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ORIGINAL RESEARCH

Genetic Effects of NDUFAF6 rs6982393 and APOE on Alzheimer's Disease in Chinese Rural Elderly: A Cross-Sectional Population-Based Study

Yingzhe Cheng[1], Yuanjing Li[2], Xiaoyan Liang[3,4], Pin Wang[3], Wenxin Fa[1], Cuicui Liu[1,3,4], Yongxiang Wang[1,3,4], Keke Liu[5], Nan Wang[3,4], Yifeng Du[1,3,4]

[1]Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, People's Republic of China; [2]Aging Research Center and Center for Alzheimer Research, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet-Stockholm University, Stockholm, Sweden; [3]Department of Neurology, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong, People's Republic of China; [4]Shandong Provincial Clinical Research Center for Neurological Diseases, Jinan, Shandong, People's Republic of China; [5]Shandong Academy of Clinical Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, People's Republic of China

Correspondence: Yifeng Du, Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324 Jingwuweiqi Road, Jinan, Shandong, 250021, People's Republic of China, Tel/Fax +86-531-68776354, Email duyifeng@email.sdu.edu.cn

Purpose: To investigate the associations of genotypes of *NDUFAF6* rs6982393 and *APOE* and their combined genotypes with the risk of Alzheimer's disease (AD) and mild cognitive impairment (MCI) in Chinese rural elderly.

Methods: This cross-sectional population-based study included 5096 older adults (age ≥60 years, 57.1% female). Genotypes of *NDUFAF6* rs6982393 and *APOE* were detected using the multiple-polymerase chain reaction amplification. We diagnosed AD following the criteria of Diagnostic and Statistical Manual of Mental Disorders, the fourth edition and diagnosed MCI following the Petersen's criteria MCI. Data were analyzed using the logistic regression model.

Results: The overall prevalence of AD and MCI was 3.57% (95% confidence interval [CI]: 0.040, 0.053) and 22.65% (95% CI: 0.223, 0.247), separately. The TT versus CC/CT genotype of *NDUFAF6* rs6982393 was related to a higher risk of AD with the multi-adjusted odds ratio (95% CI) being 1.61 (1.02, 2.54) in the total sample, 3.36 (1.48, 7.60) in those aged 60–69, and 1.24 (0.71, 2.17) in those aged 70 years and above. The interaction between genotype of *NDUFAF6* rs6982393 with age groups (60–69 versus ≥70 years) was significant on the risk of AD. The presence of *APOE* ε4 was not significantly associated with the risk of AD. Carrying both *NDUFAF6* TT and *APOE* ε4 was related to a higher risk of AD with the multi-adjusted odds ratio (95% CI) being 2.69 (1.10, 2.56). In addition, there was no significant association between the above genotypes and MCI.

Conclusion: In Chinese rural elderly, the TT versus CT/CC genotype of *NDUFAF6* rs6982393 was associated with an increased likelihood of AD; such an association only existed among young-old adults. Carrying both *NDUFAF6* rs6982393-TT and *APOE* ε4 was related to a higher risk of AD. This finding highlights the importance of considering age and combined genotype in studying the genetic profiles of AD.

Keywords: *NDUFAF6* rs6982393, *APOE*, dementia, population-based study

Introduction

By 2019, people aged 60 years and above have accounted for 17.9% in China.[1] More than 15% of these older adults lived with cognitive impairment and among them approximately 27.9% had dementia,[1] which had brought about heavy economic and labor burdens to the healthcare system.[2,3] Among the dementia profiles, Alzheimer's disease (AD) is the most common type, characterized by insidious onset, inevitable memory loss, cognitive decline in other domains, functional decline, and even increased mortality in the late stage.[4] Besides, mild cognitive impairment (MCI) is an intermediate stage between normal cognition and dementia, characterized by subjective complaints of cognitive decline, or objectively measured cognitive deterioration in specific domains, while intact daily function.[5] It has been well

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established that the presence of *APOE* ε4 could confer an increased risk of AD-type dementia and MCI in the European population.[6–8] Whereas the influence of *APOE* ε4 on AD or MCI has not achieved consistency in the Chinese elderly.[9–13]

Recently, data from the genome-wide association studies suggested that a single nucleotide polymorphism of *NDUFAF6* rs6982393 could bring about an increased risk of AD in the European population.[14] In brief, *NDUFAF6* rs6982393 encodes a protein involved in the metabolism of amyloid-β and tau protein in the etiopathology of AD.[15,16] Rare study has examined the possible influence of *NDUFAF6* rs6982393 on AD or MCI in the Chinese population. Most of the previous studies on genetic impacts on dementia have been conducted among highly educated elderly in general settings, where the findings may not be generalizable to the rural dwellers with no to little formal education. Considering that lower educational attainment serves as an independent risk factor of dementia, it is worth to explore possible genetic effect in Chinese rural elderly with limited education. Besides, it is also meaningful to investigate the possible joint effect of genotypes of NDUFAF6 rs6982393 and APOE and identified those with a higher inherited risk of MCI or AD. Furthermore, given that susceptibility genes of AD may exert differential genetic effects by age groups (such as APOE, PICALM and TOMM40 gene),[17–19] it is plausible to hypothesize that the possible above genetic influence on AD or MCI may vary by age groups.

Therefore, in this population-based study, we aimed to investigate if genotypes of *NDUFAF6* rs6982393, *APOE*, and their combined genotypes were related to the risks of AD and MCI in Chinese rural elderly with limited education attainment, and if such associations varied by age groups.

Methods

Study Population

This population-based study included participants from the ongoing Multimodal Interventions to Delay Dementia and Disability in Rural China (MIND-China).[20–22] The MIND-China study was launched by Shandong Provincial Hospital, Shandong University Cheeloo College of Medicine, in collaboration with Yanlou Town Hospital. During baseline investigation (March–September 2018), the MIND-China study targeted older residents aged 60 years and in the 52 villages of Yanlou Town, Yanggu County, western Shandong Province. A total of 5765 participants were enrolled in this study. Of these participants, 669 were excluded due to missing genotyping information (n = 250) or missing diagnosis of dementia (n = 320) or other types of dementia than AD (n = 99). Thus, the analytical sample included 5096 participants. Figure 1 shows the flowchart of study participants.

The MIND-China study was conducted in accordance with the Declaration of Helsinki and has been approved by the Ethics Committee of Shandong Provincial Hospital in Jinan, Shandong. Written informed consents were acquired from all participants, or for people with severe cognitive or functional impairment, obtained from their proxies. The MIND-China study was registered in the Chinese Clinical Trial Registry (registration no.: ChiCTR1800017758).

Data Collection and Assessment

The procedure of baseline investigation was described previously.[20,21] In brief, data were collected by trained staff through face-to-face interviews, clinical examinations, neuropsychological questionnaires, and laboratory tests. We collected data on demographics age, sex, and education), lifestyle factors (such as smoking and alcohol drinking), health status (such as hypertension, diabetes, dyslipidemia, coronary heart disease, and stroke), medical history (such as antihypertensive and hypoglycemic drugs), use of medications, and cognitive performance. All medications were classified according to Anatomical Therapeutic Chemical classification (ATC) system. Weight and height were measured in light clothes without shoes. After a rest of at least five minutes, the sitting arterial blood pressure of the right arm was measured using an electronic sphygmomanometer (hem-7127j, OMRON company, Kyoto, Japan). The 12-lead resting electrocardiogram was recorded by electrocardiograph (CM300, Shenzhen Branch, China) and then analyzed by local doctors. After an overnight fast, peripheral blood samples were taken and blood glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol were measured by automatic biochemical analyzer (cs-600b, Changchun Dirui company, China) in the laboratory of Yanlou town hospital. Alcohol drinking and smoking were divided into never, former, and current, respectively. Body mass index was calculated as weight in

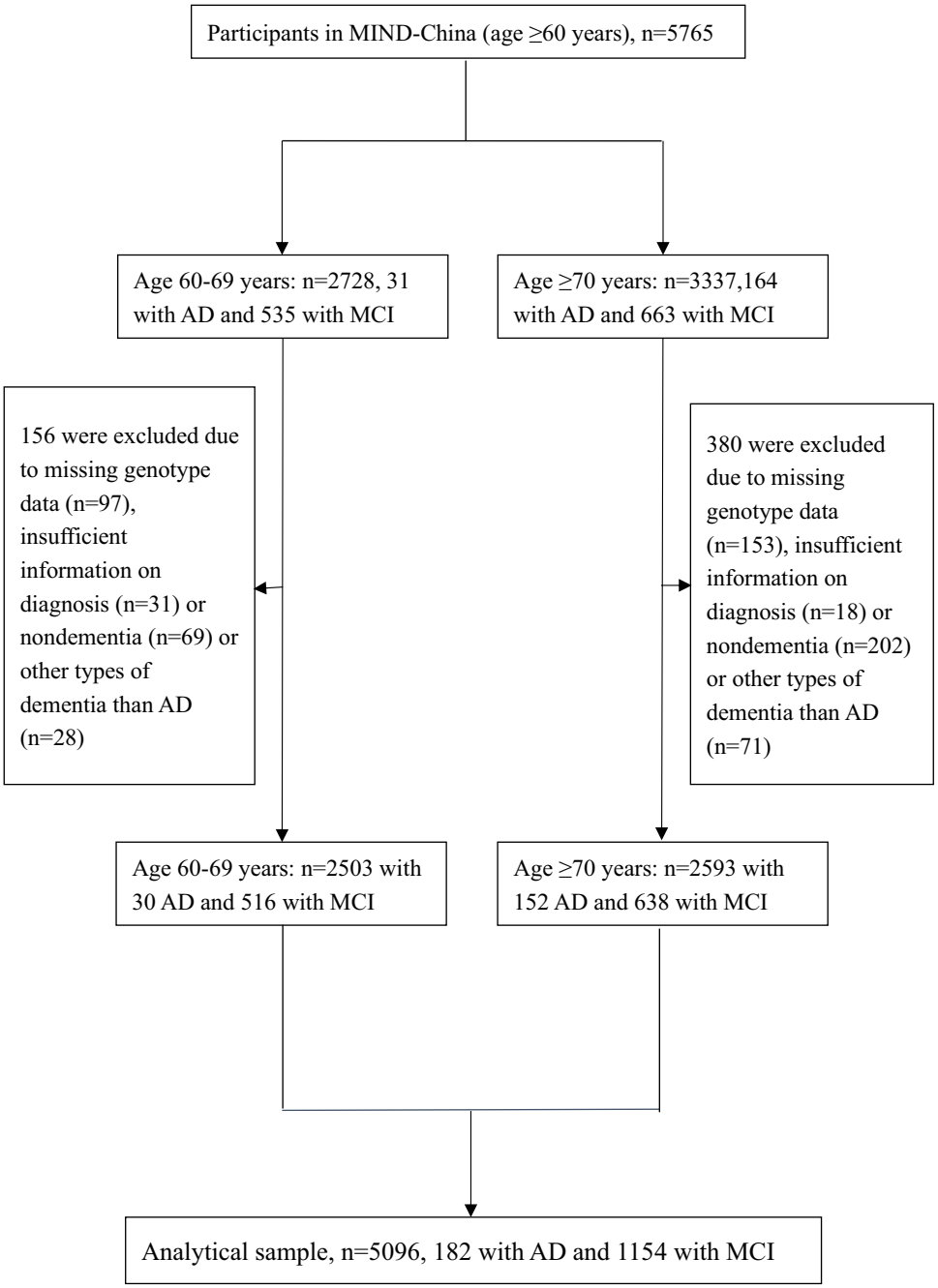

Figure 1 Flowchart of the study participants.
Abbreviations: MIND-China, Multimodal Intervention to Delay Dementia and Disability in Rural China; AD, Alzheimer's disease; MCI, mild cognitive impairment.

kilograms divided by the square of height in meters. Hypertension was defined as the systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or current use of antihypertensive drugs. Diabetes was defined as the fasting blood glucose ≥7.0 mmol/L, using hypoglycemic drugs, or a previous record of diabetes. Dyslipidemia was defined as the total cholesterol ≥6.2 mmol/L, triglyceride ≥2.3 mmol/L, low-density lipoprotein cholesterol ≥4.1 mmol/L, high-density lipoprotein cholesterol <1.0 mmol/L, or using lipid-lowering drugs.[23] Coronary heart disease was defined by a previous record or evidence from the electrocardiograph, including angina pectoris, myocardial infarction, coronary angioplasty, and coronary artery bypass grafting. Stroke was defined according to a previous record or typical neurological signs.

Genomic DNA Extraction and Genotyping

The trained staff collected venous blood from participants and extracted genomic deoxyribonucleic acid (DNA) from venous blood leukocytes using the TIANamp blood DNA kit (Tiangen, Beijing, China). Then, DNA was quantified using Nanodrop 3300 spectrometry and a total amount of 100 ng genomic DNA per sample was used for the DNA sample preparation. Subsequently, sequencing libraries were generated using MultipSeqCustom Panel (iGeneTech, Beijing, China) following standard procedures and index codes were added to each sample. Finally, qualified libraries were subjected to next-generation sequencing on a Novaseq system (Illumina), and raw data were filtered to remove low-quality reads using FastQC. Genotyping was conducted by an operator who was blinded to all clinical data.

The genotypes of *APOE* and *NDUFAF6* rs6982393 were detected using multiple-polymerase chain reaction amplification (iGeneTech Bioscience Co., Ltd, Beijing, China). The amplification of *NDUFAF6* gene used the following primers: forward, 5′- CTGGGCGCGGACATGG −3′; reverse, 5′- CACCTTGGTTGTGTGATTTGTGAA −3′. The amplification of *APOE* gene used the following primers: forward, 5′- CTGGGCGCGGACATGG −3′; reverse, 5′- ttctGCAGGTCATCGGCATC −3′. The distribution of the *NDUFAF6* rs6982393 and *APOE* ε4 conformed to the Hardy-Weinberg equilibrium.

Diagnosis on Dementia and Mild Cognitive Impairment

The diagnosis of dementia was based on the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) and followed a three-step diagnostic procedure.[24] First, trained clinicians and interviewers performed the routine clinical examinations and comprehensive neuropsychological evaluations on each participant. Then, the experienced neurologists reviewed all neuropsychological information and made a preliminary diagnosis of dementia. Finally, these neurologists conducted additional face-to-face interviews with participants suspected of having dementia, and re-evaluated their medical history, cognitive and functional records, and any possible neuroimaging data. If the participants were unable to be interviewed (approximately 13%) due to severe cognitive impairment, neurologists would visit their family members, neighbors, or village doctors for further information. After all interviews and evaluations, neurologists ascertained the final diagnosis of dementia according to DSM-IV criteria.[24] In the case of uncertainty, consultation would be provided with senior neuroscientist (L.C.) to discuss and reach a consensus diagnosis of dementia. The diagnosis of Alzheimer's disease is based on the criteria of the American Alzheimer's Association.[25]

We defined MCI following the Petersen's criteria that were operationalized in the Mayo Clinic Study of Aging:[26] (1) cognitive concern by subjects (according to memory complaints, Ascertain Dementia 8, or Clinical Dementia Rating Scale) or informants; (2) objective cognitive impairment evidenced in at least one of the four cognitive domains of memory, attention, execution, and language (according to cognitive test battery); (3) essentially preserved daily function (according to Activities of Daily Living); and (4) absence of dementia (according to DSM-IV).[24]

Statistical Analyses

First, we compared the characteristics of study participants using *t*-test for continuous variables and chi-square test for categorical variables. Next, we examined the associations of genotypes of *NDUFAF6* rs6982393 (TT versus CC/CT) and APOE genotype (with or without ε4) with AD and MCI using the multinomial logistic regression models. We report results from three models: Model 1 was adjusted for age, sex, and education; Model 2 was additionally adjusted for vascular risk factors significantly related to the risks of MCI or AD (body mass index, alcohol consumption, smoking status, and coronary heart disease) based on Model 1; model 3 was additionally adjusted for diabetes, hypertension, dyslipidemia, and stroke and *APOE* or *NDUFAF6* rs6982393 genotypes, if applicable, based on Model 2. In addition, we examined the statistical interactions of above genotypes with age groups (60–69 versus ≥70 years) on the prevalence of AD and MCI. Age-stratified analyses were performed once statistically significant interactions were detected (p for interaction <0.05). Finally, we examined the combined effect of *NDUFAF6* rs6982393-TT and *APOE* ε4 on the risks of AD and MCI using multinomial logistic regression models. IBM SPSS Statistics version 22.0 (IBM SPSS Inc., Chicago, Illinois) was used for all analyses.

Results

Characteristics of Study Participants

Of the 5096 participants, the mean age was 70.4 (SD, 5.5) years, 57.1% were women, and the average years of formal schooling was 3.3 (SD, 3.5) years. Among all participants, 1154 (prevalence: 22.65%, 95% CI: 0.223,0.247) had MCI and 182 (prevalence: 3.57%, 95% CI, 0.040,0.053) had AD. Compared to cognitively normal participants, those with AD were older, less educated, had higher female proportion and lower BMI, and were less likely to smoke or to drink, while more likely to have coronary heart disease (≤0.005). Participants with different cognitive phenotypes did not differ in the proportions of diabetes, dyslipidemia, hypertension, or genotypes (Table 1).

Associations of Polymorphisms of *NDUFAF6* rs6982393 and *APOE* with Alzheimer's Disease and Mild Cognitive Impairment

NDUFAF6 rs6982393-TT versus CC/CT genotype was significantly associated with a ~61% increased likelihood of AD after multiple adjusting, while was not significantly related to MCI (Table 2). There were significant interactions of age group (60–69 versus ≥70 years) with genotypes of *NDUFAF6* rs6982393 on the likelihood of AD (*P* for interaction = 0.038). Age-stratified analyses revealed that compared to *NDUFAF6* rs6982393-CC/CT, the TT genotype was significantly associated with a ~236% increased likelihood of AD among participants aged 60–69 years, but not among those aged ≥70 years after multiple adjusting (Figure 2). There was no significant interaction of age group with genotype of

Table 1 Characteristics of Study Participants

Characteristics	Total, N=5096	Cognitive Phenotypes			*P*-value
		Normal, n=3760	MCI, n=1154	AD, n=182	
Age (years)	70.42 (5.46)	69.83 (5.05)	71.23 (5.56)	77.51 (7.16)	<0.001
Age groups (years), n (%)					<0.001
60–69	2503 (49.10)	66.01 (2.35)	66.52 (2.14)	67.2 (1.27)	
≥70	2593 (50.9)	73.99 (3.75)	75.03 (4.46)	79.55 (5.98)	
Female, n (%)	2911 (57.1)	2001 (53.2)	382 (33.1)	138 (75.8)	<0.001
Education (years)	3.30 (3.50)	3.80 (3.63)	1.99 (2.68)	1.15 (2.30)	<0.001
BMI (kg/m^2)	24.91 (3.76)	25.02 (3.71)	24.74 (3.86)	23.81 (3.90)	<0.001
Alcohol drinking, n (%)					<0.001
Never	3161 (61.3)	213 (57.2)	829 (71.8)	148 (81.8)	
Former	481 (9.3)	345 (9.2)	139 (12.0)	11 (6.1)	
Current	1511 (29.3)	1251 (33.5)	186 (16.1)	22 (12.2)	
Smoking, n (%)					<0.001
Never	3317 (63.9)	2275 (60.5)	829 (71.8)	148 (81.3)	
Former	766 (14.8)	591 (15.7)	139 (12.0)	18 (9.9)	
Current	1110 (21.4)	893 (23.8)	186 (16.1)	16 (8.8)	
Hypertension, n (%)	3447 (66.9)	2472 (66.3)	789 (68.9)	117 (64.6)	0.647
Diabetes, n (%)	747 (14.4)	531 (14.1)	161 (14.0)	29 (15.9)	0.494
Dyslipidemia, n (%)	1250 (24.1)	888 (23.6)	279 (24.2)	48 (26.4)	0.393
Coronary heart disease, n (%)	1098 (21.1)	767 (20.4)	252 (21.8)	53 (29.1)	0.005
Stroke, n (%)	745 (15.8)	465 (13.8)	190 (16.5)	26 (14.3)	0.868
***APOE* ε4, n (%)**	830 (16.0)	589 (15.7)	190 (16.5)	34 (18.7)	0.276
***NDUFAF6* rs6982393**					0.089
CC/CT	4583 (88.2)	3336 (88.7)	1007(87.3)	154 (84.6)	
TT	612 (11.8)	424 (11.3)	147 (12.7)	28 (15.4)	

Notes: Data were means (standard deviations), unless otherwise specified. Missing numbers were 26 for BMI, 42 for alcohol drinking, for 2 for smoking, 37 for hypertension, 490 for stroke. In the subsequent analyses, a dummy variable was created for each of the covariates with missing values.

Abbreviations: *APOE*, Apolipoprotein E gene; BMI, body mass index; *NDUFAF6*, NADH dehydrogenase (ubiquinone) complex I assembly factor 6; MCI, mild cognitive impairment; AD, Alzheimer's disease.

Table 2 Associations of Polymorphism of *NDUFAF6* rs6982393 with Mild Cognitive Impairment and Alzheimer's Disease

Genotype of *NDUFAF6* rs6982393	Total No.	Odds Ratios (95% Confidence Intervals)							
		Mild Cognitive Impairment				Alzheimer's Disease			
		No.	Model 1	Model 2	Model 3	No.	Model 1	Model 2	Model 3
CC/CT TT	4498 598	1007 147	1.00 (Ref.) 1.16 (0.94, 1.43)	1.00 (Ref.) 1.16 (0.94, 1.43)	1.00 (Ref.) 1.17 (0.95, 1.43)	154 28	1.00 (Ref.) 1.62 (1.03, 2.54)[a]	1.00 (Ref.) 1.61 (1.03, 2.54)[a]	1.00 (Ref.) 1.61 (1.02, 2.54)[a]
P for interaction of polymorphism of *NDUFAF6* rs6982393 with age groups									
	5096	1154	0.660	0.660	0.576	182	0.028	0.031	0.038

Notes: Model 1 was adjusted for age, sex, and education; Model 2 was additionally adjusted for body mass index, alcohol consumption, smoking status, and coronary heart disease based on Model 1; Model 3 was additionally adjusted for diabetes, hypertension, dyslipidemia, stroke, and APOE genotypes based on Model 2. [a]$P < 0.05$.
Abbreviations: No., number; *NDUFAF6*, NADH dehydrogenase (ubiquinone) complex I assembly factor 6; *APOE*, Apolipoprotein E gene; MCI, mild cognitive impairment; AD, Alzheimer's disease.

NDUFAF6 rs6982393 on the risk of MCI (Table 2). In addition, the presence of APOE ε4 was not significantly associated with the risk of AD or MCI (Supplementary Table 1).

Associations of Combined Effect Between Genotypes of NDUFAF6 Rs6982393 and APOE on Alzheimer's Disease and Mild Cognitive Impairment

Carrying both *NDUFAF6 rs6982393*-TT and *APOE* ε4 was related to a ~69% increased risk of AD after multiple adjusting (Table 3). Carrying *NDUFAF6 rs6982393*-TT while not *APOE* ε4 was related to a higher risk of MCI after adjusting for age, sex, and education. Whereas, such association was not significant after adjusted for vascular risk factors (Table 3).

Discussion

In this population-based study of rural-dwelling Chinese older adults, we found that *NDUFAF6* TT (versus CC/CT) genotype was associated with an increased likelihood of AD, especially among young-old people. The presence of *APOE*

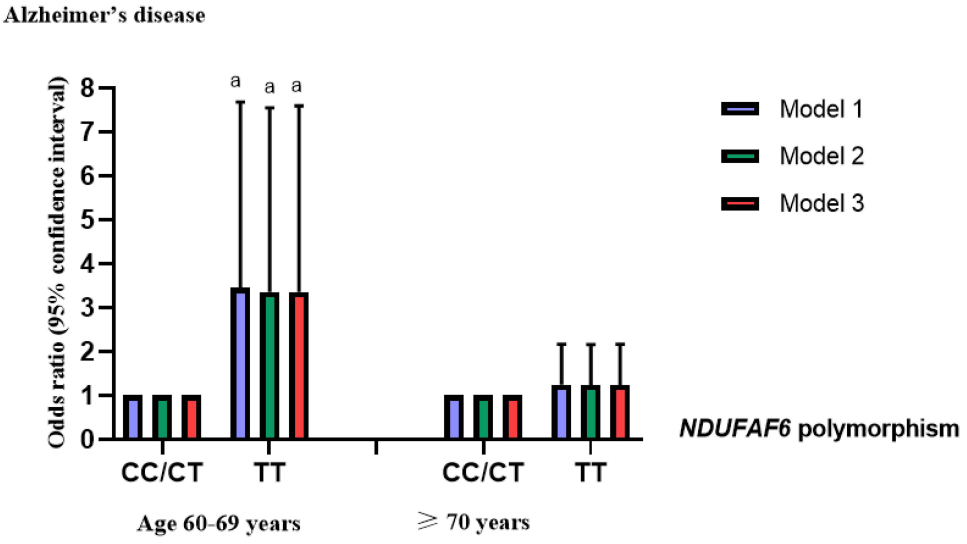

Figure 2 Associations between genotypes of *NDUFAF6* rs6982393 and Alzheimer's disease by age groups.
Notes: Model 1 was adjusted for age, sex, and education. Model 2 was additionally adjusted for body mass index, alcohol consumption, smoking status and coronary heart disease. Model 3 was additionally adjusted for hypertension, diabetes, dyslipidemia, stroke and *APOE* genotypes. [a]$p < 0.05$.
Abbreviation: *NDUFAF6*, NADH dehydrogenase (ubiquinone) complex I assembly factor 6.

Table 3 Associations of Combined Effect of Genotypes of *NDUFAF6* rs6982393 with *APOE* on Mild Cognitive Impairment and Alzheimer's Disease

Genotypes of APOE	Genotypes of *NDUFAF6* rs6982393	Total No.	Odds Ratios (95% Confidence Intervals)							
			Mild Cognitive Impairment				Alzheimer's Disease			
			No.	Model 1	Model 2	Model 3	No.	Model 1	Model 2	Model 3
ε4 (-)	CC/CT	3768	832	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	128	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
ε4 (-)	TT	514	131	1.25 (1.01, 1.56)[a]	1.25 (1.00, 1.56)	1.23 (0.99, 1.54)	20	1.51 (0.90, 2.52)	1.51 (0.90, 2.54)	1.44 (0.85, 2.44)
ε4 (+)	CC/CT	729	175	1.17 (0.97, 1.42)	1.14 (0.94, 1.39)	1.15 (0.94, 1.40)	27	1.36 (0.86, 2.15)	1.33 (0.84, 2.11)	1.31 (0.82, 2.08)
ε4 (+)	TT	89	16	0.86 (0.49, 1.52)	0.84 (1.48, 1.49)	0.85 (0.48, 1.50)	7	2.71 (1.11, 6.61)[a]	2.55 (1.04, 6.26)[a]	2.69 (1.10, 2.56)[a]

Notes: Model 1 was adjusted for age, sex, and education; Model 2 was additionally adjusted for body mass index, alcohol consumption, smoking status, and coronary heart disease based on Model 1; Model 3 was additionally adjusted for diabetes, hypertension, dyslipidemia, and stroke based on Model 2. [a]$P < 0.05$.
Abbreviations: No., number; *NDUFAF6*, NADH dehydrogenase (ubiquinone) complex I assembly factor 6; *APOE*, Apolipoprotein E gene.

ε4 was not related to the risks of AD or MCI. Moreover, the combined genotype of *NDUFAF6* TT and *APOE* ε4 was associated with a higher risk of AD.

To the best of our knowledge, this is the first population-based study to investigate the association of *NDUFAF6* rs6982393 with AD and MCI. Our results were in line with the previous genome-wide association study in European countries.[14] *NDUFAF6*-TT, encodes a protein involved in the assembly of mitochondrial respiratory chain complex I, which might contribute to oxidative stress, and further contribute to amyloid-β aggregation and tau phosphorylation.[27] A slight inhibition of the activity of complex I would improve the cognitive function by inhibiting amyloid-β aggregation and tau phosphorylation in animal models.[28] In addition, previous studies summarized the genome-wide association studies and found that *NDUFAF6* rs6982393 might be the susceptibility gene of both type II diabetes and AD,[14] which suggested that *NDUFAF6* rs6982393 might confer risk of AD by pathophysiological mechanisms relevant to diabetes. Taken above, these findings supported the association between genotypes of *NDUFAF6* rs6982393 and AD. On the other hand, we found that the association of *NDUFAF6* rs6982393 TT genotype with AD was only evident in young-old, which was in accordance with the view that the potential genetic influence on the risk of AD might be weakened by advancing age.[19,29] Moreover, the selective survival bias should be taken into consideration when interpreting the genetic effect in people aged 70 years and above.

We did not find a strong association between *APOE* ε4 and the risk of AD or MCI, which was consistent with other Chinese cohort studies.[30,31] The frequency of the *APOE* ε4 allele differ between Asian and Caucasian population,[10,32,33] which might lead to different statistical powers. In addition, the various molecular signatures of *APOE* region across ethics may also account for the different association between the *APOE* ε4 allele and AD among race/ethnic groups.[34]

Our study revealed a combined effect between genotypes of *NDUFAF6* rs6982393 and *APOE* variants on AD. Previous studies via biochemical assays and proteomic profiling of mice neurons indicated Apolipoprotein E4 would lead to neurotoxicity by interfering with mitochondrial normal functions.[35] The post-mortem studies in patients with AD found the isoform of Apolipoprotein E would affect the natural structure and functions of mitochondrion, which would further lead to increased level of oxidative stress, synaptic dysfunction, and cognitive decline.[36] Taking into account the fact that *NDUFAF6* rs6982393 might also lead to cognitive decline via interfering with mitochondrial function, the combined effect of *NDUFAF6* and *APOE* risk variants on AD may be partly attributed to the common pathological mechanisms such as mitochondrial dysfunction.

The strength of our study was the population-based design and the rural-dwelling sample of Chinese older adults, integrating epidemiological, clinical, and genetic data. Our study also has limitations. First, in the cross-sectional design, the selective survival bias might be inevitable, which usually leads to an underestimated association. Second, the sample

size of the study was not large enough for genetic research which might limit the statistical power in analyses. Thirdly, the study participants were recruited from a single rural area in north China, and characterized by lower educational attainment, which should be kept in mind when extending our findings to the other population.

Conclusion

In conclusion, among rural-dwelling Chinese older adults, the *NDUFAF6* rs6982393-TT (versus CC/CT) genotype was related to a higher risk of AD especially in young-old people. The combined genotype of *NDUFAF6* rs6982393-TT and *APOE* ε4 might be associated with a higher risk of AD. It is worth to explore the pathophysiological role of *NDUFAF6* rs6982393 in the general population in the future studies. Besides, this finding highlights the importance of considering age and combined genotypes in studying the genetic profiles of AD.

Abbreviations

AD, Alzheimer's disease; MCI, Mild cognitive impairment; *NDUFAF6*, NADH dehydrogenase (ubiquinone) complex I assembly factor 6; *APOE*, Apolipoprotein E; ATC, Anatomical Therapeutic Chemical; BMI, body mass index; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; MIND-China, Multimodal Intervention to Delay Dementia and Disability in Rural China; NIA-AA, National Institute on Aging-Alzheimer's Association.

Data Sharing Statement

Data supporting the findings from this study will be available from the corresponding authors upon approval by the data management committee of MIND-China.

Ethics Approval and Informed Consent

MIND-China was approved by the Ethics Committee of Shandong Provincial Hospital in Jinan, Shandong. Written informed consent was obtained from all participants, or in the case of severely cognitively impaired participants, from informants. MIND-China was registered in the Chinese Clinical Trial Registry (registration no.: ChiCTR1800017758).

Consent for Publication

All authors confirm that the work described has not been published before, that it is not under consideration for publication elsewhere, and that its publication has been approved by all co-authors.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, agreed to the submitted journal, and agreed to be accountable for all aspects of the work.

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Disclosure

The authors declared no conflicts of interest in connection with this study.

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