Received: 9 February 2020 | Revised: 24 May 2020 | Accepted: 28 June 2020

DOI: 10.1002/alz.12153

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

RESEARCH ARTICLE

The *APOE ε4* exerts differential effects on familial and other subtypes of Alzheimer's disease

Longfei Jia[1] | Hui Xu[1] | Shuoqi Chen[1] | Xiu Wang[1] | Jianwei Yang[1] | Min Gong[1] | Cuibai Wei[1] | Yi Tang[1] | Qiumin Qu[2] | Lan Chu[3] | Lu Shen[4] | Chunkui Zhou[5] | Qi Wang[1] | Tan Zhao[1] | Aihong Zhou[1] | Ying Li[1] | Fangyu Li[1] | Yan Li[1] | Hongmei Jin[1] | Qi Qin[1] | Haishan Jiao[1] | Yan Li[1] | Heng Zhang[1] | Diyang Lyu[1] | Yuqing Shi[1] | Yang Song[1] | Jianping Jia[1,6,7,8]

[1] Innovation Center for Neurological Disorders and Department of Neurology, Xuanwu Hospital, Capital Medical University, National Clinical Research Center for Geriatric Diseases, Beijing, China

[2] Department of Neurology, The First Affiliated Hospital of Xi'an Jiaotong University, Xian, China

[3] Department of Neurology, The Affiliated Hospital of Guizhou Medical University, Guiyang, China

[4] Department of Neurology, Xiangya Hospital Central South University, Changsha, China

[5] Department of Neurology, The First Teaching Hospital of Jilin University, Changchun, China

[6] Beijing Key Laboratory of Geriatric Cognitive Disorders, Beijing, China

[7] Clinical Center for Neurodegenerative Disease and Memory Impairment, Capital Medical University, Beijing, China

[8] Center of Alzheimer's Disease, Beijing Institute for Brain Disorders, Beijing, China

Correspondence
Jianping Jia, Innovation Center for Neurological Disorders and Department of Neurology, Xuanwu Hospital, Capital Medical University, National Clinical Research Center for Geriatric Diseases, Beijing 100053, China.
Email: jjp@ccmu.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Number: 81530036; National Key Scientific Instrument and Equipment Development Project, Grant/Award Number: 31627803; Mission Program of Beijing Municipal Administration of Hospitals, Grant/Award Number: SML20150801; Beijing Scholars Program; Beijing Brain Initiative from Beijing Municipal Science & Technology Commission, Grant/Award Number: Z161100000216137; Project for Outstanding Doctor with Combined Ability of Western and Chinese Medicine; and Beijing Municipal Commission of Health and Family Planning, Grant/Award Number: PXM2019_026283_000003

Abstract

Introduction: The genetic risk effects of apolipoprotein E (*APOE*) on familial Alzheimer's disease (FAD) with or without gene mutations, sporadic AD (SAD), and normal controls (NC) remain unclear in the Chinese population.

Methods: In total, 15 119 subjects, including 311 FAD patients without *PSEN1*, *PSEN2*, *APP*, *TREM2*, and *SORL1* pathogenic mutations (FAD [unknown]); 126 FAD patients with *PSENs/APP* mutations (FAD [*PSENs/APP*]); 7234 SAD patients; and 7448 NC were enrolled. The risk effects of *APOE ε4* were analyzed across groups.

Results: The prevalence of the *APOE ε4* genotype in FAD (unknown), FAD (*PSENs/APP*), SAD, and NC groups was 56.27%, 26.19%, 36.23%, and 19.54%, respectively. Further, the *APOE ε4* positive genotype had predictive power for FAD (unknown) risk (odds ratio: 4.51, 95% confidence interval: 3.57–5.45, $P < .001$).

Discussion: *APOE ε4* positive genotype may cause familial aggregation, and the investigation of multiple interventions targeting *APOE* pathological function to reduce the risk for this disease warrants attention.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2020 The Authors. *Alzheimer's & Dementia* published by Wiley Periodicals, Inc. on behalf of Alzheimer's Association

KEYWORDS
Alzheimer's disease, *APOE*, dementia, gene mutation, genetic risk effect, prevalence

1 | INTRODUCTION

The aging population in China has reached an unprecedented level. Dementia, especially Alzheimer's disease (AD), has become a serious social and family burden.[1] AD is classified into familial Alzheimer's disease (FAD) and sporadic Alzheimer's disease (SAD). FAD is almost entirely genetically determined, with heritability ranging from 92% to 100%.[2] It is characterized by an early age of onset and pedigree clustering. FAD has been widely researched over the years since its first identification. The pathogenic mutations in the amyloid precursor protein (*APP*),[3] presenilin 1 (*PSEN1*),[4] and presenilin 2 (*PSEN2*)[5] genes involved in the amyloid beta (Aβ) peptide processing, leads to development of FAD. However, these mutations underlie FAD in only a small proportion of cases, leaving a large group of familial subtypes genetically unexplained.[6] Advances in sequencing techniques have enabled the identification of rare mutations and variants with moderate-to-strong risk effects on this complex disease. Recently, next-generation sequencing studies have identified new loci such as sortilin-related receptor 1 (*SORL1*)[7] and triggering receptor expressed on myeloid cells 2 (*TREM2*),[8] suggesting the role of functional pathways other than Aβ processing in AD pathogenesis. These new genes and mechanistic pathways could inspire new diagnostic concepts or therapeutic targets for AD.

Apolipoprotein E (*APOE*) is regarded as the greatest risk gene for AD; among the three isoform alleles ($\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$), *APOE* $\varepsilon 4$ is considered the primary genetic risk factor.[9] Homozygous *APOE* $\varepsilon 4$ carriers usually exhibit earlier onset of the disease than heterozygous carriers, highlighting the dose-effect of the $\varepsilon 4$ allele on AD. One copy of the $\varepsilon 4$ allele increases the risk of AD by $\approx$2 to 6 times, and the presence of two copies increases the risk by 7.2 to 21.8 times (African Americans 7.2, Hispanics 8.6, white 11.8, Japanese 21.8).[9-14] The general frequency of the *APOE* $\varepsilon 4$ allele ranges from 9% to 23% in diverse ethnic populations (Asian 9%, Hispanic 12%, white 14%, African descent 19%, other/mixed 23%), but dramatically increases in AD patients (Hispanic 24%, Asian 28%, African descent 35%, white 38%, other/mixed 45%).[15] The frequency of the *APOE* $\varepsilon 4$ allele also varies among these subtypes.[16,17] A recent study in a cohort of 404 Chinese subjects with FAD showed that among patients without *PSENs/APP* mutations, 44.31% carried one *APOE* $\varepsilon 4$ allele, while 14.85% carried two *APOE* $\varepsilon 4$ alleles.[18] This suggests that *APOE* $\varepsilon 4$ plays a major role in cases of FAD without *PSENs/APP* mutations. These results challenge the role of *APOE* as a risk factor mainly for SAD development. Therefore, a large-scale, multicenter study is necessary to investigate the effects of *APOE* in FAD, especially in those without *PSENs/APP* mutations.

Our aim was to explore the distribution and genetic effects of the *APOE* $\varepsilon 4$ genotype in FAD without *APP*, *PSEN1*, *PSEN2*, *TREM2*, and *SORL1* mutations. In addition, we compared the frequency of *APOE* in the Chinese population with data available from other countries. The outcomes of this study provide more clarity regarding the regulation of AD by the *APOE* $\varepsilon 4$ positive genotype.

2 | METHODS

2.1 | Participants

In total, 15 119 individuals, comprising 437 FAD, 7234 SAD patients, and 7448 normal controls (NC), were included in this study between January 2013 and May 2019.

FAD patients were enrolled from the Chinese Familial Alzheimer's Disease Network (CFAN), which is a multicenter nationwide longitudinal study (www.chinacfan.org). All individuals from families with AD underwent *APOE* genotyping and testing for *PSEN1*, *PSEN2*, *APP*, *TREM2*, and *SORL1* mutations. Depending on whether an individual carried a pathogenic mutation in an AD-associated gene, the cohort was divided into the following two subgroups: FAD without *PSEN1*, *PSEN2*, *APP*, *TREM2*, and *SORL1* mutations (FAD [unknown]), and FAD with *PSEN1*, *PSEN2*, and *APP* mutations (FAD [*PSENs/APP*]). In total, 311 patients with FAD (unknown) and 126 patients with FAD (*PSENs/APP*) were included in this study. The samples of SAD were derived from the China Cognition and Aging Study (COAST), which is a multicenter cohort study comprising clinical diagnosis, disease progression, genetic regulation, and drug trials across 30/31 provinces in China. To investigate the prevalence of the *APOE* $\varepsilon 4$ genotypes and allele frequencies in AD among the Chinese population, we excluded individuals for whom *APOE* genotype data were not available. The protocol of this study was approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University. Written informed consent was obtained from the participants or their legal guardians prior to any study procedures.

2.2 | Procedures

The participants from the CFAN or COAST studies were initially assessed using the Mini-Mental State Examination (MMSE). Participants with the MMSE score <26 points underwent an additional structured clinical visit, during which their demographic information, family history, and medical history were collected, and neurological physical examination and neuropsychological assessments were performed. Four cognitive domains, namely memory, executive function, language, and visuo-constructive skills, were assessed with a battery of neuropsychological scales, including the Montreal Cognitive Assessment, the Chinese Version of the World Health Organization University of California-Los Angeles, Auditory Verbal Learning Test (WHO-UCLA AVLT), Trail Making Test, Digit Span Test, and Boston Naming Test. Any neuropsychiatric symptoms were detected using

the Neuropsychiatric Inventory. Functional ability was assessed by the Activities of Daily Living. Overall cognitive function was evaluated using a clinical dementia rating (CDR); CDR global scores (range 0-3) and CDR sum of boxes (SOB) scores (range 0-18) were recorded.

The following four groups were included in this study: FAD (unknown), FAD (*PSENs/APP*), SAD, and NC. The FAD (unknown) group comprised individuals with at least two first-degree relatives affected by AD across two successive generations and without missense mutations in *PSEN1*, *PSEN2*, *TREM2*, and *SORL1*. The FAD (*PSENs/APP*) group comprised individuals with at least two first-degree relatives affected by AD across two successive generations and with missense mutations in the *PSEN1*, *PSEN2*, or *APP* genes. The NC group comprised individuals with normal cognition with an MMSE score ≥26 points from the China COAST. For FAD (unknown), FAD (*PSENs/APP*), and SAD groups, dementia status was diagnosed according to the criteria described by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision.[19] The diagnosis of AD was made using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association[20] or the National Institute on Aging-Alzheimer's Association criteria.[21] The detailed inclusion and exclusion criteria are provided in Table S1 in supporting information.

Blood samples were drawn by venipuncture at baseline and DNA was extracted by salting-out procedures, as previously described.[22] The Sanger sequencing method was used to determine the *APOE* genotypes. Exon 4 of the *APOE* gene was amplified by PCR using the following primers: *APOE* sense, 5′-AGACGCGGGCACGGCTGTCCAAGGA-3′; and *APOE* antisense, 5′-CCCTCGCGGGCCCCGGCCTGGTACAC-3′. *PSENs/APP* mutation genotyping was performed by screening exons 3-12 of *PSEN1*, exons 3-12 of *PSEN2*, and exons 16-17 of *APP* genes with the flanking intron sequences being amplified by PCR, using specific primers.[23,24] The PCR products were sequenced using an ABI3730xl DNA Analyzer (Sangon Biotech Co., Ltd., Shanghai, China). The DNA sequences were analyzed using Chromas (Chromas version 2.33, Technelysium Pty Ltd, USA). The pathogenicity of the detected mutations in *PSEN1*, *PSEN2*, *APP*, *TREM2*, or *SORL1* was assessed using the AD Mutation Database (http://www.molgen.ua.ac.be/ADMutations/), AlzForum (http://www.alzforum.org/), PubMed (http://www.ncbi.nlm.nih.gov/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.mutationtaster.org).

2.3 | Statistical analyses

Descriptive statistics were used to summarize the participant characteristics, including the variables of age, age at onset, sex, years of education, MMSE scores, and CDR scores. The means ± standard deviation were used to describe the quantitative variables. The prevalence of the *APOE* allele and genotype in each group was analyzed by Pearson's χ^2 and Fisher's exact test (as necessary), with post hoc Bonferroni corrections. Binary logistic regression models were conducted to evaluate the predictive effect of the *APOE* genotypes and ε4- or ε2- positive genotype status on the cases (FAD [unknown], FAD [*PSENs/APP*], and SAD) compared to the NC after adjusting for age, sex, education, and region of subjects. We further calculated the risk of *APOE* genotype for AD across different ages in the FAD (unknown) group and SAD group using a binary logistic regression model after adjusting for sex and education. According to the Framingham risk scoring method described by Sullivan et al.,[25] we established a FAD (unknown) prediction model. The FAD (unknown) risk prediction model is based on a binary logistic regression model, and *APOE* genotype, age, sex, and education were included as risk factors. The disease risk score was estimated according to the regression coefficient of the significant risk factors, and the risk heat map was drawn according to the risk prediction probability. The prediction accuracy of FAD (unknown) risk prediction model was estimated by the area under curve (AUC) using a logistic regression model. All statistical analyses were performed using SPSS version 22.0 (SPSS Inc, Chicago, IL, USA). $P < .05$ was considered to indicate significant results.

RESEARCH IN CONTEXT

1. **Systematic review**: Although the apolipoprotein E (*APOE*)-*ε4* positive genotype has been widely studied as a risk factor for sporadic Alzheimer's disease, few studies have focused on its impact on familial AD (FAD) with unknown mutations (FAD [unknown]).
2. **Interpretation**: The results of this study involving 15 119 subjects showed that the genetic risk effect of the *APOE ε4* positive genotype differed across subtypes of AD. Among these, the FAD (unknown) was the most affected. We propose that the *APOE ε4* positive genotype may cause familial aggregation; therefore, the *APOE ε4* gene plays a major role in this clinical phenomenon.
3. **Future direction**: Future research in this field should aim to elucidate the mechanisms by which the *APOE ε4* positive genotype exerts genetic risk effects on familial clustering of the FAD (unknown) subtype. In addition, studies should seek to identify therapeutic agents targeting the pathological function of *APOE ε4* in order to develop viable therapeutic options for patients with FAD (unknown).

3 | RESULTS

3.1 | Demographics

The demographics and clinical characteristics of each diagnostic group are presented based on the results obtained from the three regions across the China (Figure 1A). On an average, the participants included in the analyses (n = 15 119) were older than 65 years, except for

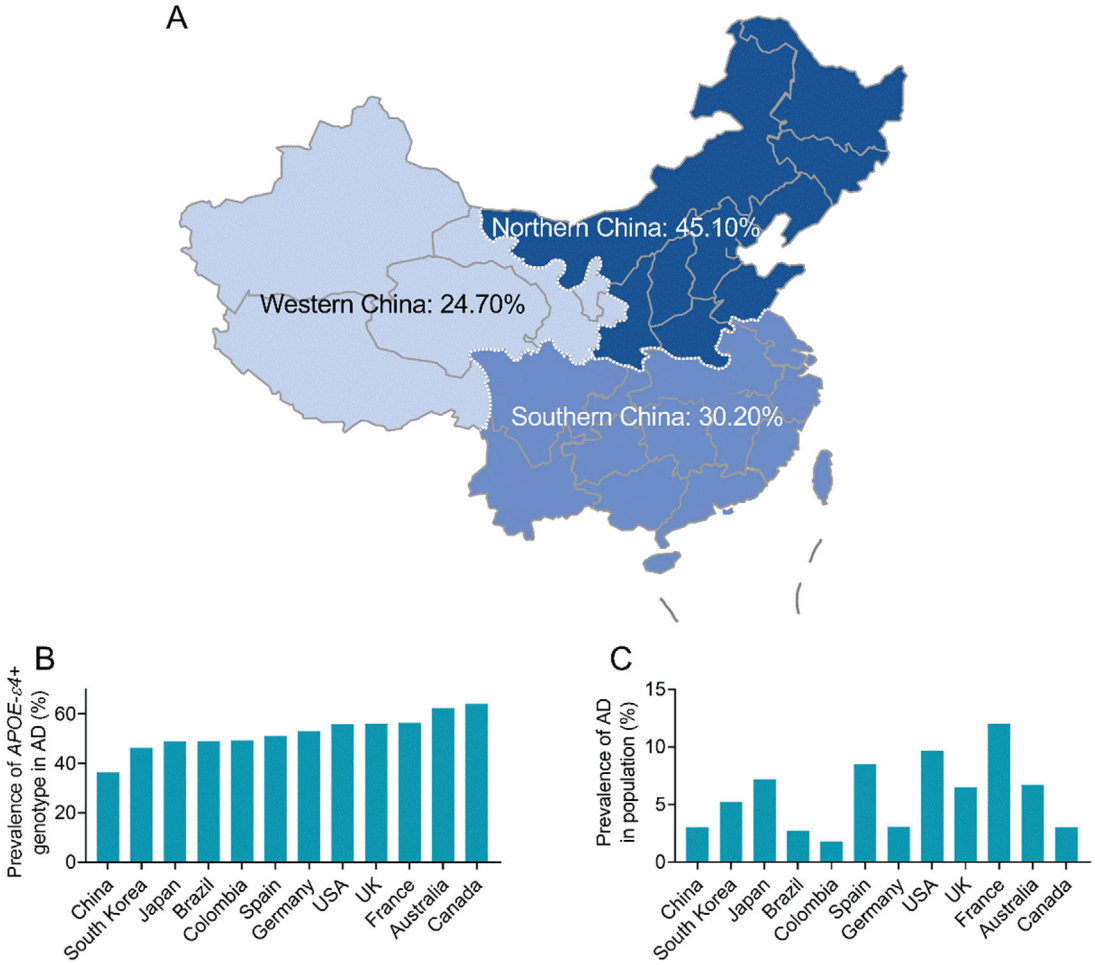

FIGURE 1 Distribution of 15 119 participants in China, prevalence of apolipoprotein E (*APOE*) *ε4* positive genotypes among Alzheimer's disease (AD) patients, and the prevalence of AD among normal populations in China and other countries. A, Proportion of study participants from various geographical areas in China (45.10% from northern, 30.20% from southern, and 24.70% from western China.) B, The prevalence of *APOE ε4* positive genotype shows a rising trend in the order of China (36.23%), South Korea (46.25%), Japan (48.92%), Brazil (49%), Colombia (49.37%), Spain (51.15%), Germany (53.04%), America (55.84%), United Kingdom (56.16%), France (56.36%), Australia (62.44%), and Canada (64.20%).[26,27] China is positioned at the lowest prevalence level. C, The prevalence of AD in the population in France is presented the highest (12%), followed by the United States (9.7%), Spain (8.5%), Japan (7.2%), Australia (6.7%), UK (6.5%), South Korea (5.2%), Germany (3.06%), Canada (3%), China (3%), Brazil (2.7%), and Colombia (1.8%)[28-35]

those in the FAD (*PSENs/APP*) group. The average years of education in each group ranged from 7 to 12 years. The differences in the MMSE, CDR, and CDR SOB scores among the various groups are presented in Table 1.

3.2 | APOE allele frequencies across the diagnostic groups

The *ε3* allele was the most frequent allele identified across all the groups (58.68% FAD [unknown], 80.56% FAD [*PSENs/APP*], 73.44% SAD, and 81.38% NC), followed by the *ε4* allele (36.50% FAD [unknown], 13.89% FAD [*PSENs/APP*], 20.98% SAD, and 10.38% NC) and the *ε2* allele (4.82% FAD [unknown], 5.56% FAD [*PSENs/APP*], 5.58% SAD, and 8.24% NC; Table 2).

3.3 | APOE *ε4* positive genotype carriers across the diagnosed groups

The prevalence of the *APOE ε4* in SAD patients in China was the lowest among 12 countries (Figure 1B);[26,27] however, the prevalence of SAD in China was not the lowest (Figure 1C).[28-35] Further, we found that the prevalence of the *APOE ε4* positive genotype was higher (56.27% FAD [unknown], 26.19% FAD [*PSENs/APP*], 36.23% SAD, and 19.54% NC, all $P < .001$) than in the NC group (Table 2, and Table S2 in supporting information). In addition, the prevalence of the different *APOE* genotypes across the diagnostic groups is shown in Figure 2A. Interestingly, the prevalence of the *APOE ε4* positive genotype in the FAD (unknown) group was higher than that in the SAD or FAD (*PSENs/APP*) groups ($P < .001$), while the prevalence of the *APOE ε4* positive genotype in the SAD group was higher than that in the FAD (*PSENs/APP*) group

TABLE 1 Participant characteristics

Characteristics	FAD (unknown) n = 311	FAD (*PSENs/APP*) n = 126	SAD n = 7234	NC n = 7448
Age, mean ± SD	65.20 ± 11.30	49.97 ± 14.45	71.58 ± 10.46	65.04 ± 11.45
AAO, mean ± SD	64.35 ± 9.24	48.52 ± 8.93	68.27 ± 10.38	_[a]
Female, N (%)	148 (44.44)	69 (54.76)	3556 (54.31)	4104 (55.10)
Education, mean ± SD	8.92 ± 4.52	10.96 ± 5.30	8.03 ± 6.19	8.8 ± 4.52
MMSE, mean ± SD	18.95 ± 7.43	20.42 ± 8.82	17.27 ± 7.11	27.05 ± 2.59
CDR global, mean ± SD	1.11 ± 0.78	0.98 ± 0.96	1.63 ± 1.76	_[a]
CDR SOB, Mean ± SD	5.28 ± 4.69	5.39 ± 5.59	5.54 ± 4.71	_[a]

Abbreviations: AAO, age at onset; *APP*, amyloid precursor protein; CDR, Clinical Dementia Rating; FAD (*PSENs/APP*), familial Alzheimer's disease with *PSEN1*, *PSEN2*, or *APP* mutations; FAD (unknown), familial Alzheimer's disease without *PSEN1*, *PSEN2*, *APP*, *TREM2*, and *SORL1* mutations; FAD, familial Alzheimer's disease; MMSE, Mini-Mental State Examination; NC, normal control; *PSEN*, presenilin; SAD, sporadic Alzheimer's disease; SOB, sum of boxes.
[a]**NOTE.** Data not available.

TABLE 2 Prevalence of the *APOE* alleles and genotypes in each diagnostic group

Group (N)	FAD (unknown) n = 311	FAD (*PSENs/APP*) n = 126	SAD n = 7234	NC n = 7448
Allele				
ε2	30 (4.82)	14 (5.56)	808 (5.58)	1228 (8.24)
ε3	365 (58.68)	203 (80.56)	10625 (73.44)	12122 (81.38)
ε4	227 (36.50)	35 (13.89)	3035 (20.98)	1546 (10.38)
Genotype				
APOE ε3/ε3	111 (35.69)	82 (65.08)	3966 (54.82)	4935 (66.26)
APOE ε2/ε2	0 (0.00)	0 (0.00)	39 (0.54)	35 (0.47)
APOE ε2/ε3	25 (8.04)	11 (8.73)	608 (8.4)	1023 (13.74)
APOE ε2/ε4	5 (1.61)	3 (2.38)	122 (1.69)	135 (1.81)
APOE ε3/ε4	118 (37.94)	28 (22.22)	2085 (28.82)	1229 (16.50)
APOE ε4/ε4	52 (16.72)	2 (1.59)	414 (5.72)	91 (1.22)
APOE ε4+	175 (56.27)	33 (26.19)	2621 (36.23)	1455 (19.54)
APOE ε4-	136 (43.73)	93 (73.81)	4613 (63.77)	5993 (80.46)
APOE ε2+	30 (9.65)	14 (11.11)	769 (10.63)	1193 (16.02)
APOE ε2-	281 (90.35)	112 (88.89)	6465 (89.37)	6255 (83.98)

NOTE. Data are presented as N (percentage).
Abbreviations: *APOE*, apolipoprotein E; *APP*, amyloid precursor protein; FAD (*PSENs/APP*), familial Alzheimer's disease with *PSEN1*, *PSEN2*, or *APP* mutations; FAD (unknown), familial Alzheimer's disease without *PSEN1*, *PSEN2*, *APP*, *TREM2*, and *SORL1* mutations; FAD, familial Alzheimer's disease; NC, normal control; *PSEN*, presenilin; SAD, sporadic Alzheimer's disease.

($P = .02$). The *APOE* genotypes were classified as *APOE ε4* heterozygous or homozygous, and their distribution in each group is shown in Figure 2C,D.

3.4 | Predictive effect of APOE ε4 in AD

To explore the predictive effect of *APOE* in the different AD subtypes, we used binary logistic regression models adjusted for age, sex, education, and region of subjects (Table 3). We found that the *APOE ε4* positive genotype had predictive power for the risk of FAD (unknown) (odds ratio [OR]: 4.51, 95% confidence interval [CI]: 3.57-5.45, $P < .001$), and SAD (OR: 2.26, 95% CI: 2.17-2.35, $P < .001$; Figure 2B). We also examined the effect of *APOE ε4* gene dosage on disease risk, and found that the OR values for two *APOE ε4* copies (homozygous) were higher than those for a single copy (heterozygous) in the FAD (unknown) and SAD groups; this was especially evident in the FAD (unknown) group (*APOE ε4* heterozygous: OR: 3.26, 95% CI: 2.61-3.91, $P < .001$; *APOE ε4* homozygous: OR: 22.13, 95% CI: 15.79-28.47, $P < .001$; Figure 2C,D).

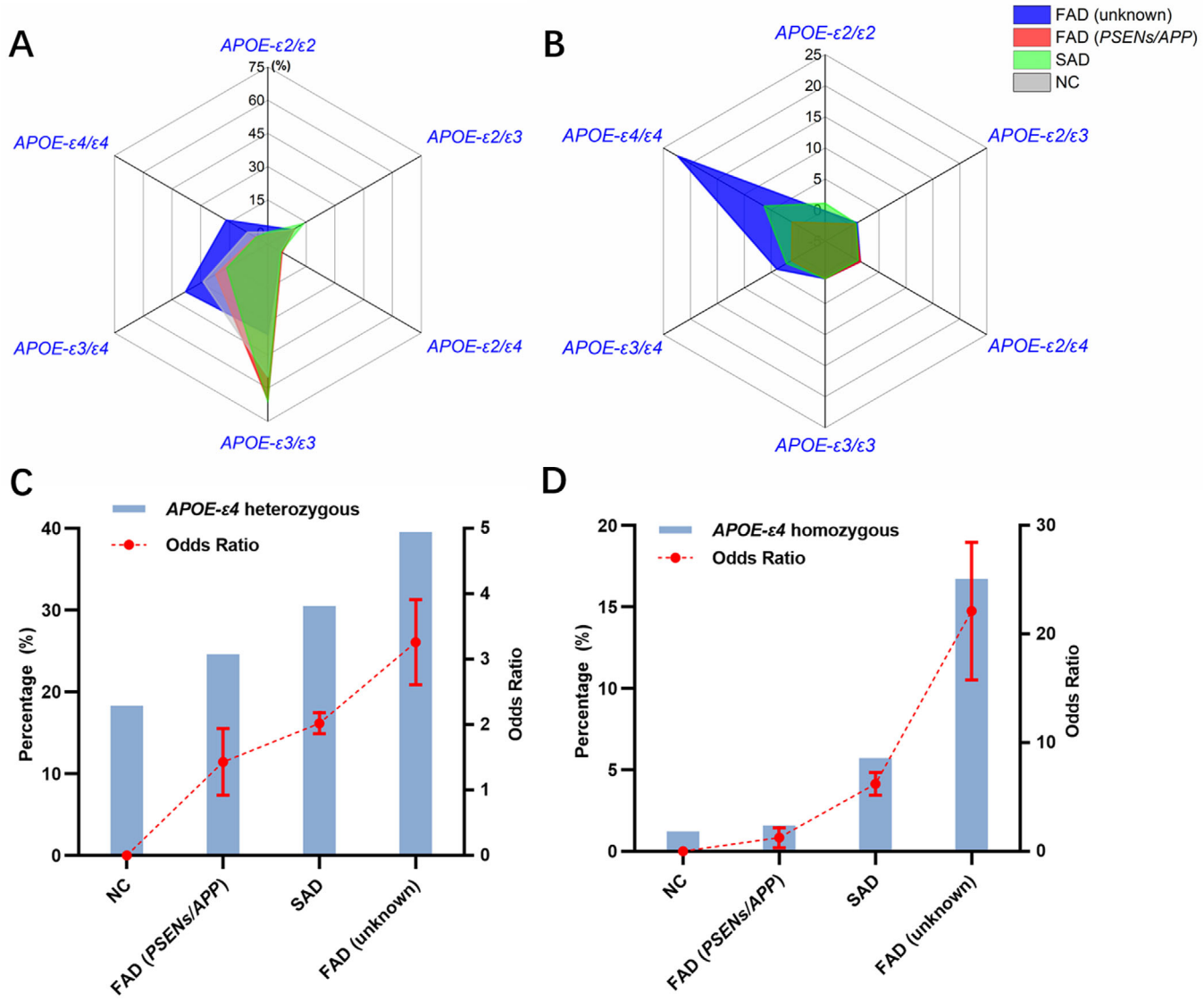

FIGURE 2 The prevalence and predictive effect of apolipoprotein E (*APOE*) genotypes. The radar charts show the prevalence of *APOE* genotypes in Alzheimer's disease (AD) subtypes and in normal population (A) and the odds ratio (OR) of *APOE* genotypes in AD subtypes (B). The prevalence and OR value of the *APOE ε4/ε4* genotype was higher in the FAD (unknown) group compared to that in other groups. The bar graphs with the trendline indicates the rising frequencies and OR value in *APOE ε4* heterozygous groups (C). The rising frequencies and OR in *APOE ε4* homozygous genotypes in the FAD (unknown) group were also significantly higher than that in other groups (the OR value was presented with 95% confidence interval) (D). FAD (unknown), familial Alzheimer's disease without *PSEN1*, *PSEN2*, *APP*, *TREM2*, and *SORL1* mutations; FAD, familial Alzheimer's disease; NC, normal control

Risk effect of APOE genotype for AD across different ages in the FAD (unknown) and SAD groups

We estimated the OR values of the *APOE ε4* positive genotype in comparison with that for the *APOE ε4* negative genotype across different ages in the FAD (unknown) groups and SAD group by binary logistic regression models adjusted for sex and education (Figure 3A,B). We found that, in the same age range, the OR value increased with the number of *APOE ε4* alleles in both groups. Moreover, we found that the risk due to the ε4 allele in both groups was age-dependent. In the FAD (unknown) group, the risk due to the ε4 allele gradually increased with age, and decreased gradually after 70 to 79 years of age. Similarly, in the SAD group, the risk for AD due to the ε4 allele gradually increased with age, and decreased after 65 to 74 years of age.

3.5 | Risk prediction probability model of FAD (unknown)

Our results using the logistic regression model showed that *APOE* genotype and age were significant risk factors for FAD (unknown). We calculated the risk scores based on different age ranges and the number of *APOE ε4* copies. The total risk score range was 0 to 15 points, and each score corresponded to a risk prediction probability score; these

TABLE 3 Logistic regression analysis for predictive effect of *APOE* on AD subtypes

	FAD (unknown) N = 311	FAD (*PSENs/APP*) N = 126	SAD N = 7234
Genotype			
ε3/ε3[a]	1	1	1
ε2/ε2	[b]	[b]	1.11 (0.72-1.50), *P* = .493
ε2/ε3	0.93(0.58-1.28), *P* = .843	0.56 (0.32-0.80), *P* = .253	0.76 (0.64-0.88), *P* < .001
ε2/ε4	1.52 (0.71-2.33), *P* = .267	1.55 (0.45-2.65), *P* = .552	1.11 (0.76-1.46), *P* = .580
ε3/ε4	3.96 (2.95-4.97), *P* < .001	1.23 (0.82-1.64), *P* = .112	2.17 (1.95-2.39), *P* <.001
ε4/ε4	22.36 (16.43-28.29), *P* < .001	1.13 (0.36-1.90), *P* = .87	6.32 (5.02-7.62), *P* < .001
APOE ε4 positive genotype			
ε4(-)[a]	1	1	1
ε4(+)	4.51 (3.57-5.45), *P* < .001	1.33 (0.97-1.69), *P* = .068	2.26 (2.17-2.35), *P* < .001
APOE ε2 positive genotype			
ε2(-)[a]	1	1	1
ε2(+)	0.56 (0.43-0.69), *P* = .002	0.57 (0.21-0.93), *P* = .213	0.58 (0.46-0.70), *P* < .001
Number of *APOE ε4* copies			
No *ε4*[a]	1	1	1
One *ε4*	3.26 (2.61-3.91), *P* < .001	1.43 (0.92-1.94), *P* = .072	2.02 (1.86-2.18), *P* < .001
Two *ε4*	22.13 (15.79-28.47), *P* < .001	1.25 (0.33-2.17), *P* = .8623	6.22 (5.17-7.27), *P* < .001

NOTE. Data are presented as OR (95% CI) and *P* value.
Abbreviations: *APP*, amyloid precursor protein; CI, confidence interval; FAD (*PSENs/APP*), familial Alzheimer's disease with *PSEN1*, *PSEN2*, or *APP* mutations; FAD (unknown), familial Alzheimer's disease without *PSEN1*, *PSEN2*, *APP*, *TREM2*, and *SORL1* mutations; FAD, familial Alzheimer's disease; NC, normal control *APOE*, apolipoprotein E; OR, odds ratio; *PSEN*, presenilin; SAD, sporadic Alzheimer's disease.
[a]Represents reference group.
[b]Represents the lack of data in this group and were excluded from the model.

are shown in Table S3 in supporting information. The result showed that *APOE 4* copies as predictors, the prediction accuracy (AUC) was 73% (*P*<.001, 95% CI: 0.70–0.76). A risk heat map was drawn to depict the risk prediction probability of FAD (unknown) (Figure 3C). The figure displayed that with the increase in the number of alleles and age, the risk of FAD (unknown) increased significantly, particularly for the *APOE ε4* homozygotes after 45 years of age.

4 | DISCUSSION

To our knowledge, this is the first multicenter study to examine *APOE* in the Chinese population and involved 15 119 individuals. We described the prevalence of the *APOE* alleles and genotypes in FAD (unknown), FAD (*PSENs/APP*), SAD, and NC groups and compared the genetic effects of *APOE ε4* among these groups. We found that the *APOE ε4* positive genotype increased the risk of AD, enhanced the familial aggregation, and showed a peak risk effect between 70 and 79 years of age in FAD (unknown) patients. Additionally, we found that the prevalence of the *APOE ε4* positive genotype was clearly lower among the SAD patients in China than among those in Europe and the United States, indicating that ethnic background is an important factor in the risk of AD development.[26,27]

The prevalence of the *APOE ε4* positive genotype displayed the following trend: FAD (unknown) (56.27%) > SAD (36.23%) > FAD (*PSENs/APP*) (26.19%) > NC (19.54%). The results revealed an uneven modulation across the groups. The results indicated that the *APOE ε4* carriers in the FAD (unknown) group represented the largest proportion and had the highest OR values among all AD subgroups. However, the frequency of the *APOE ε4* allele in the FAD (*PSENs/APP*) group was the lowest among AD groups, indicating that mutations in *PSEN1*, *PSEN2*, and *APP* genes promote disease development. This observation is consistent with those from previous studies.[36,37] The high frequency of *APOE ε4* is mainly attributed to the FAD (unknown) group rather than the FAD (*PSENs/APP*) group, suggesting that *APOE ε4* plays an important role in the prevalence of FAD (unknown). We speculated that *APOE* may act as a major gene with incomplete penetrance, rather than a risk gene, in unknown mutant FAD. Furthermore, we found that the *APOE ε4/4* positive genotype was prevalent in one third of the FAD (unknown) patients (*APOE ε4/4* 16.72% vs *APOE ε4* positive genotype 56.27%) and in approximately one sixth of the SAD patients (*APOE ε4/4* 5.72% vs *APOE ε4* positive genotype 36.23%). Moreover, the OR values for the two *APOE ε4* copies (increase in risk by 22.13-fold) were higher than those for a single copy (by 3.26-fold) in the FAD (unknown) group. These values were ≈2-fold higher than those reported in previous studies.[9-11] Thus, based on our study, we propose that patients

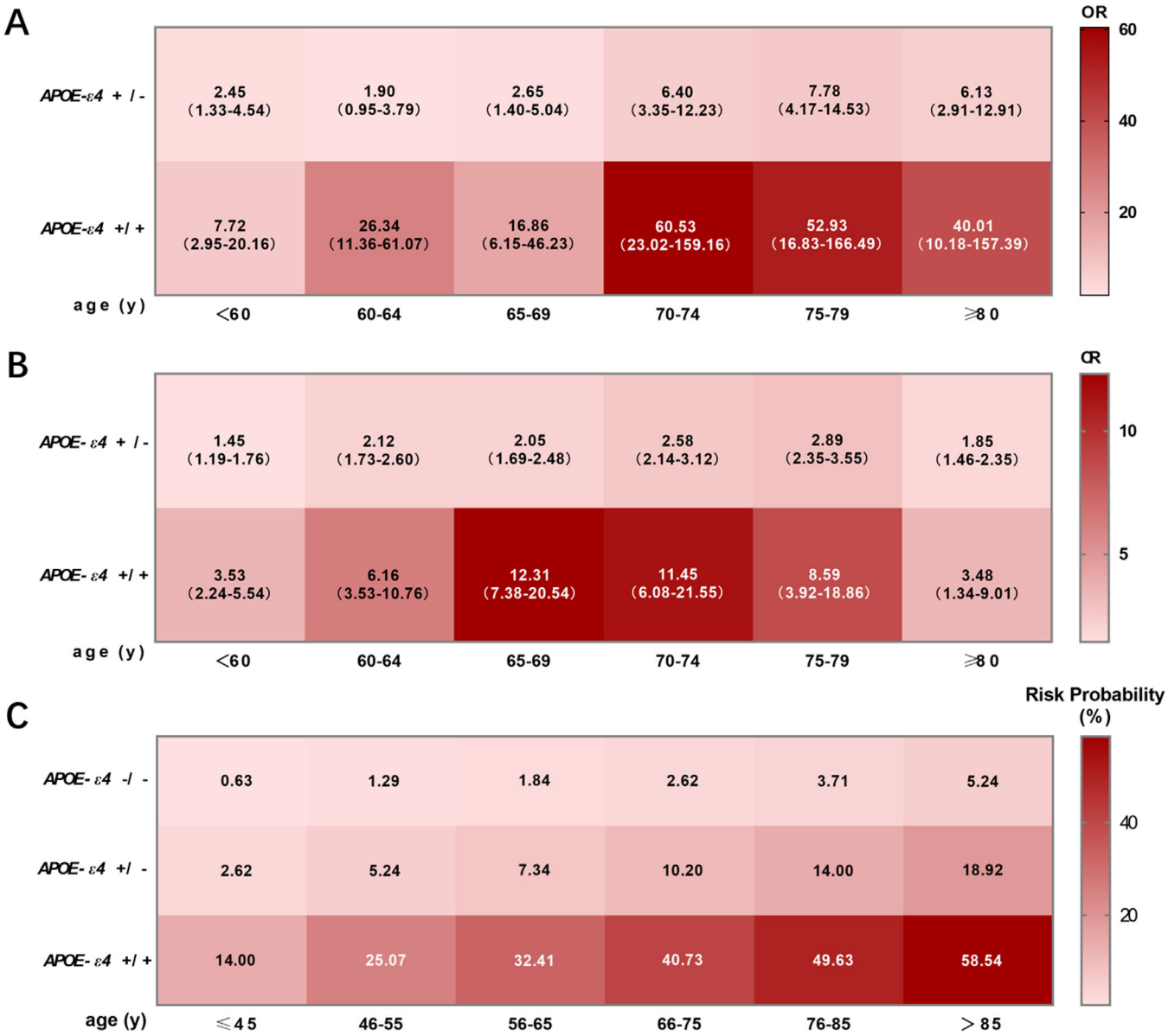

FIGURE 3 The odds ratio (OR) and risk probability for Alzheimer's disease (AD) development among different apolipoprotein E (*APOE*) *ε4* genotypes with different age ranges. In the FAD (unknown) group (A), and SAD group (B), the odds ratio (OR) value for developing AD was higher in the *APOE ε4* +/+ group than in the *APOE ε4* +/- group, irrespective of the age range. In the FAD (unknown) group, the OR value increased with age, and decreased gradually after 70 to 79 years of age. In the SAD group, similar to FAD (unknown), the risk effect of the *ε4* allele for AD gradually increased till 65 to 74 years of age and decreased thereafter (the OR value was presented with 95% confidence interval). C, The heat maps show the risk probability of FAD (unknown). The increasing dosage of the *APOE ε4* allele and increasing age significantly impact the risk probability of FAD (unknown). FAD (unknown), familial Alzheimer's disease without *PSEN1*, *PSEN2*, *APP*, *TREM2*, and *SORL1* mutations; FAD, familial Alzheimer's disease; NC, normal control; SAD, sporadic Alzheimer's disease

with two *APOE ε4* alleles are more likely to develop FAD (unknown) than those with a single ε4 allele and other subtypes of AD. This phenomenon called the *APOE ε4* diploid enhancement of familial aggregation has been suggested in previous studies,[38,39] indicating that an increased *APOE ε4* gene dosage may promote the development of the familial form of the disease. In addition, the prevalence of *APOE ε4* homozygous was not significantly different between early-onset and late-onset patients with FAD (unknown) (data not shown), suggesting that this *APOE ε4* diploid enhancement effect may play a similar role in both, which awaits further exploration. Our results have expanded the understanding of the gene dosage effect on FAD (unknown) and the harmful effects of *APOE ε4* on all subtypes of AD, strongly indicating a relationship between *APOE* and AD.

In both SAD and FAD (unknown), we observed that risk effect of *APOE ε4* positive genotype for AD was age dependent. The maximum risk was at the age of 65 to 74 years for SAD, and at the age of 70 to 79 years for FAD (unknown). It is indicated that risk factors other than *APOE ε4* positive genotype may play an important role in disease onset

at a younger or older age. At the younger age range, these unidentified genetic variants may interact with each other or act independently, leading to AD. In the older age group, the increased mortality rates of *APOE ε4* carriers with AD might contribute to the lower risk.[40] Furthermore, several studies have demonstrated that the effects of the *APOE ε4* allele diminish at a very old age.[41,42] To date, the biological mechanisms underlying these effects have not been elucidated. Additionally, the risk prediction model shows that the risk of FAD (unknown) increased with the gene dosage of *APOE ε4* and age, respectively. In *APOE ε4* homozygotes, the risk of FAD (unknown) was significantly higher after 45 years of age. This observation may be clinically important. For instance, the administration of therapeutic interventions, such as *APOE*-targeted AD therapeutic strategies or comprehensive treatment modalities, before an at-risk individual reaches the highest-risk age range might reduce the risk of development of AD. Because the risk associated with the *APOE ε4* allele varies by age, clinical trials investigating the prevention of AD in *APOE ε4* carriers should be designed considering the effects of *APOE ε4* at different ages.

Our study had some inherent limitations. First, the number of patients with FAD was relatively small owing to its low incidence compared to SAD. Another reason is that the life span for FAD patients is usually shorter, and as such, we managed to enroll few patients over 70 years, which affects our result regarding the effects of age. We plan to expand this sample size in our future research. Second, although we believe that *APOE ε4* may be a pathogenic gene with an incomplete penetrance in FAD (unknown), we did not perform functional in vitro or in vivo studies to verify this hypothesis in the FAD subtype. Third, we found that *APOE ε4* had different genetic effects in different AD subtypes; however, this finding should be validated in longitudinal cohorts in the future. Fourth, although our model has capacity to predict the risk of FAD (unknown), there would be other factors such as family history that contribute to the development of FAD (unknown). Therefore, we should include more factors to improve the prediction efficiency in the future. Fifth, previous studies have identified some risk genes for AD, such as *APOE*, *BIN1*, *CD33*, *EPHA1*, *SORL1*, *TOMM40* and so on.[43-45] We only tested *APP*, *PSEN1*, *PSEN2*, *TREM2*, and *SORL1* mutations in our study and did not test the deletion/duplication, which may lead to the loss of genetic information. We will include this content in future research.

5 | CONCLUSIONS

This is the largest multicenter study investigating the association between *APOE* and AD in the Chinese population. This study revealed that the *APOE ε4* positive genotype was associated with different AD subtypes and showed an increasing trend in NC, FAD (*PSENs/APP*), SAD, and FAD (unknown) groups, in that order. The high frequency of *APOE ε4* in FAD (unknown) suggests that it plays an important role in familial aggregation. Future studies to identify therapeutic strategies for FAD (unknown) subtype should consider the age and *APOE ε4* genotype. This might lead to identification of potential viable therapeutic options for patients in the FAD (unknown) family.

ACKNOWLEDGMENTS

We thank all participants in this study and all neurologists at relevant academic centers for their help in the recruitment of subjects. In particular, we thank the participating hospitals including The First Affiliated Hospital of Xi'An JiaoTong University; Xiangya Hospital, Central South University; Huashan Hospital, Fudan University; The Second Affiliated Hospital of Zhejiang University School of Medicine; The First Affiliated Hospital of Chongqing Medical University; The Affiliated Hospital of Qingdao University; Guang Zhou Huiai Hospital; Henan Provincial People's Hospital; Tianjin Medical University General Hospital; No. 88 Hospital of PLA; The First Affiliated Hospital of Wenzhou Medical University; The First Bethune Hospital of Jilin University; University Hospital of Hubei University for Nationalities; The Second Hospital of Shandong University; The First Affiliated Hospital of Anhui Medical University; Beijing Chaoyang Hospital, Capital Medical University.

This study was supported by the Key Project of the National Natural Science Foundation of China (81530036); the National Key Scientific Instrument and Equipment Development Project (31627803); Mission Program of Beijing Municipal Administration of Hospitals (SML20150801); Beijing Scholars Program; Beijing Brain Initiative from Beijing Municipal Science & Technology Commission (Z161100000216137); Project for Outstanding Doctor with Combined Ability of Western and Chinese Medicine; and Beijing Municipal Commission of Health and Family Planning (PXM2019_026283_000003).

AUTHOR CONTRIBUTIONS

Conceptualization: Jianping Jia, Longfei Jia, Hui Xu. Data curation: Jianping Jia, Longfei Jia, Cuibai Wei, Yi Tang, Aihong Zhou, Yan Li. Formal analysis: Hui Xu. Funding acquisition: Jianping Jia. Investigation: Jianping Jia, Longfei Jia. Methodology: Hui Xu. Project administration: Jianping Jia, Longfei Jia, Cuibai Wei, Yi Tang, Aihong Zhou, Yan Li. Resources: Jianping Jia, Qiumin Qu, Lan Chu, Lu Shen, Chunkui Zhou, Qi Wang, Lina Zhao, Hongmei Jin, Ying Li, Fangyu Li, Hui Xu, Shuoqi Chen, Xiu Wang, Jianwei Yang, Min Gong, Lu Shi, Tan Zhao, Qin Qi. Supervision: Jianping Jia, Longfei Jia. Validation: Jianping Jia, Longfei Jia. Visualization: Longfei Jia, Hui Xu. Writing—original draft: Longfei Jia, Hui Xu, Shuoqi Chen, Xiu Wang, Jianwei Yang, Min Gong, Cuibai Wei, Yi Tang, Qiumin Qu, Lan Chu, Lu Shen, Chunkui Zhou, Qi Wang, Lu Shi, Tan Zhao, Aihong Zhou, Ying Li, Fangyu Li, Yan Li, Lina Zhao, Hongmei Jin, Qi Qin, Haishan Jiao, Yan Li, Heng Zhang, Diyang Lyu, Yuqing Shi, Yang Song, Jianping Jia. Writing—review & editing: Jianping Jia, Longfei Jia.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Jia L, Quan M, Fu Y, et al. Dementia in China: epidemiology, clinical management, and research advances. *Lancet Neurol* 2020;19(1):81-92.
2. Wingo TS, Lah JJ, Levey AI, Cutler DJ. Autosomal recessive causes likely in early-onset Alzheimer disease. *Arch Neurol*. 2012;69:59-64

3. Sherrington R, Rogaev EI, Liang Y, et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*. 1995;375:754-60
4. Levy-Lahad E, Wasco W, Poorkaj P, et al. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science*. 1995;269:973-77
5. Goate A, Chartier-Harlin MC, Mullan M, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*. 1991;349:704-06.
6. Jarmolowicz AI, Chen HY, Panegyres PK. The patterns of inheritance in early-onset dementia: Alzheimer's disease and frontotemporal dementia. *Am J Alzheimers Dis Other Demen*. 2015;30:299-306.
7. Kunkle BW, Grenier-Boley B, Sims R, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat Genet*. 2019;51:414-30.
8. Guerreiro R, Wojtas A, Bras J, et al. TREM2 variants in Alzheimer's disease. *N Engl J Med*. 2013;368:117-27.
9. Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein e and Alzheimer disease: Risk, mechanisms and therapy. *Nat Rev Neurol*. 2013;9:106-18.
10. Verghese PB, Castellano JM, Holtzman DM. Apolipoprotein E in Alzheimer's disease and other neurological disorders. *Lancet Neurol*. 2011;10:241-52.
11. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: A meta-analysis. *J Am Med Assoc*. 1997;278:1349-56.
12. Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. *Nat Genet*. 2007;39(1):17-23.
13. Murrell JR, Price B, Lane KA, et al. Association of apolipoprotein E genotype and Alzheimer disease in African Americans. *Arch Neurol*. 2006;63(3):431-434.
14. Blue EE, Horimoto A, Mukherjee S, Wijsman EM, Thornton TA. Local ancestry at APOE modifies Alzheimer's disease risk in Caribbean Hispanics. *Alzheimers Dement*. 2019;15(12):1524-1532.
15. AlzGene meta-analysis for APOE (e2/3/4) Available at: http://www.alzgene.org/meta.asp?geneID=83. Accessed May 23, 2020.
16. Olarte L, Schupf N, Lee JH, et al. Apolipoprotein E ε4 and age at onset of sporadic and familial Alzheimer disease in Caribbean Hispanics. *Arch Neurol*. 2006;63:1586-1590.
17. Cruchaga C, Del-Aguila JL, Saef B, et al. Polygenic risk score of sporadic late-onset Alzheimer's disease reveals a shared architecture with the familial and early-onset forms. *Alzheimer's Dement*. 2018;14:205-214.
18. Jia L, Fu Y, Shen L, et al. PSEN1, PSEN2, and APP mutations in 404 Chinese pedigrees with familial Alzheimer's disease. *Alzheimers Dement*. 2020;16:178-191.
19. Association AP. *Diagnostic and Statistical Manual of Mental Disorders*. 4th text revision ed. Washington, DC: American Psychiatric Association; 2000;553-557.
20. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. 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;34:939-944.
21. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement*. 2011;7:263-269.
22. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16:1215.
23. Cruts M, Van Duijn CM, Backhovens H, et al. Estimation of the genetic contribution of presenilin-1 and -2 mutations in a population-based study of presenile Alzheimer disease. *Hum Mol Genet*. 1998;7:43-51.
24. Fidani L, Rooke K, Chartier-harlin MC, et al. Screening for mutations in the open reading frame and promoter of the β-amyloid precursor protein gene in familial alzheimer's disease: Identification of a further family with APP717 val→Ile. *Hum Mol Genet*. 1992;1:165-8.
25. Sullivan LM, Massaro JM, D'Agostino RB. Presentation of multivariate data for clinical use: The Framingham Study risk score functions. *Stat Med*. 2004;23(10):1631-1660.
26. Ward A, Crean S, Mercaldi CJ, et al. Prevalence of Apolipoprotein E4 genotype and homozygotes (APOE e4/4) among patients diagnosed with alzheimer's disease: A systematic review and meta-analysis. *Neuroepidemiology*. 2012;38(1):1-17.
27. Vieira RN, Ávila R, de Paula JJ, et al. Association between DCHS2 gene and mild cognitive impairment and Alzheimer's disease in an elderly Brazilian sample. *Int J Geriatr Psychiatry*. 2016;31(12):1337-1344.
28. Ohara T, Hata J, Yoshida D, et al. Trends in dementia prevalence, incidence, and survival rate in a Japanese community. *Neurology*. 2017;88(20):1925-32.
29. Pérès K, Brayne C, Matharan F, et al. Trends in prevalence of dementia in French farmers from two epidemiological cohorts. *J Am Geriatr Soc*. 2017;65(2):415-420
30. Kalaria RN, Maestre GE, Arizaga R, et al. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. *Lancet Neurol*. 2008;7(9):812-26.
31. Plassman BL, Langa KM, Fisher GG, et al. Prevalence of Dementia in the United States: The Aging, Demographics, and Memory Study. *Neuroepidemiology*. 2007;29(1-2):125-132.
32. Tola-Arribas MA, Yugueros MI, Garea MJ, et al. Prevalence of dementia and subtypes in Valladolid, northwestern Spain: the DEMINVALL study. *PLoS One*. 2013;8(10):e77688.
33. Kosteniuk JG, Morgan DG, O'Connell ME, et al. Simultaneous temporal trends in dementia incidence and prevalence, 2005-2013: A population-based retrospective cohort study in Saskatchewan, *Canada*. *Int Psychogeriatr*. 2016;28(10):1643-1658.
34. Doblhammer G, Fink A, Fritze T. Short-term trends in dementia prevalence in Germany between the years 2007 and 2009. *Alzheimers Dement*. 2015;11(3):291-299.
35. Alzheimer's Disease International. World Alzheimer Report 2015: The Global Impact of Dementia. Available at: https://www.alz.co.uk/research/world-report-2015. Accessed May 13, 2020.
36. Hsu S, Gordon BA, Hornbeck R, et al. Discovery and validation of autosomal dominant Alzheimer's disease mutations. *Alzheimer's Res Ther*. 2018;10:67.
37. Aguirre-Acevedo DC, Lopera F, Henao E, et al. Cognitive decline in a colombian kindred with autosomal dominant Alzheimer disease a retrospective cohort study. *JAMA Neurol*. 2016;73:431-8.
38. Martinez M, Campion D, Brice A, et al. Apolipoprotein E ε4 allele and familial aggregation of Alzheimer disease. *Arch Neurol*. 1998;55:810-6.
39. Huang W, Qiu C, von Strauss E, Winblad B, Fratiglioni L. Genotype, family history of dementia, and Alzheimer disease risk. *Arch Neurol*. 2004;61:1930-4.
40. Tilvis RS, Strandberg TE, Juva K. Apolipoprotein E phenotypes, dementia and mortality in a prospective population sample. *J Am Geriatr Soc*. 1998;46:712-5.
41. Corrada MM, Paganini-Hill A, Berlau DJ, Kawas CH. Apolipoprotein e genotype, dementia, and mortality in the oldest old: The 90+ Study. *Alzheimer's Dement*. 2013;9:12-8.
42. Rebeck GW, Perls TT, West HL, Sodhi P, Lipsitz LA, Hyman BT. Reduced apolipoprotein ε4 allele frequency in the oldest old alzheimer's patients and cognitively normal individuals. *Neurology*. 1994;44:1513-6.
43. Li WW, Wang Z, Fan DY, et al. Association of Polygenic Risk Score with Age at Onset and Cerebrospinal Fluid Biomarkers of Alzheimer's Disease in a Chinese Cohort. *Neurosci Bull*. 2020;36(7):696-704.
44. Jung YJ, Kim YH, Bhalla M, Lee SB, Seo J.Y. Genomics: new light on Alzheimer's disease research. *Int J Mol Sci*. 2018; 19(12): 3771.

45. Tosto G, Reitz C. Genomics of Alzheimer's disease: value of high-throughput genomic technologies to dissect its etiology. *Mol Cell Probes*. 2016;30(6):397-403.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Jia L, Xu H, Chen S, et al. The *APOE ε4* exerts differential effects on familial and other subtypes of Alzheimer's disease. *Alzheimer's Dement*. 2020;1–11. https://doi.org/10.1002/alz.12153