

Cite this article as: Neural Regen Res. 2012;7(20):1570-1577.

# Smoking and Alzheimer's disease among Mongolian and Han Chinese aged 55 years and over living in the Inner Mongolia farming area of China<sup>★</sup>

Chunyu Zhang<sup>1</sup>, Lin Da<sup>2</sup>, Shigang Zhao<sup>1</sup>, Desheng Wang<sup>3</sup>, Guangming Niu<sup>1</sup>, Huriletemuer<sup>1</sup>

<sup>1</sup>Department of Neurology, First Affiliated Hospital of Inner Mongolia Medical College, Hohhot 010050, Inner Mongolia Autonomous Region, China

<sup>2</sup>School of Mathematical Sciences, Inner Mongolian University, Hohhot 010021, Inner Mongolia Autonomous Region, China

<sup>3</sup>Department of Neurology, First Affiliated Hospital, Harbin Medical University, Harbin 150001, Heilongjiang Province, China

## Abstract

Residents aged 55 years or older from 27 communities and two settlements in Xilingol League of Inner Mongolia were selected for participation in an Alzheimer's disease epidemiological investigation from June 2008 to June 2009, including 3 259 Mongolians and 5 887 Han Chinese. The Mongolian subjects in the Alzheimer's disease group were at age of 55 years or older (on average), and more of them were male, illiterate and/or had a history of coronary artery disease and/or diabetes compared with the Mongolian subjects in the non-Alzheimer's disease group. The Han Chinese subjects in the Alzheimer's disease group were at age of 55 years or older (on average) and more of them were women, illiterate and/or had a history of coronary artery disease, and less of them had a history of alcohol consumption compared with the non-Alzheimer's disease group. Non-conditional multivariate stepwise logistic regression identified that male gender, increasing age and having a history of diabetes and/or coronary heart disease were associated with higher odds of Alzheimer's disease among Mongolians while having an educational background was associated with lower odds ( $OR = 0.259$ , 95%CI 0.174–0.386). Among the Han Chinese subjects, male gender, increasing age and having a history of coronary heart disease and/or hypertension was associated with higher odds of Alzheimer's disease, while having an educational background was associated lower odds ( $OR = 0.271$ , 95%CI 0.192–0.381). The results also indicated that extremely heavy smoking may be a risk factor for Alzheimer's disease in Mongolian males aged over 55 years. There was no significant difference in smoking habits between the Mongolian and Han Chinese subjects with Alzheimer's disease.

## Key Words

Alzheimer's disease; Mongolian; Han Chinese population; elderly; epidemiology; risk factor; protection factor

## Research Highlights

(1) This large-scale epidemiological investigation of Alzheimer's disease showed that male gender, increasing age, and a history of diabetes and/or coronary heart disease were independent Alzheimer's disease risk factors for Mongolians, while having an educational background was a protective factor. (2) Among the Han Chinese subjects, male gender, increasing age and a history of coronary heart disease and hypertension were independent Alzheimer's disease risk factors, while having an educational background was a protective factor.

Chunyu Zhang<sup>★</sup>, Master, Associate professor, Department of Neurology, First Affiliated Hospital of Inner Mongolia Medical College, Hohhot 010050, Inner Mongolia Autonomous Region, China

Chunyu Zhang and Lin Da contributed equally to this work.

Corresponding author: Guangming Niu, M.D., Professor, Department of Neurology, First Affiliated Hospital of Inner Mongolia Medical College, Hohhot 010050, Inner Mongolia Autonomous Region, China; Huriletemuer, M.D., Associate chief physician, Department of Neurology, First Affiliated Hospital of Inner Mongolia Medical College, Hohhot 010050, Inner Mongolia Autonomous Region, China  
gmniu@sina.com;  
hurile1992@163.com

Received: 2011-12-13  
Accepted: 2012-05-21  
(N20111026005/YJ)

Zhang CY, Da L, Zhao SG, Wang DS, Niu GM, Huriletemuer. Smoking and Alzheimer's disease among Mongolian and Han Chinese aged 55 years and over living in the Inner Mongolia farming area of China. Neural Regen Res. 2012;7(20):1570-1577.

www.crter.cn  
www.nrronline.org

doi:10.3969/j.issn.1673-5374.2012.20.007

## INTRODUCTION

Studies have investigated the relationship between smoking and dementia<sup>[1-3]</sup>. However, there are few data regarding Alzheimer's disease epidemiology and its related risk factors in the western part of China, especially in the regions inhabited by minority ethnic groups, which leads to limited Alzheimer's disease prevention in these regions. The Inner Mongolia Autonomous Region is one such region where no Alzheimer's disease epidemiological investigation has been conducted.

The present study is the first large-scale Alzheimer's disease epidemiological investigation of the population aged 55 years or older in the Inner Mongolia Autonomous Region farming area undertaken to investigate the correlation between smoking and Alzheimer's disease.

## RESULTS

### Quantitative analysis of experimental subjects

10 035 individuals were recruited for the study. Of them, 9 266 completed the survey and 769 withdrew from the study, including 561 Han Chinese, 192 Mongolian and 16 from other nationalities. Of the 9 266 individuals who completed the survey, data from 120 individuals of nationalities other than Mongolian and Han Chinese were excluded. Therefore, 3 256 Mongolian and 5 887 Han Chinese were included in the final analysis. All subjects were aged 55 years or older.

### Comparison of the demographics of Mongolian and Han Chinese subjects

No significant differences were found between the Mongolian and Han Chinese subjects in terms of sex distribution, blood pressure or proportion of subjects with normal body mass, but there were significant differences in the history of alcohol intake and smoking (Table 1).

### Comparison of Alzheimer's disease risk factors between Mongolians and Han Chinese subjects

Among the Mongolian subjects, compared with the non-Alzheimer's disease group, those in the Alzheimer's disease group were older (on average), more were illiterate, more had history of coronary artery disease and/or diabetes and fewer were male ( $P < 0.01$ , Table 2).

Among the Han Chinese subjects, compared with the non-Alzheimer's disease group, those in the Alzheimer's disease group were older (on average), more were illiterate, more had a history of coronary artery disease, and fewer were male and/or had a history of alcohol intake ( $P$

$< 0.01$ ; Table 3).

Table 1 Comparison of the demographics of Mongolian and Han Chinese subjects  $\geq 55$  years old living in the Inner Mongolia farming area

Item	Mongolian (n = 3 259)	Han Chinese (n = 5 887)	P
Age (mean $\pm$ SD, year)	65.6 $\pm$ 7.5	66.6 $\pm$ 7.8	0.000
Male [n (%)]	1 141(44.2)	2 568(43.6)	0.737
History of alcohol intake [n (%)]	897(27.5)	1 396(23.7)	0.000
Smoking [n (%)]	1 234(37.9)	1 927(32.7)	0.000
Systolic blood pressure (mean $\pm$ SD, mm Hg)	139.8 $\pm$ 22.9	138.7 $\pm$ 21.5	0.025
Diastolic blood pressure (mean $\pm$ SD, mm Hg)	86.8 $\pm$ 13.2	86.3 $\pm$ 12.5	0.067
Hypertension [n (%)]	1 726(53.0)	3 222(54.7)	0.109

1 mm Hg = 0.133 kPa.

Table 2 Comparison of clinical data between Alzheimer's disease (AD) and non-AD groups among the Mongolian subjects  $\geq 55$  years old living in the Inner Mongolia farming area

Item	AD (n = 151)	Non-AD (n = 3 108)	P
Age (mean $\pm$ SD, year)	72.3 $\pm$ 7.1	65.3 $\pm$ 7.4	0.000
Male [n (%)]	46(30.5)	1 395(44.9)	0.000
Illiteracy [n (%)]	112(74.2)	1 006(32.4)	0.000
History of alcohol intake [n (%)]	40(26.5)	858(27.6)	0.771
Systolic blood pressure (mean $\pm$ SD, mm Hg)	140.9 $\pm$ 27.9	139.7 $\pm$ 22.7	0.534
Diastolic blood pressure (mean $\pm$ SD, mm Hg)	85.7 $\pm$ 14.4	86.91 $\pm$ 13.1	0.254
Hypertension [n (%)]	71(47.0)	1 462(47.0)	0.996
Coronary artery disease [n (%)]	31(20.5)	334(10.8)	0.000
Diabetes [n (%)]	8(5.3)	63(2.0)	0.007

1 mm Hg = 0.133 kPa.

Table 3 Comparison of clinical data between Alzheimer's disease (AD) and non-AD groups among Han Chinese subjects  $\geq 55$  years old living in the Inner Mongolia farming area

Item	AD (n = 288)	Non-AD (n = 5 599)	P
Age (mean $\pm$ SD, year)	75.4 $\pm$ 7.2	66.2 $\pm$ 7.5	0.000
Male [n (%)]	79(27.4)	2 489(44.4)	0.000
Illiteracy [n (%)]	242(84.0)	5 586(40.8)	0.000
History of alcohol intake [n (%)]	50(17.4)	1 346(24.0)	0.009
Systolic blood pressure (mean $\pm$ SD, mm Hg)	139.4 $\pm$ 22.6	138.7 $\pm$ 21.5	0.543
Diastolic blood pressure (mean $\pm$ SD, mm Hg)	85.6 $\pm$ 12.4	86.4 $\pm$ 12.5	0.268
Hypertension [n (%)]	140(48.6)	2 525(45.1)	0.243
Coronary artery disease [n (%)]	33(11.5)	4.7(7.3)	0.008
Diabetes [n (%)]	15(5.2)	270(4.8)	0.776

1 mm Hg = 0.133 kPa.

### Correlation between smoking and Alzheimer's disease

The smoking rate was 37.86% and 32.70% among the Mongolians and the subjects of Han Chinese ethnicity. Smoking habits of Mongolian Alzheimer's disease and non-Alzheimer's disease groups by sex is shown in Table 4.

Table 4 Results of univariate unconditional logistic regression analysis of the relationship between smoking and Alzheimer's disease among Mongolian subjects  $\geq 55$  years old

Item	B	SE	Wald	Sig	OR	95%CI
<b>Male</b>						
Mild smoking	-0.230	0.430	0.286	0.593	0.794	0.342-1.846
Moderate smoking	0.301	0.453	0.441	0.507	1.351	0.556-3.286
Heavy smoking	0.002	0.561	0.000	0.998	1.002	0.334-3.006
Extremely heavy smoking	1.058	0.421	6.301	0.012	2.880	1.261-6.576
<b>Female</b>						
Mild smoking	0.291	0.273	1.138	0.286	1.338	0.784-2.284
Moderate smoking	0.694	0.392	3.136	0.077	2.001	0.929-4.311
Heavy smoking	0.860	0.450	3.645	0.056	2.363	0.977-5.711
Extremely heavy smoking	0.244	0.741	0.108	0.742	1.276	0.299-5.456

Mild smoking:  $\leq 26.7$  pack-years; moderate smoking:  $> 26.7-40.5$  pack-years; heavy smoking:  $> 40.5-55.5$  pack-years; extremely heavy smoking:  $> 55.5-156$  pack-years.

Extremely heavy smoking was identified as a risk factor of Alzheimer's disease ( $OR = 2.880$ ) among male Mongolian subjects. Among female Mongolian subjects, there were borderline associations between moderate smoking ( $P = 0.077$ ,  $OR = 2.001$ ,  $95\% CI 0.929-4.311$ ), heavy smoking ( $P = 0.056$ ,  $OR = 2.363$ ,  $95\% CI 0.977-5.711$ ) and Alzheimer's disease risk. Among the Han Chinese subjects of both sexes, there were no significant associations between smoking habit and Alzheimer's disease risk (Table 5).

Table 5 Univariate unconditional logistic regression analysis of the relationship between smoking and Alzheimer's disease among Han Chinese subjects  $\geq 55$  years old

Item	B	SE	Wald	Sig	OR	95%CI
<b>Male</b>						
Mild smoking	0.117	0.291	0.162	0.688	1.124	0.635-1.989
Moderate smoking	0.332	0.404	0.674	0.412	0.718	0.325-1.585
Heavy smoking	0.190	0.349	0.297	0.586	1.209	0.610-2.398
Extremely heavy smoking	0.186	0.388	0.230	0.632	1.204	0.563-2.578
<b>Female</b>						
Mild smoking	-0.012	0.307	0.002	0.968	0.988	0.541-1.804
Moderate smoking	-0.001	0.522	0.000	0.998	0.999	0.359-2.777
Heavy smoking	0.066	0.600	0.012	0.912	0.936	0.289-3.034
Extremely heavy smoking	0.861	0.626	1.892	0.169	2.365	0.694-8.064

Mild smoking:  $\leq 26.7$  pack-years; moderate smoking:  $> 26.7-40.5$  pack-years; heavy smoking:  $> 40.5-55.5$  pack-years; extremely heavy smoking:  $> 55.5-156$  pack-years.

#### Relationship between Alzheimer's disease and smoking among the subjects of the Mongolian and

#### the Han Chinese groups

The distribution of smoking habits in the Alzheimer's disease and the non-Alzheimer's disease groups by nationality is shown in Table 6.

Table 6 Distribution of smoking habits by Alzheimer's disease (AD) and non-AD group and nationality [ $n$  (%)]

Item	Mongolian		Han Chinese	
	AD group	Non-AD group	AD group	Non-AD group
Non-smoking	89(58.9)	1 936(62.3)	216(75.0)	3 748(66.9)
Mild smoking	26(17.2)	597(19.2)	33(11.5)	792(14.1)
Moderate smoking	15(9.9)	253(8.1)	12(4.2)	424(7.6)
Heavy smoking	10(6.6)	185(6.0)	15(5.2)	372(6.6)
Extremely heavy smoking	11(7.3)	137(4.4)	12(4.2)	263(4.7)

Number in bracket represents percent.

There was no significant difference in the number of mild smokers, heavy smokers or extremely heavy smokers between the Mongolian and Han Chinese subjects. However, there was a borderline significant difference in the number of non-smokers and moderate smokers ( $\chi^2 = 3.081$ ,  $P = 0.079$ ;  $\chi^2 = 3.642$ ,  $P = 0.056$ ).

#### Multivariate logistic regression analysis of factors associated with Alzheimer's disease

To gain further insight into the relationship between smoking, other relevant factors and Alzheimer's disease, the present study conducted a non-conditional multivariate stepwise logistic regression. The independent variables tested were age, gender, educational background, alcohol intake, blood pressure, history of diabetes and/or coronary heart disease. Male gender, increasing age, and having a history of diabetes and/or coronary heart disease were associated with higher odds of Alzheimer's disease, while having an educational background was associated with lower odds of Alzheimer's disease among the Mongolians (Table 7). Among the Han Chinese group, male gender, increasing age and a history of coronary heart disease and/or hypertension were associated with higher odds of Alzheimer's disease, while having an educational background was associated with lower odds of Alzheimer's disease (Table 8).

## DISCUSSION

Many factors are related to the pathogenesis of Alzheimer's disease. Known risk factors include increasing age, and a family history of Alzheimer's disease. Not yet fully established risk factors include traumatic brain injury,

gender, educational level<sup>[4]</sup>, marital status, occupation, region and race, mild cognitive impairment, vascular risk factors<sup>[5-10]</sup>, inflammation, economic level, lifestyle, occupational exposure, estrogen, heavy metals, trace elements, and stress<sup>[11-14]</sup>.

Table 7 Multivariate logistic regression analysis of factors associated with Alzheimer's disease among Mongolian subjects aged  $\geq 55$  years

Item	B	SE	Wald	Sig	OR	95%CI
Gender	0.972	0.235	17.057	0.000	2.643	1.667-4.193
Age	0.096	0.012	68.638	0.000	1.101	1.076-1.126
Educational background	-1.349	0.203	44.190	0.000	0.259	0.174-0.386
Alcohol intake	-0.432	0.250	2.990	0.084	0.649	0.397-1.059
Diabetes	1.342	0.418	10.276	0.001	3.825	1.684-8.686
Coronary heart disease	0.446	0.225	3.921	0.048	1.562	1.005-2.430
High blood pressure	-0.089	0.178	0.250	0.617	0.915	0.645-1.298
Smoking						
Mild	0.011	0.246	0.002	0.965	1.011	0.624-1.639
Moderate	0.479	0.316	2.297	0.130	1.613	0.869-2.994
Heavy	0.320	0.367	0.758	0.384	1.377	0.670-2.827
Extremely heavy	0.245	0.378	0.421	0.516	1.278	0.610-2.678

Educational background: primary school education or higher.

Table 8 Multivariate logistic analysis of factors associated with Alzheimer's among Han Chinese subjects aged  $\geq 55$  years

Item	B	SE	Wald	Sig	OR	95%CI
Gender	0.906	0.188	23.317	0.000	2.475	1.713-3.575
Age	0.132	0.009	223.203	0.000	1.141	1.122-1.161
Educational background	-1.307	0.175	56.045	0.000	0.271	0.192-0.381
Alcohol intake	-0.134	0.207	0.410	0.522	0.876	0.584-1.314
Diabetes	0.405	0.296	1.877	0.171	1.500	0.840-2.676
Coronary heart disease	0.562	0.210	7.145	0.008	1.754	1.162-2.649
High blood pressure	-0.322	0.131	5.998	0.016	1.369	1.061-1.767
Smoking						
Mild	0.111	0.223	0.249	0.619	1.113	0.722-1.729
Moderate	-0.277	0.338	0.670	0.413	0.759	0.391-1.471
Heavy	0.225	0.306	0.539	0.463	1.252	0.687-2.281
Extremely heavy	0.007	0.348	0.000	0.985	1.007	0.509-1.991

Educational background: primary school education or higher.

In addition, toxic substances contained in tobacco such as carbon monoxide, nicotine, tar and a large number of free radicals, lead to vasoconstriction and enhance monocyte adhesion to endothelial cells which further increase oxidative damage and vitamin metabolism, thus increasing the risk of Alzheimer's disease<sup>[15-21]</sup>. The rela-

tionship between nicotine and cognitive decline in Alzheimer's disease has been extensively studied<sup>[22]</sup>. Continued nicotine consumption in human and animal models can lead to brain amyloid accumulation in specific regions and the presence of amyloid beta accumulation has been found<sup>[22]</sup>. Nicotine also has large effects on brain development<sup>[23]</sup>. Nicotine not only causes destruction of the coordination of cell proliferation and differentiation, but also interferes with normal cell division such as neurite growth, cell migration and recognition<sup>[24]</sup>. Some researchers believe that nicotine in cigarettes can improve cognitive function<sup>[25]</sup>, increase nicotine receptor sites and resist the formation of A $\beta$ -fiber neurons, playing a protective role for nerve cells. It can also increase release of acetylcholine, dopamine, norepinephrine, glutamate and other neurotransmitters to promote learning and memory and improve cognitive performance<sup>[26-28]</sup>. There has been great controversy regarding the relationship between smoking and Alzheimer's disease. The mechanism of increased or decreased risks of dementia associated with cigarette smoking remains unclear. Advanced studies are required to investigate whether smoking is a protective factor against Alzheimer's disease, or whether smokers die earlier because of other systemic diseases, consequently affecting their prevalence of Alzheimer's disease. In a cohort study with a 5-year follow-up by Wang *et al*<sup>[29]</sup>, the mortality rate of smokers who developed dementia was 3.4 times that of non-smokers with dementia. That is, smokers died earlier after being diagnosed with dementia, so that non-smokers with Alzheimer's disease are more prone to being assigned to the Alzheimer's disease group in a cross-sectional or case-control study because of their longer life-span. This would lead to a higher proportion of non-smokers than smokers in the Alzheimer's disease group, and the conclusion that "smoking has a protective effect on the development of Alzheimer's disease" could be drawn, a so-called "survival bias". Launer *et al*<sup>[30]</sup> summarized the results of four prospective studies from Denmark, France, Netherlands and the UK regarding the relation between smoking and Alzheimer's disease. The study included 13 147 individuals with a 2-year follow-up. The results showed that compared with never-smokers, among former smokers the relative risk of developing Alzheimer's disease did not increase ( $RR = 1.2$ , 95%  $CI$  0.8-1.5), while the risk was increased among current smokers ( $RR = 1.7$ , 95%  $CI$  1.2-2.5). However, some researchers believe that nicotine in the cigarettes could enhance cognitive performance<sup>[31]</sup>. Wang *et al*<sup>[32]</sup> conducted a case-control study in Stockholm that included 198 patients with Alzheimer's disease and 343 non-demented elderly individuals as controls. The results showed that compared with the age-matched control group, smoking had a protective effect against

Alzheimer's disease ( $OR = 0.6$ , 95%  $CI$ : 0.4–1.1). However, smokers did not have decreased risk of developing Alzheimer's disease ( $HR = 1.1$ , 98%  $CI$ : 0.5–2.4) compared with non-smokers. Further analyses showed that the smokers' mortality rate was higher than that of the non-smokers among the Alzheimer's disease patients ( $HR = 3.5$ , 98%  $CI$  1.4–8.8). In the normal control group, the smokers' mortality rate was not different to that of non-smokers ( $HR = 0.8$ , 98%  $CI$ : 0.5–1.2). Deng *et al*<sup>[33]</sup> conducted a 2-year follow-up study of 2820 people older than 60 years from Chongqing, China. There were 121 patients with dementia, including 84 (69%) patients with Alzheimer's disease, 16 (13%) with vascular dementia, and 21 (17%) with other dementia. After adjusting for potential confounders (age, sex, educational level, blood pressure, and alcohol consumption), current smokers had higher risks of developing Alzheimer's disease ( $RR = 2.72$ ; 95%  $CI$  1.63–5.42) and vascular dementia ( $RR = 1.98$ ; 95%  $CI$  1.53–3.12) compared with people who had never smoked. Compared with non-smokers, heavy smokers had the highest relative risk of developing Alzheimer's disease ( $RR = 3.03$ ; 95%  $CI$  1.25–4.02); followed by moderate smokers ( $RR = 2.56$ ; 95%  $CI$  1.65–5.52). Another study by Wang *et al*<sup>[34]</sup> demonstrated a double-edged effect of smoking on Alzheimer's disease. On one hand, smoking had a protective effect against developing Alzheimer's disease; on the other hand, smoking had interactions with vascular diseases, which could increase the risk of developing Alzheimer's disease by affecting the risk of vascular events. In the present study, among the Mongolian subjects, 55.59% were male and 37.86% were smokers. Among the Han Chinese subjects, 62.03% were male and 32.70% were smokers. Few studies have focused on the relationship between the extent of smoking and Alzheimer's disease. Extremely heavy smoking was identified as an Alzheimer's disease risk factor ( $OR = 2.880$ ) among the male Mongolian subjects. Among the female Mongolian subjects, moderate ( $P = 0.077$ ,  $OR = 2.001$ ) and heavy smoking ( $P = 0.077$ ,  $OR = 1.276$ ) had borderline significantly higher odds of Alzheimer's disease than non-smokers. Among the Han Chinese subjects of both sexes, there were no statistically significant differences in the extent of smoking and the prevalence of Alzheimer's disease. We speculate that the increased extent of smoking among male Mongolians lead to the increased risk of Alzheimer's disease. Extremely heavy smoking was identified as an Alzheimer's disease risk factor in the present study. Similar trends were also found in the female Mongolian population, in that moderate and heavy smoking had borderline significance in relation to Alzheimer's disease risk. However, among both male and female Han Chinese subjects, there were no statistically significant differences between the Al-

zheimer's disease group and non-Alzheimer's disease group in terms of smoking habits. There was a borderline significant difference between the numbers of Mongolian and Han Chinese subjects in the non-smoking and moderate smoking groups. However, there are still many unknown issues about the nature and mechanism of the effects of smoking on dementia, and the effects can either protect against or increase the risk of developing dementia. Differences in effects may also be found among different ethnic groups and races. To gain insight into the relationship between smoking, other factors and Alzheimer's disease, we analyzed the effects of smoking, age, gender, educational background, social population factor, alcohol intake, blood pressure, and history of diabetes and/or coronary heart disease on Alzheimer's disease risk by non-conditional multivariate stepwise logistic regression. The results show that male gender, increasing age, and having a history of diabetes and/or coronary heart disease were independently associated with higher odds of Alzheimer's disease among the Mongolians, while having an educational background was identified as a protective factor. Similarly, for the Han Chinese subjects, male gender, increasing age, and having a history of coronary heart disease and/or hypertension was associated with higher odds, and having an educational background with lower odds, of Alzheimer's disease. Smoking can increase the risk of developing cardiovascular and cerebrovascular diseases, and is a definite risk factor for vascular diseases. The risk of developing cerebrovascular diseases among smokers is dramatically higher compared with non-smokers. In at least some populations, e.g., in Mongolian men and women, smoking might predispose the occurrence of Alzheimer's disease by increasing vascular events, thus increasing smokers' risk of developing Alzheimer's disease.

---

## SUBJECTS AND METHODS

---

### Design

Epidemiological investigation using stratified randomized and multistage cluster sampling.

### Time and setting

The experiment was conducted in the Inner Mongolia Autonomous Region, China from June 2008 to June 2009.

### Subjects

The study extracted four banners and one city from Xilingol League of Inner Mongolia as first-level sampling clusters, then extracted four sub-district offices, eight towns and two sumu from the first-level sampling clusters as the second-level sampling clusters. Using stratified

random and stratified multistage cluster sampling, residents aged 55 years or older were selected from 27 communities and two settlements for participation in this Alzheimer's disease epidemiological investigation. Inclusion criteria: Resident population of the town or sumu, and non-residents who had lived at least one month in the town or sumu; those aged 55 years or older; those who gave their informed consent to participate and who were able to cooperate with the investigation. Exclusion criteria: Non-resident population of the town or sumu and those who had lived there less than 1 month; those not completing the investigation. Ten experienced neurologists or physicians of internal medicine composed the investigators. The diagnostic process included neuropsychological test forms, questionnaires and clinical investigations.

## Methods

### Survey methods

Each subject received a screening questionnaire (mini-mental state examination) and an interview during the first phase of the study<sup>[35]</sup>. We obtained a detailed medical history from family members and neighbors of subjects who were suspected to have cognitive impairment. Individuals completed the second phase of diagnostic and neuropsychological testing if they met one of the following criteria: scores on the mini-mental state examination indicating that they were illiterate ( $\leq 19$  points), had primary school education ( $\leq 22$  points), or at least middle school education ( $\leq 26$  points); failed to complete the mini-mental state examination because of another disease; or, had a normal mini-mental state examination score, but whose screening personnel or family members believed that the individual displayed significant cognitive impairment.

The assessment tools used in the second phase of the study included the following: Pfeffer's Outpatient Disability Questionnaire, Fuld Object-Memory Evaluation, Rapid Verbal Recall, Digit Span, Block Design, the Hachinski Ischemic Scale and the Hamilton Depression Scale. With subjects for whom the above mentioned scales could not be used to verify their cognitive impairment, the following assessment tools were used: the Clinical Memory Scale, Painting Bell, Complex Figure Test, Clinical Dementia Rating and the Cobsm Index (*i.e.*, an assessment of behaviors and psychological state). We re-evaluated and confirmed the diagnoses for a randomly selected 4% of the samples who had a mini-mental state examination score in the normal range and for whom their families had provided information about their cognitive and functional abilities.

The third phase of the study began 6 months after the first phase. In this phase, we analyzed the assessment results and made a diagnosis. We scheduled a head CT

scan, MRI or other related laboratory tests for the patients who had ambiguous data or when it was difficult to make an accurate diagnosis. We ensured that each diagnosis was based on a detailed medical history, physical examination, test scores, observed trends, repeated discussions and analyses, a secondary inspection and a follow-up observation.

### Classification of smoking

According to the status of smoking or not, the participants were divided into a non-smoking group or a smoking group. Non-smoking was defined as no active or passive smoking throughout one's lifetime. Smoking was defined as smoking every day and more than one cigarette per day. Participants who smoked occasionally or smoked less than one cigarette per day were excluded from the study. According to different smoking habits, the participants were classified into four groups: mild smoking ( $\leq 26.7$  pack-years), moderate smoking ( $> 26.7-40.5$  pack-years), heavy smoking ( $> 40.5-55.5$  pack-years), and extremely heavy smoking ( $> 55.5-156$  pack-years). The years of smoking, packs smoked per day, and the age at which smoking was initiated were recorded. The quantity of smoking was calculated with pack-years [packs per day (pack)  $\times$  number of days of smoking per year, if a participant smoked every day, then this would be: packs per day (pack)  $\times 365$ ]<sup>[36]</sup>.

### Classification of alcohol intake

The investigation of alcohol intake included assessments of whether subjects drink alcohol or not, drinking age, and drinking category. Drinking alcohol indicated that subjects drink alcohol at least once a week. Drinking alcohol only during the holidays was not viewed as alcohol intake<sup>[37]</sup>.

### Diabetes and heart disease classification criteria

Coronary heart disease was diagnosed according to the criteria of the 2005 Prevention Guide of Coronary Heart Disease<sup>[38]</sup>. Either of the following was viewed as coronary heart disease: (1) related disease diagnosed at rank 1 of class 2 hospitals or higher; (2) a prior definite diagnosis of coronary heart disease.

Diabetes was also diagnosed according to the criteria of the 2005 Prevention Guide of Coronary Heart Disease<sup>[38]</sup>. Either of the following was viewed as diabetes: (1) related disease diagnosed at rank 1 of class 2 hospitals or higher; (2) a prior definite diagnosis of diabetes.

### Diagnosis of dementia

Dementia was diagnosed according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4<sup>th</sup> edition, by the American Psychological Society<sup>[39]</sup>.

### Diagnosis of Alzheimer's disease

Alzheimer's disease was diagnosed according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association<sup>[40]</sup>.

### Statistical analysis

The database was established with EPIdata3.1 software (EpiData Association, Odense M, Denmark). All the questionnaires were carefully double-checked, and the data were input in duplicate by two persons, to ensure accuracy. Logic and spot checks were used to identify data entry errors. When errors in data input were identified, the data were carefully reentered. The database was locked and managed by a designated person. Non-Alzheimer's disease participants were assigned as the control group. The correlation between smoking and Alzheimer's disease was investigated among people of Mongolian and Han Chinese ethnicity living in the Inner Mongolian farming area who were aged 55 years or older. Measurement data are expressed as mean  $\pm$  SD, and enumeration data are expressed as rates. Comparisons of enumeration data between two groups or among multiple groups were performed with chi-square tests or Fisher's exact tests. Comparisons of measurement data between two groups were performed with single-sample *t*-tests or Mann-Whitney tests. Measurement data of multiple groups were compared with one-way analysis of variance and appropriate *post-hoc* tests were used for intergroup comparisons. Interaction between smoking and Alzheimer's disease was analyzed using logistic stepwise regression, with a variable inclusion and exclusion level of 0.1. Missing values were replaced with expectation and maximization using SPSS 13.0 software (SPSS, Chicago, IL, USA).

**Acknowledgments:** We thank the Xilingol League Administrative Office, Center for Disease Control and Prevention and Sonid Right Banner, Inner Mongolia Autonomous Region, China for their help.

**Funding:** This work was supported by grants from the Science and Technology Research Project at University of Inner Mongolia Autonomous Region, No. NJ09116; Important Project of Inner Mongolia Medical College Affiliated Hospital, No. NYFY ZD 2006001; Social Development of Inner Mongolia Autonomous Region, No. KJT10JHN; the Natural Science Foundation of Inner Mongolia Autonomous Region, No. 2010MS1121; and the Program of Higher-Level Talents of Inner Mongolia University, No. SPH-IMU, Z200901002.

**Author contributions:** Guangming Niu and Huriletemuer conceived and designed the study and revised the manuscript. Chunyu Zhang drafted the manuscript and contributed to statistical analysis. Lin Da, Shigang Zhao and Desheng Wang provided, integrated and analyzed experimental data.

**Conflicts of interest:** None declared.

**Ethical approval:** The study was approved by the Medical Ethics Committee, Affiliated Hospital of Inner Mongolia Medical College, China.

### REFERENCES

- [1] Peters R, Poulter R, Warner J, et al. Smoking, dementia and cognitive decline in the elderly, a systematic review. *BMC Geriatr.* 2008;8:36.
- [2] Demirovic J, Prineas R, Loewenstein D, et al. Prevalence of dementia in three ethnic groups: the South Florida program on aging and health. *Ann Epidemiol.* 2003;13(6): 472-478.
- [3] Jorm AF, Henderson AS. Dementia in Australia. Canberra: Australian Government Publishing Service. 1998.
- [4] Roe CM, Xiong C, Miller JP, et al. Education and Alzheimer disease without dementia: support for the cognitive reserve hypothesis. *Neurology.* 2007;68(3): 223-228.
- [5] Haan MN, Miller JW, Aiello AE, et al. Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging. *Am J Clin Nutr.* 2007;85(2):511-517.
- [6] Evan der Flier WM, Scheltens P. Epidemiology and risk factors of dementia. *J Neurol Neurosurg Psychiatry.* 2005;76 Suppl 5:v2-7.
- [7] Irie F, Fitzpatrick AL, Lopez OL, et al. Enhanced risk for Alzheimer disease in persons with type 2 diabetes and APOE epsilon4: the Cardiovascular Health Study Cognition Study. *Arch Neurol.* 2008;65(1):89-93.
- [8] Xu W, Qiu C, Gatz M, et al. Mid- and late-life diabetes in relation to the risk of dementia: a population-based twin study. *Diabetes.* 2009;58(1):71-77.
- [9] Dickstein DL, Walsh J, Brautigam H, et al. Role of vascular risk factors and vascular dysfunction in Alzheimer's disease. *Mt Sinai J Med.* 2010;77(1):82-102.
- [10] de la Torre JC. Vascular risk factor detection and control may prevent Alzheimer's disease. *Ageing Res Rev.* 2010; 9(3):218-225.
- [11] Morris MC, Schneider JA, Tangney CC. Thoughts on B-vitamins and dementia. *J Alzheimers Dis.* 2006;9(4): 429-433.
- [12] Amantea D, Russo R, Bagetta G, et al. From clinical evidence to molecular mechanisms underlying neuroprotection afforded by estrogens. *Pharmacol Res.* 2005;52(2):119-132.
- [13] Tran TT, Srivareerat M, Alkadhi KA. Chronic psychosocial stress triggers cognitive impairment in a novel at-risk model of Alzheimer's disease. *Neurobiol Dis.* 2010;37(3): 756-763.
- [14] Frisardi V, Solfrizzi V, Capurso C, et al. Aluminum in the diet and Alzheimer's disease: from current epidemiology to possible disease-modifying treatment. *J Alzheimers Dis.* 2010;20(1):17-30.
- [15] Kadoya C, Domino EF, Matsuoka S. Relationship of electroencephalographic and cardiovascular changes to plasma nicotine levels in tobacco smokers. *Clin*

- Pharmacol Ther. 1994;55(4):370-377.
- [16] Luchsinger JA, Reitz C, Honig LS, et al. Aggregation of vascular risk factors and risk of incident Alzheimer disease. *Neurology*. 2005;65(4):545-551.
- [17] Hanna ST. Nicotine effect on cardiovascular system and ion channels. *J Cardiovasc Pharmacol*. 2006;47(3):348-358.
- [18] Chierakul N, Wongwisutikul P, Vejbaesya S, et al. Tumor necrosis factor-alpha gene promoter polymorphism is not associated with smoking-related COPD in Thailand. *Respirology*. 2005;10(1):36-39.
- [19] Tanriverdi H, Evrengul H, Kuru O, et al. Cigarette smoking induced oxidative stress may impair endothelial function and coronary blood flow in angiographically normal coronary arteries. *Circ J*. 2006;70(5):593-599.
- [20] Anbarasi K, Vani G, Balakrishna K, et al. Effect of bacoside A on brain antioxidant status in cigarette smoke exposed rats. *Life Sci*. 2006;78(12):1378-1384.
- [21] Stuerenburg HJ, Ganzer S, Arlt S, et al. The influence of smoking on plasma folate and lipoproteins in Alzheimer disease, mild cognitive impairment and depression. *Neuro Endocrinol Lett*. 2005;26(3):261-263.
- [22] Oddo S, Caccamo A, Green KN, et al. Chronic nicotine administration exacerbates tau pathology in a transgenic model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2005;102(8):3046-3051.
- [23] Zhao Z, Reece EA. Nicotine-induced embryonic malformations mediated by apoptosis from increasing intracellular calcium and oxidative stress. *Birth Defects Res B Dev Reprod Toxicol*. 2005;74(5):383-391.
- [24] Levin ED, McClernon FJ, Rezvani AH. Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. *Psychopharmacology (Berl)*. 2006;184(3-4):523-539.
- [25] Maskos U, Molles BE, Pons S, et al. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature*. 2005;436(7047):103-107.
- [26] Newhouse PA, Potter A, Singh A. Effects of nicotinic stimulation on cognitive performance. *Curr Opin Pharmacol*. 2004;4(1):36-46.
- [27] Hernandez CM, Terry AV Jr. Repeated nicotine exposure in rats: effects on memory function, cholinergic markers and nerve growth factor. *Neuroscience*. 2005;130(4):997-1012.
- [28] Mousavi M, Hellström-Lindahl E, Guan ZZ, et al. Protein and mRNA levels of nicotinic receptors in brain of tobacco using controls and patients with Alzheimer's disease. *Neuroscience*. 2003;122(2):515-520.
- [29] Wang HX, Fratiglioni L, Frisoni GB, et al. Smoking and the occurrence of Alzheimer's disease: cross-sectional and longitudinal data in a population-based study. *Am J Epidemiol*. 1999;149(7):640-644.
- [30] Launer LJ, Andersen K, Dewey ME, et al. Rates and risk factors for dementia and Alzheimer's disease: results from EURODEM pooled analyses. EURODEM Incidence Research Group and Work Groups. *European Studies of Dementia*. *Neurology*. 1999;52(1):78-84.
- [31] Maskos U, Molles BE, Pons S, et al. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature*. 2005;436(7047):103-107.
- [32] Wang HX, Karp A, Winblad B, et al. Late-life engagement in social and leisure activities is associated with a decreased risk of dementia: a longitudinal study from the Kungsholmen project. *Am J Epidemiol*. 2002;155(12):1081-1087.
- [33] Deng J, Zhou HD, Li JC, et al. Study of the relationship between cigarette smoking and dementia. *Chongqing Yixue*. 2006;35(10):921-923.
- [34] Wang QH, Zhang ZX, Tang MN, et al. Smoking, alcohol and tea drinking on Alzheimer's disease. *Zhonghua Shenjing Ke Zazhi*. 2004;37(3):234-238.
- [35] Zhang ZX, Zahner GE, Román GC, et al. Dementia subtypes in China: prevalence in Beijing, Xi'an, Shanghai, and Chengdu. *Arch Neurol*. 2005;62(3):447-453.
- [36] Tyas SL, White LR, Petrovitch H, et al. Mid-life smoking and late-life dementia: the Honolulu-Asia Aging Study. *Neurobiol Aging*. 2003;24(4):589-596.
- [37] Giovannucci E, Colditz G, Stampfer MJ, et al. The assessment of alcohol consumption by a simple self-administered questionnaire. *Am J Epidemiol*. 1991;133(8):810-817.
- [38] Hypertension Prevention Guide Revision Committee. *Hypertension Prevention Guide*. Beijing: People's Medical Press. 2006:1-31.
- [39] American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4<sup>th</sup> (DSM-IV). Washington: American Psychiatric Association. 1994:143-147.
- [40] McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34(7):939-944.

(Edited by Jiang B, Zhu FQ/Su LL/Song LP)