongcun Town, Shandong Province, China. Baseline examination was conducted from October 2017 to October 2018, during which information on demographics, socioeconomics, personal and family medical history, and lifestyle factors was collected through face-to-face interviews, physical examination, and blood tests. Asymptomatic ICAS was initially screened using transcranial Doppler examination and then diagnosed using magnetic resonance angiography. Atherosclerosis in carotid arteries was diagnosed via carotid ultrasonography. High-resolution magnetic resonance imaging was further used to evaluate the vessel wall of aICAS. Neuropsychological assessments were performed in the participants diagnosed with aICAS and the age- and sex-matched controls.

Findings to date: Of the 2311 participants, 2027 (87.7%) completed the diagnostic procedure and aICAS was detected in 154 persons, resulting in an overall prevalence of 7.6%. The prevalence of aICAS increased with advancing age from 5.1% in participants aged 40-49 years to 12.7% in those aged ≥70 years ($P<0.001$). Asymptomatic ICAS was detected in 305 intracranial arteries, including 221 (72.5%) in the anterior circulation and 84 (27.5%) in the posterior circulation ($P<0.001$). In addition, major CRFs were highly prevalent among middle-aged and elderly rural dwellers who were free of clinical stroke.

Future plans: Follow-up examinations will be performed every 3 years following the baseline examination. This study will increase our knowledge about the natural history of aICAS and facilitate studies of aICAS-associated disorders among rural-dwelling Chinese adults, such as ischaemic stroke and vascular cognitive impairment.

Strengths and limitations of this study

- This population-based study integrated TCD and MRA to detect aICAS among residents living in rural communities, which is feasible to investigate prevalence of aICAS in rural residents.
- High-resolution MRI scans were used to further identify characteristics of aICAS.
- The use of TCD as a screening tool for aICAS might have missed some cases and underestimated the prevalence of aICAS.
- The study sample was selected from only one rural area, and thus, cautiousness is needed when generalizing the study findings to other rural populations in China.

INTRODUCTION

Intracranial artery stenosis (ICAS) due to atherosclerosis is a leading cause of ischaemic stroke worldwide, especially in Asian populations[1]. In addition, intracranial atherosclerosis has been associated with cognitive impairment[2]. Atherosclerotic lesions may develop and progress silently over years, subsequently impair cognitive function, or even suddenly cause clinical stroke. Therefore, the detection of ICAS in the asymptomatic phase may facilitate early preventive and therapeutic interventions, and thus delay the progression to symptomatic stroke and cognitive impairment[3, 4]. Several population-based studies that investigated the prevalence of asymptomatic ICAS (aICAS, ie, ICAS without a history of stroke or transient ischaemic attack [TIA]) by using non-invasive tools, such as transcranial ultrasound or magnetic resonance angiography (MRA), suggested that the prevalence of aICAS ranged from around 9% in Caucasian populations[5, 6] and 12% in African-Americans[6] to as high as 24.5% in Asian populations[7-10]. The substantial racial differences in the prevalence and distribution of aICAS could be due partly to different genetic and environmental factors[11]. Therefore, the study involving Asian populations who are more vulnerable to ICAS or aICAS may shed light on the effects of genetic backgrounds, circumstances or social customs, and habits difference on the development of ICAS or aICAS. Moreover, data remain limited with regard to the prevalence of aICAS among rural residents in China, where cardiovascular risk factors (CRFs) are highly prevalent and poorly controlled[12].

Furthermore, the natural history and prognosis of aICAS remains poorly understood. The Warfarin versus Aspirin Symptomatic Intracranial Disease trial showed that the risk of stroke from aICAS was relatively low among patients with co-existing symptomatic ICAS and aICAS[13]. In addition, asymptomatic atherosclerotic stenosis of the middle cerebral artery (MCA) is apparently not a malignant disease[14, 15]. These studies suggest that aICAS may have a benign long-term prognosis. It is worth noting that these previous studies have either focused on selective patient groups (co-existence of asymptomatic and symptomatic stenosis) or targeted special vessels (e.g., MCA), and the general population-based studies that aimed at investigating the prognosis of aICAS remain scarce. Indeed, a population-based prospective study of 2807 healthy volunteers (mean age, 62.0 years) in Japan found that aICAS was a risk factor for clinical stroke[16]. However, the predictors for the conversion and progression from aICAS to the symptomatic stage remain largely unclear.

Symptomatic ICAS (i.e., ICAS with a history of stroke or TIA) is an independent risk factor for cognitive decline. However, whether aICAS is associated with subsequent cognitive decline remains to be explored. Recently, a clinical-based study suggested that in participants with asymptomatic severe MCA stenosis, poor collateral circulation was associated with declines in multiple cognitive domains characterized by vascular cognitive impairment[17]. The association between cognitive function and aICAS in other intracranial arteries remains to be clarified.

Therefore, we initiated the Kongcun Town aICAS (KT-aICAS) study in Shandong Province, China. This cohort profile aimed to describe the establishment of the study cohort, baseline assessments, and some of the findings from this cohort.

Cohort description

Study Design and Purposes

KT-aICAS is designed as a population-based prospective cohort study. The purposes of the KT-aICAS were: (1) to investigate occurrence (e.g., prevalence and incidence) and distribution of aICAS among middle-aged and older adults living in rural communities; (2) to explore CRFs and clinical biomarkers of aICAS that may predict the progression from aICAS to ischaemic stroke; (3) to assess the association between aICAS and cognitive impairment; and (4) to build a comprehensive database that facilitates longer-term multidisciplinary research regarding the biomedical, clinical, neuroimaging, and epidemiological aspects of aICAS. At baseline, a cross-sectional study was conducted to detect the prevalence and distribution of aICAS. A subsequent follow-up assessment will be performed to determine the incidence rates of aICAS, examine the association between aICAS and ischaemic stroke, and explore a variety of CRFs, clinical features, imaging markers or biomarkers for the conversion from aICAS to symptomatic ICAS, clinical stroke or TIA, and the potential cognitive consequences.

Study Communities and Population

The KT-aICAS Study targeted all registered residents who were aged ≥40 years, free of a history of stroke or TIA, and living in the Kongcun Town of Pingyin county in Shandong Province in October 2017. Self-report of a physician diagnosis and/or clinical examinations showing typical symptoms were consider as a history of stroke or TIA, which was determined during the interviews and clinical examination. Kongcun Town is a rural township that is located ~76 km west of Jinan, the capital of Shandong Province, China. Kongcun Town includes ~30000 residents in an area of ~5000 hectares. The majority of people in the town are farmers. Since 2009, the School of Public Health at Shandong

University has been working closely with the town government and local health professionals to conduct epidemiological surveys on health conditions of local residents every 3 years. This helped improve the cooperation from local residents (e.g., a high participation rate at baseline and possibly high adherence to future follow-up assessments) for implementing this project. Following the baseline assessment in 2017-2018, all participants will be invited to undertake a follow-up assessment every 3 years with the aim of detecting patients with aICAS and identifying incident clinical stroke and cognitive impairment. Figure 1 shows the basic design of the KT-aICAS Study and the flowchart of the study population and assessments.

Ethics Approval and Consent to Participate

The protocol of the KT-aICAS Study was reviewed and approved by the ethical standards committees on human experimentation at Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University. Written informed consent was obtained from all of the participants. The study was conducted in accordance with the ethical principles for medical research involving human subjects expressed in the Declaration of Helsinki. The face-to-face interviews were scheduled at the most convenient times for the participants. Respondents were free to refuse participation or withdraw from the study at any time. Any case of serious illness that had not been detected previously, was urgently referred to the local hospital for further examinations. All participant-related information was de-identified from the database to preserve privacy.

Baseline Assessments

Baseline assessments were performed in October 2017-October 2018, which included a two-phase design as described below.

Phase 1

From October to November 2017, we performed a phase 1 assessment, a screening phase that aimed to collect baseline data and identify people at high risk of aICAS. During this phase, all participants underwent a structured questionnaire survey, a transcranial Doppler (TCD) examination, and a colour Doppler ultrasound examination (neck) on the same day.

Face-to-face interviews. A standardized questionnaire was used to collect data on demographic features, health and family history (e.g., chronic diseases), CRFs (e.g., hypertension, diabetes, and obesity), lifestyle factors (e.g., diet habits, smoking, alcohol consumption, tea consumption, and physical activity), menstrual and reproductive history, quality of life, and current use of medication[18, 19]. Typically, the interview took approximately 30 minutes. Then, all participants underwent physical and clinical examinations, which included blood pressure and anthropometric measurements, carotid artery B-mode ultrasonography, and TCD examination.

Blood pressure and anthropometric measurements. All measurements were performed by trained nurses. After a 5-min rest, arterial blood pressure was measured twice at a 5-min interval using an automated sphygmomanometer with the participant in the seated position. The mean value of the two measurements was used in the analysis. Hypertension was defined as blood pressure ≥140/90 mmHg or use of antihypertensive drugs. Height was measured without shoes using a standard right-angle device and a fixed measurement tape (to the nearest 0.5 cm). Body weight without heavy clothing was measured. We defined obesity as a body mass index (BMI) ≥28 kg/m^2 and overweight as a BMI of 24-27.9 kg/m^2.

TCD ultrasound examination. Participants were examined with a portable machine (VIASYS Companion III, Nicolet, Washington, United States) by two experienced physicians with expertise in sonography after a 5-min rest in a quiet and comfortable room. The bilateral MCA, anterior cerebral artery (ACA), posterior cerebral artery (PCA), vertebral artery (VA), and basilar artery (BA) were examined with a 2 MHz probe through a temporal window, occipital window, and eye window, respectively. The depth, peak systolic velocity (PSV), mean flow velocity (MFV), end diastolic velocity, and pulsatility index were measured for each artery. The following MFV cut-offs were used for the diagnosis of ≥50% intracranial stenosis of various arteries according to the criteria described in the Stroke Outcomes and Neuroimaging of Intracranial Atherosclerosis (SONIA) trial[20]: MFV ≥100 cm/s for MCA stenosis, MFV ≥80 cm/s for VA stenosis, and MFV ≥80 cm/s for BA stenosis. Because the ACA and PCA were not evaluated in the SONIA trial, we followed previously validated criteria to define ≥50% stenosis in these vessels[21]: MFV ≥80 cm/s and ≥30% different when compared to the contralateral ACA segment for ACA stenosis and MFV ≥80 cm/s for PCA stenosis. The criteria for ≥70% stenosis were as follows[22]: MCA: MFV ≥120 cm/s or a stenotic/prestenotic ratio ≥3 or low velocity; VA and BA: MFV ≥110 cm/s or a stenotic/prestenotic ratio ≥3. In addition, the presence of turbulence or musical sounds and an abnormal segmental velocity were considered for the diagnosis of stenosis.

Carotid ultrasonography examination. Carotid ultrasound examination was performed in the head-straight flat supine position by two experienced physicians to measure intimal medial thickness (IMT), PSV, and EDV of the common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) with a 7-MHz linear transducer (Siemens ACUSON P500). First, the structure of the vascular wall of the full-length of the CCA and the bifurcation and the initial segment of the ICA as well as atherosclerotic plaques and intravascular echo were measured in a cross-section. Then, the IMT of the distal segment of the CCA (1 to 1.5 cm below the bifurcation level), the internal diameter of the ICA, and the distal segment of the CCA were measured in longitudinal sections. The diameter was defined as the vertical distance between the upper edge of the posterior wall of the vessel and the inferior border of the anterior wall. When vessel stenosis occurred, the residual diameter and original diameter were measured. The thickness of the IMT was defined as the vertical distance between the upper edge of the posterior wall of the vessel and the upper edge of the outer membrane. Location, morphology, and integrity of the fibrous cap and the acoustic characteristics of the plaque were observed, and the size of the plaque was measured. Intimal thickening was defined as intimal-middle film thickness ≥1.0 mm, and plaque was defined as focal intimal-middle film thickness ≥1.5 mm[23].

Laboratory tests. After overnight fasting, participants came to the local clinic, and peripheral venous blood samples were taken in the morning. The blood was then allowed to clot in a 37 °C water bath for 30 minutes before it was centrifuged at 3,000 rpm for 15 minutes. Then, the serum supernatant was collected and separated into five aliquots (200 μL per aliquot) and immediately stored in liquid nitrogen. Blood tests were conducted at the certified clinical laboratory of the Shandong Provincial Hospital Affiliated to Shandong University. We measured a complete blood count in the whole blood as well as serum fasting blood glucose

(FBG), liver function, renal function, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglyceride, apolipoprotein genotyping, homocysteine, supersensitivity C-reactive protein, and insulin. The remaining serum samples were stored at -80 °C until further analyses. Diabetes was defined as FBG ≥7.0 mmol/l or current use of blood glucose-lowering agents or insulin injection. High cholesterol was defined as total cholesterol ≥6.20 mmol/l or current use of hypolipidaemic drugs, and dyslipidaemia was defined as total cholesterol ≥6.20 mmol/l, triglycerides ≥1.8 mmol/l, HDL-C <1.11 mmol/l or LDL-C ≥3.36 mmol/l or current use of hypolipidaemic drugs.

Phase 2

From April to October 2018, participants who were screened positive, and a group of age- and sex-matched controls (1:1) who were screened negative for aICAS by TCD in Phase 1, underwent Phase 2 assessment in our hospital, which included neuropsychological assessments, structural brain MRI scans, and MRA.

MRI protocol and acquisition. All invited participants underwent brain MRI scans on a 3.0T scanner (Achieve; Philips Medical Systems, Best, The Netherlands) with an 8-channel head coil. The core MRI protocol included MRA, dynamic susceptibility contrast-enhanced perfusion-weighted imaging (DSC-PWI), diffusion tensor imaging (DTI), T1-weighted imaging with 3D variable refocusing flip angle volume isotropic turbo spin-echo acquisition (3D T1VISTA), 3D T2VISTA, fluid-attenuated inversion recovery images, and contrast-enhanced T1VISTA. The post-enhanced T1VISTA parameters were the same as those used for pre-T1VISTA. A gadolinium contrast medium (Gd-DTPA, Magnevist, Beijing BeiLu Pharmaceutical Co., Ltd., Beijing, China) was intravenously injected at the third acquisition

(0.2 mL/kg or 4.5 mL/s followed immediately by a 30 mL physiological saline flush). The extent of stenosis in the five evaluated arteries (bilateral MCA, bilateral intracranial ICA, and BA) was classified into five grades by consensus as normal, mild (signal reduction <50%), moderate (signal reduction ≥50% and <70%), severe (signal reduction ≥70%), or occlusion (focal signal loss with the presence of distal signal). The diagnosis and grading of stenosis was performed by a neurologist specializing in stroke and a clinical neuroradiologist.

Neuropsychological evaluation. Neuropsychological assessments were performed by experienced neuropsychologists trained specifically for this project and included tests of global cognitive function and specific cognitive domains. The Chinese versions of the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) were used to assess global cognitive function[24]. Specific cognitive domains (e.g., episodic memory, verbal fluency, attention, and executive function) were assessed using the following neuropsychological tests: (1) the Rey Auditory Verbal Learning Test Learning (RAVLT: direct recall, delayed recall, and word list recognition) for episodic memory[25], the Fuld-object Memory Evaluation (FOM) for object memory[26], (2) the Category Verbal Fluency test (CVF) for verbal performance and executive function[27], (3) the Trail Making Test (TMT A&B) for executive function[28], (4) the Wechsler Memory Scale-III Forward Digit Span (FDS) and Backward Digit Span(BDS) for attention and executive function[29], and (5) the Wechsler block design test and Clock Drawing Test (CDT) for visuospatial and motor skills[30]. In addition, depressive symptoms were assessed using the Hamilton depression Scale (HAMD)[31]. The Barthel index was used to assess basic activities of daily living (ADL)[32].

Quality control procedure. Before the beginning of the study, all staff were centrally trained and required to demonstrate competency to perform different assessments, including interviews, biospecimen acquisition, cerebrovascular assessments, and cognitive testing. Psychologists, neuroradiologists, and neurologists attended a standardization meeting to ensure the consistency of application of the evaluation criteria. The data collection and processing were checked and verified by at least two people, and the entry of the collected data was completed via integral double entry.

Statistical Analysis

The data are presented as frequencies (%) for categorical variables and as the mean (SD) or median (interquartile range, IQR) for continuous variables. Characteristics of the study participants by sex were compared using the general linear regression model for continuous variables and chi-square test for categorical variables. We describe the distribution of major CRFs and aICAS. We used the statistical packages R (http://www.r-project.org; version 3.4.3) and EmpowerStats (www.empowerstats.com; X&Y Solutions Inc.) for all analyses.

Follow-up assessments

All survivors of the baseline participants will be invited to undertake a follow-up evaluation in the third year. The same questionnaire used for the baseline survey will be used during the follow-up examination for collecting epidemiological, neuropsychological, and clinical data. Analysis of biomarkers as well as TCD test, carotid ultrasonography examination, and brain MRI scan will also be performed.

Patient and public involvement

No patients or public were involved in the development of research questions and the design of the project. Participants were informed of the examination procedures and the main results of the examination, and contributed to the data used for this study. We plan to disseminate the key findings from the project among participants and local residents as part of health education and promotion program.

Findings to date

In this ongoing prospective cohort study of middle-aged and older adults who were free of clinical stroke, we have demonstrated the feasibility of a two-phase procedure to study the epidemiology and nature history of aICAS in the remote rural communities by integrating epidemiological approaches with clinical, neuroimaging, and neuropsychological techniques.

The characteristics of baseline participants in KT-aICAS by sex are reported in table 1. Compared with men, women were older, less educated, and less likely to be single, and had higher levels of total cholesterol, triglycerides, LDL-C, and BMI, but lower levels of diastolic pressure and HDL-C. There was no significant sex difference in levels of FBG or systolic pressure and prevalence of hypertension. Men were much more likely to smoke and consume alcohol than women across all age groups, whereas women had a higher prevalence of diabetes, obesity and a higher cholesterol level than men mainly in groups over 60 years of age (figure 2). The sociocultural tradition in China, especially in rural regions, could explain the sex differences in behavioural factors such as smoking and alcohol consumption[33]. In addition, the differences in health conditions between men and women might be partially attributable to sex differences in factors such as educational achievement, socioeconomic position, and other unmeasured lifestyle factors (e.g., diet). Taken together, our data showed that major CRFs were highly prevalent among the rural-dwelling middle-aged and elderly people in western Shandong Province, China and that there were substantial sex differences in certain lifestyle factors. We have reported that these CRFs, especially when coexisting, are strongly correlated with aICAS[34]. Among the subsample of people who received neuropsychological tests, men had a higher score than women in the MMSE test (P=0.001), but not in MoCA test (P=0.088), in which higher educational attainments in men than in women may partly contribute to the sex differences in the global cognitive tests because MMSE test is more sensitive to education than MoCA test. The potential cognitive consequence of CRFs and aICAS will be fully analysed in a future study. Therefore, given the well-established aetiological relationship of CRFs with atherosclerosis and clinical stroke in the literature as well as findings from our study, active interventions to target these modifiable risk factors are urgently needed to counteract the huge burden of stroke in rural areas of China.

Of the 2311 participants, 2027 (87.7%) completed the diagnostic procedure for aICAS. Out of the 2027 participants, 437 (21.5%) participants have poor temporal window, and 154 persons (92 women and 62 men) were diagnosed with aICAS, which corresponded to the overall prevalence of 7.6% (8.7% in women vs. 6.4% in men, P=0.055). The overall prevalence of aICAS increased with advancing age, from 5.1% in individuals aged 40-49 years to 12.7% in those aged ≥70 years (p for trend <0.001) (figure 3). There was a sex difference in the patterns of age-specific prevalence of aICAS, such that the prevalence increased steadily with age in men (from 2.8% in 40-49 years of age to 12.9% in people aged

≥70 years) (p for trend <0.001), but in women the prevalence was relatively stable from 40 to 60 years of age (just over 8%), and then substantially increased (12.8% in people aged ≥70 years) (p for trend =0.141). The reason for the sex differences is not fully understood, but the fast increase in the prevalence of aICAS with age after 60 years of age might be partially due to a decrease in estrogen levels among postmenopausal women[35]. Indeed, evidence has suggested that oestrogens have atheroprotective effect via anti-inflammation, protection of vascular endothelial cells, and other mechanisms[36, 37], and that menopause has an unfavorable impact on several cardiovascular risk factors, structure of large arteries, and atherosclerosis[38].

A total of 305 intracranial arteries were diagnosed with aICAS; 221 (72.5%) were involved in ACA, MCA or ICA, and 84 (27.5%) were involved in PCA, VA or BA (table 2). The distributions of aICAS by regional arteries were as follows: ACA, 55 (18.0%) arteries; MCA, 95 (31.1%) arteries; ICA, 71 (23.3%) arteries; PCA, 38 (12.5%) arteries; VA, 25 (8.2%) arteries; BA, 21 (6.9%) arteries. The arteries in the anterior circulation were more frequently involved than arteries in the posterior circulation.

Most previous studies have used either TCD or MRA alone to diagnose aICAS, and different detection approaches and procedures may contribute to the substantial variations in the reported prevalence of aICAS. For example, the prevalence of aICAS was reported to be 13.2% among coal mine workers aged ≥40 years in a study that used only TCD to detect aICAS, whereas another study of people aged ≥40 years (mean age, 58.1 years) from Korea reported that the prevalence of aICAS was 24.5% in all intracranial vessels[7]. We used a two-phase approach that included a screening phase with TCD examination followed by a diagnostic phase with MRA, and we found that the overall prevalence of aICAS was 7.6% in this rural-dwelling middle-aged and elderly population. TCD examination, when used as a tool for detecting arterial stenosis, has the advantage of convenience and non-invasiveness but also has a high negative predictive value (~90%); thus, TCD is suitable for screening arterial stenosis in a large-scale population study. However, the positive predictive value of TCD examination is only ~50%. In contrast, MRA provides a good positive predictive value for ICAS but may not be suitable for large-scale community-based studies due to its high costs and inconvenience[20]. In this study, we combined TCD with MRA to detect aICAS, which is feasible especially among people who are living in the remote rural communities. This approach will allow us to obtain a more accurate estimate of aICAS prevalence than that was achieved in previous studies used either TCD or MRA alone.

We also found that the distribution of aICAS was more common in the anterior circulation than in the posterior circulation, in accordance with a study from Japan[39]. The variations in the regional arterial distribution of aICAS are likely due to differences in the anatomical and haemodynamic characteristics between the anterior and posterior circulation. For example, in the anterior circulation, the higher number of vascular branches may increase the risk of atherosclerosis[40]. In addition, TCD has relatively low sensitivity in detecting posterior circulation stenosis[41], and this may have contributed, in part, to the differences observed in the regional arterial distribution of aICAS.

In summary, in the community-based KT-aICAS study, aICAS affected nearly one-tenth of the rural-dwelling middle-aged and elderly residents, and its prevalence increased with advanced age. Anterior circulation arteries were most vulnerable to atherosclerotic lesions. In addition, major CRFs were highly prevalent among middle-aged and elderly rural dwellers who were free of stroke. These findings may have important implications for the primary prevention of aICAS, clinical stroke, and cognitive impairment. Future follow-up data from this cohort may contribute to the understanding of the epidemiology and natural history as well as cognitive consequences of aICAS among rural residents in China.

Strengths and Limitations

This cohort study targeted residents living in rural communities in China, to whom the research community has so far paid little attention. Second, we integrated TCD and MRA into a two-phase procedure to detect aICAS in a large-scale population study, and this approach is likely to allow us to obtain a relatively accurate estimate of the prevalence of aICAS. Furthermore, high-resolution MRI scans could help further evaluate the characteristics of arterial stenosis and avoid the contamination of ICAS cases with cases involving other non-atherosclerotic diseases[42]. In addition, high-resolution MRI can be used to detect intraplaque haemorrhage, lipid cores, and artery fibrous caps, thus providing further insight into the long-term prognosis of aICAS. To the best of our knowledge, this is the first community-based study of aICAS among an Asian population that used high-resolution MRI as well as PWI images and DTI images.

Our study also has limitations. First, we used TCD as a screening tool for arterial stenosis, which might have missed some cases of aICAS, and thus, have underestimated the overall prevalence of aICAS. Furthermore, in case of a poor or incomplete echo window for TCD, people without any stenosis in the VA, BA, and ACA were considered to be non-stenotic. This might also have resulted in the underestimation of the true prevalence of aICAS in this population. Finally, our study sample was selected from only one rural area and the cohort might differ from other rural populations given that the local residents previously underwent health surveys, which should be kept in mind when generalizing our findings to other rural populations.

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Author Contributions QS, FX, and YD conceived the research question and designed the study. XW and FX conducted the statistical analysis. XW, CQ, and QS wrote the first and subsequent drafts of the manuscript. XJ, SwS, SS, YZ, PY, SL, JL, GW, and ML contributed to the epidemiological investigation. All authors contributed to data interpretation and critical revisions of the manuscript, and approved the final version of the submitted manuscript.

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Table 1. Characteristics of the study participants by gender

Characteristics[b]	Total	Men	Women	P value[a]
No. of participants (%)	2311 (100)	1076 (46.6)	1235 (53.4)	--
Age (years), mean (SD)	57.6(10.5)	56.3(10.3)	58.8(10.5)	<0.001
Age group (years), n(%)				<0.001
40-49	613(26.6)	353(32.9)	260(21.1)	
50-59	733(31.8)	334(31.1)	399(32.4)	
60-69	607(26.3)	240(22.3)	367(29.8)	
≥70	352(15.3)	147(13.7)	205(16.7)	
Marital status[b], n (%)				<0.001
Married	2058(89.1)	1003(93.3)	1055(85.4)	
Single	252(10.9)	72(6.7)	180(14.6)	
Educational level[b], n (%)				<0.001
Illiterate	354(15.3)	36(3.3)	318(25.8)	
Elementary school	679(29.4)	247(23.0)	432(35.0)	
Middle school	976(42.3)	582(54.1)	394(31.9)	
High school and above	301(13.0)	211(19.6)	90(7.3)	
Body mass index (kg/m^2)[b], mean (SD)	25.2(3.8)	24.8(3.2)	25.5(4.1)	<0.001
Systolic pressure (mm Hg)[b], mean (SD)	145.0(22.4)	144.5(21.3)	145.4(23.4)	0.365
Diastolic pressure (mm Hg)[b], mean (SD)	88.3(12.7)	89.9(13.0)	86.9(12.2)	<0.001
Fasting blood glucose (mmol/l)[b], mean (SD)	6.1(1.8)	6.0(1.8)	6.1(1.9)	0.084
LDL-C (mmol/l)[b], mean (SD)	3.0(0.7)	2.9(0.7)	3.1(0.7)	<0.001
HDL-C (mmol/l)[b], mean (SD)	1.6(0.4)	1.6(0.4)	1.6(0.3)	0.031
Triglycerides (mmol/l)[b], mean (SD)	1.4(0.9)	1.3(0.9)	1.5(0.9)	<0.001
Total cholesterol (mmol/l)[b], mean (SD)	5.4(1.0)	5.2(0.9)	5.5(1.0)	<0.001
Dyslipidemia[b], n (%)	922(41.7)	378(36.2)	544(46.7)	<0.001
Smoking[b], n (%)	504(21.9)	489(45.6)	15(1.2)	<0.001
Alcohol consumption[b], n (%)	727(31.5)	672(62.5)	55(4.5)	<0.001
Obesity[b], n (%)	437(19.2)	165(15.5)	272(22.5)	<0.001
Hypertension[b], n (%)	1294(56.6)	592(55.3)	702(57.8)	0.220

Diabetes[b], n (%)	725(32.9)	319(30.5)	406(34.9)	0.028
Carotid stenosis[b], n (%)	59(2.9)	33(3.4)	26(2.5)	0.202
aICAS[b], n (%)	154(7.6)	62(6.4)	92(8.6)	0.055
MMSE score[c], mean (SD)	23.4(5.4)	24.7(4.4)	22.5(5.9)	0.001
MoCA score[c], mean (SD)	17.9(5.3)	18.6(4.8)	17.5(5.6)	0.088

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; aICAS: asymptomatic intracranial artery stenosis; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment.

[a]P values are for the test of differences between male and female.

[b]The number of participants with missing value were one for marital status and educational level, 38 for body mass index and obese status, 29 for blood pressure, 175 for fasting blood glucose, 106 for lipid measurements (LDL-C, HDL-C, triglycerides, and total cholesterol), eight for smoking, two for alcohol consumption, 102 for dyslipidemia, 26 for hypertension, 104 for diabetes, 284 for carotid stenosis, and 284 for aICAS (of the 2311 participants, 2027 completed the diagnostic procedure for aICAS).

[c]MMSE and MoCA tests were performed in a subsample of 308 participants, including 154 with aICAS and 153 age- and sex-matched controls.

Table 2. Arterial distribution of asymptomatic intracranial atherosclerotic stenosis (aICAS) according to severity of stenosis (Total number of arteries with aICAS = 305)

Arteries	Severity of intracranial arterial stenosis, n (%)				
	Mild	Moderate	Severe	Occlusion	Total
ICA	28 (9.2)	20 (6.6)	17 (5.6)	6 (2.0)	71(23.3)
MCA	45 (14.8)	21 (6.9)	23 (7.6)	6 (2.0)	95(31.2)
ACA	22 (7.2)	17 (5.6)	16 (5.3)	0 (0.0)	55(18.0)
PCA	10 (3.3)	9 (3.0)	18 (5.9)	1 (0.3)	38(12.5)
VA	4 (1.3)	2 (0.7)	8 (2.6)	11 (3.3)	25(8.2)
BA	13 (4.3)	3 (1.0)	5 (1.6)	0 (0.0)	21(6.9)

MCA, middle cerebral artery; ACA, anterior cerebral artery; ICA, intracranial internal carotid artery; PCA, posterior cerebral artery; VA, vertebral artery; BA, basilar artery.

Figure Legends

Figure 1 Flowchart of the study participants

Figure 2 Age- and gender-specific prevalence (per 100 population) of cardiovascular risk factors

Figure 3 Age- and gender-specific prevalence (per 100 population) of aICAS

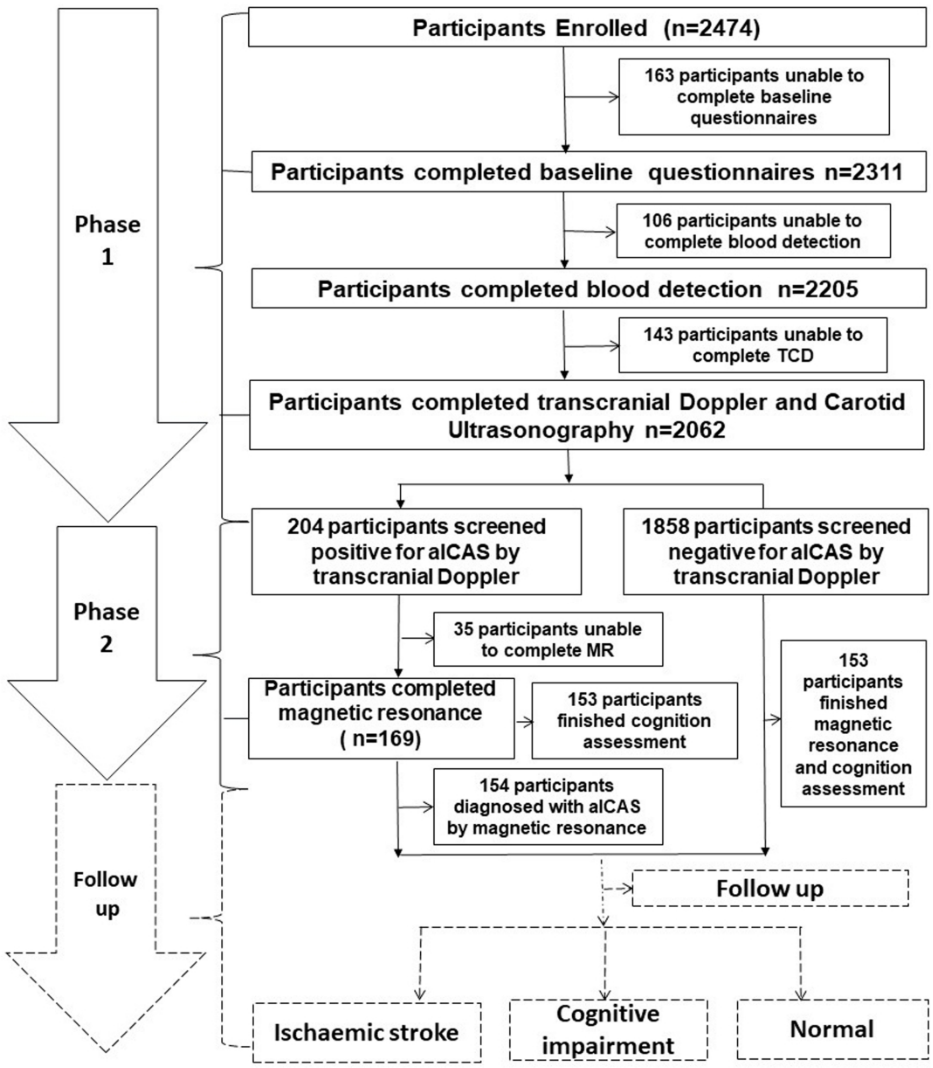


Figure 1 Flowchart of the study participants

236x263mm (300 x 300 DPI)

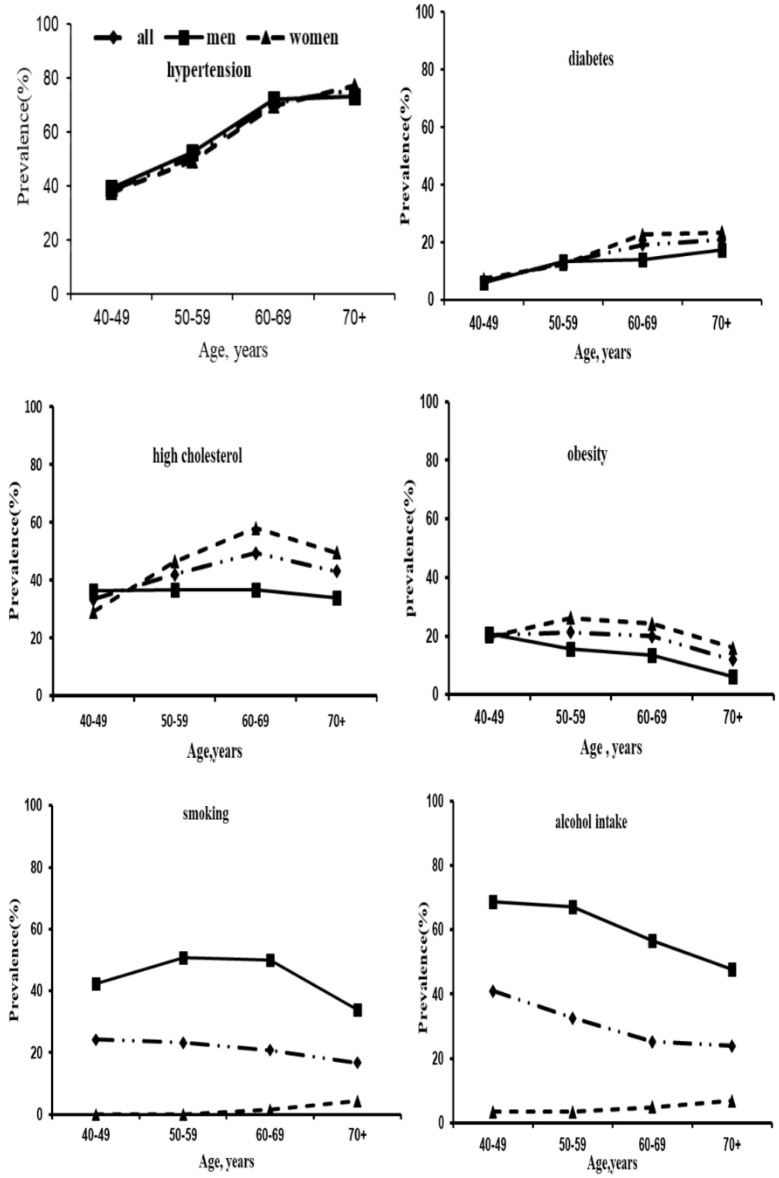
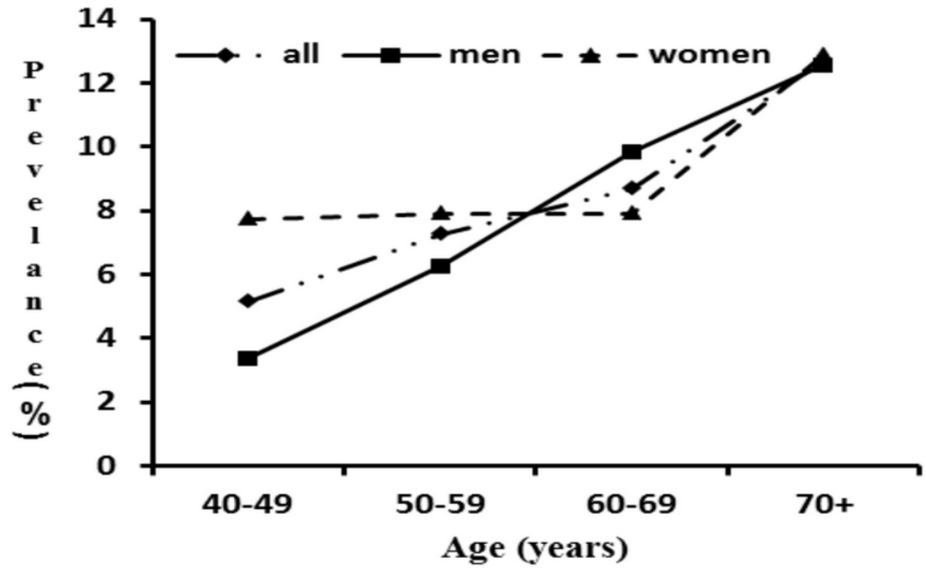


Figure 2 Age- and gender-specific prevalence (per 100 population) of cardiovascular risk factors

101x145mm (300 x 300 DPI)



all: P for trend<0.001; Men: P for trend<0.001; Women: P for trend =0.140

Figure 3 Age- and gender-specific prevalence (per 100 population) of aICAS

78x56mm (300 x 300 DPI)