

FEATURED ARTICLE

PSEN1, PSEN2, and APP mutations in 404 Chinese pedigrees with familial Alzheimer's disease

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Abstract

Introduction: The *PSENs/APP* mutation distribution in Chinese patients with familial Alzheimer's disease (FAD) remains unclear. We aimed to analyze the genetic features of Chinese FAD pedigrees with and without *PSENs/APP* mutations.

Methods: In total, 1330 patients with Alzheimer's disease (AD) or mild cognitive impairment in 404 pedigrees were enrolled from the Chinese Familial Alzheimer's Disease Network. *PSENs/APP* mutations and *APOE* frequencies were determined.

Results: In total, 13.12% of pedigrees carried *PSENs/APP* missense mutations, 3.71% carried *PSENs/APP* synonymous/untranslated region variants, and 83.17% did not carry *PSENs/APP* mutations. Eleven missense mutations were first identified. In patients without *PSENs/APP* mutations, 44.31% carried one *APOE*ε4 allele, and 14.85% two *APOE*ε4 alleles.

Discussion: The new *PSENs/APP* mutations indicate heterogeneity in AD pathogenesis between Chinese and other ethnic groups. The low mutation rate suggests the involvement of other genes/factors in Chinese FAD. *APOE*ε4 might be a major gene for some FAD without *PSENs/APP* mutations.

KEYWORDS

Alzheimer's disease, *APOE*ε4, familial Alzheimer's disease, mild cognitive impairment, *PSENs/APP* mutations

1 | BACKGROUND

Alzheimer's disease (AD) is the most common type of dementia and the number of patients with AD in China is currently 8.75 million.¹ This large patient population costs China US \$167.74 billion² per year, and

thus has an immense social impact and heavy economic burden. AD can be categorized as sporadic AD or familial AD (FAD) based on family history. FAD requires a patient with AD to have at least one first-degree relative diagnosed with AD or mild cognitive impairment (MCI) due to AD, and was reported to account for 12.50% to 25.00%^{3,4} of all AD

cases. FAD greatly contributes to AD research. First, it encompasses the physiological and pathological characteristics of all cognitive stages from preclinical to MCI to dementia. Second, it represents an ideal population, with a possible predictable age at onset (AAO), which is relatively young and has minimal complications (such as cerebrovascular disease), and facilitates exploration of the pathogenesis of AD. Third, it promotes the development of transgenic animal models with which anti-dementia drugs are tested.

FAD includes autosomal dominant AD, caused by mutations of genes⁵ including the amyloid precursor protein gene (*APP*), presenilin 1 gene (*PSEN1*), and presenilin 2 gene (*PSEN2*), which have been targeted by the Dominantly Inherited Alzheimer Network (<https://dian.wustl.edu/>); the E280A Antioquia cohort study⁶; and FAD cohorts, including those in France,⁷ England,⁸ Japan,⁹ South Korea,¹⁰ Finland,¹¹ and so on. To date, 289 *PSEN1*, 48 *PSEN2*, and 58 *APP* mutations have been identified (<https://www.alzforum.org/mutations>), most of which were first reported in Caucasians. In Chinese, our team reported the first variant¹² (NM_000021.3:c.289G>T V97L in *PSEN1*). Several studies have since investigated the genetics of FAD: one study¹³ identified four variants in *PSEN1* and one variant in *APP*, and the detection rate of variants in *PSENs/APP* was 18.75%. Another¹⁴ identified two variants in *APP*, eight variants in *PSEN1*, and three variants in *PSEN2*, and the detection rate of variants in *PSENs/APP* was 8.80%. However, these cohorts were not multicentric and the sample sizes were relatively small; thus the findings did not fully represent the mutation spectrum of FAD in China.

Therefore, we established the Chinese Familial Alzheimer's Disease Network (CFAN). The CFAN is a nationwide, multicenter, longitudinal cohort used to collect the hereditary and clinical profile of FAD, to analyze the genetic features, and to compare genetic heterogeneity and/or homogeneity between Chinese and other ethnic groups with FAD, with and without *PSENs/APP* mutations. The present study enhances our understanding of the pedigrees with and without *PSENs/APP* mutations.

2 | METHODS

2.1 | Study design and participants

We recruited 404 FAD pedigrees with 1330 patients from the CFAN between January 2013 and September 2019. The CFAN is a multicenter study including 69 medical centers in 26 of the 34 provinces and regions of China (<https://www.clinicaltrials.gov/>, NCT03657732). In this study, individuals who met the following criteria were recruited: (1) of Chinese ethnicity, (2) at least one first-degree relative in addition to the patient himself/herself within the family who exhibited objective cognitive decline suggestive of AD, (3) >18-years-old, and (4) written informed consent was obtained from each individual either directly or from his/her legal guardian. The exclusion criteria were the following: (1) other causes of cognitive impairment supported by magnetic resonance imaging (MRI) or laboratory tests (vitamin B12, folic acid, liver and renal function, blood glucose, lipid profile, thyroid function, HIV,

RESEARCH IN CONTEXT

1. Systematic review: The literature was reviewed using traditional (eg, Google Scholar; PubMed) sources and the AlzForum mutation dataset (<https://www.alzforum.org/mutations>) to identify studies of mutation profiles in familial Alzheimer's disease (FAD). Most of the mutations in *PSENs/APP* were first reported in Caucasians and few were reported in Chinese populations.
2. Interpretation: We found 40 missense mutations, 8 *PSENs/APP* synonymous variants, and 3 untranslated region variants. Eleven missense mutations and one synonymous variant were first identified herein. Our findings provide a general mutation profile of FAD in China, and highlight the importance of the exploration of pedigrees without *PSENs/APP* gene mutations.
3. Future directions: We wish to conduct a functional study of the mutations first identified herein. The large proportion of pedigrees without *PSENs/APP* mutations in the Chinese population necessitates in-depth studies exploring novel pathogenic genes involved in AD. The genetic impact of *APOEε4* in FAD will be investigated.

and syphilis); (2) individuals who had a medical or psychiatric disorder that would interfere with completion of the assessments; and (3) individuals who had a modified Hachinski Ischemic Score of >4.¹⁵

The diagnosis of AD was determined according to the National Institute of Neurological and Communicative Disorders and Stroke-AD and Related Disorders Association criteria¹⁶ or the National Institute on Aging and Alzheimer's Association criteria¹⁷; MCI due to AD was diagnosed by other criteria as described previously.¹⁸ For each pedigree, cognitively normal was defined as no evidence of significant cognitive impairment beyond the AAO of AD/MCI for that mutation or in that pedigree. This study protocol was approved by the ethics committee of Xuanwu Hospital and was carried out in accordance with the principles of the Declaration of Helsinki.

2.2 | Procedures

We collected comprehensive data (demographic characteristics, family history, physical examination, and neuropsychological evaluation) of the participants using a unified case report form (CRF) from each hospital. Brain structural MRI scans and peripheral blood samples were required for all subjects who visited the clinics. Some cases further underwent fluorodeoxyglucose-positron emission tomography, ¹¹C-Pittsburgh compound B-positron emission tomography, or lumbar puncture. Cerebrospinal fluid and blood samples were taken to the central laboratory for further analysis.

Genomic DNA was extracted from the peripheral blood samples using a salting-out procedure.¹⁹ Exons 3 to 12 of *PSEN1*, exons 1 to 12 of *PSEN2*, and exons 16 to 17 of *APP* genes were amplified by polymerase chain reaction (PCR) using specific primers^{20,21} (Supplementary Table A.1) and determined by Sanger sequencing.²² In addition, the primers covered parts of the 3' and 5' untranslated regions (UTRs) of *PSENs* (Supplementary Table A.2). PCR product sequencing was performed using the ABI3730xl DNA Analyzer (Sangon Biotech Co., Ltd., Shanghai, China). The DNA sequences were analyzed using Chromas (ver. 2.33; Technelysium Pty. Ltd., South Brisbane, Australia). Apolipoprotein E (*APOE*) genotypes were also determined by Sanger sequencing,²² and the primers are presented in Supplementary Table A.1.

Based on the genetic test results, we divided the pedigrees into three groups: pedigrees with *PSENs/APP* missense mutations, which meet the criteria of co-segregation; pedigrees with *PSENs/APP* synonymous/UTR variants; and pedigrees without *PSENs/APP* gene mutations (Figure 1). Early onset (EO) cases were defined as AAO of <65 years. The missense mutations in *PSEN1*, *PSEN2*, or *APP* were defined as first identified in the CFAN if they were not reported previously in an FAD pedigree by other groups in the AD Mutation Database (<http://www.molgen.ua.ac.be/ADMutations/>), AlzForum (<http://www.alzforum.org/>), and PubMed (<http://www.ncbi.nlm.nih.gov/>), or if they were at a reported site with a novel amino acid substitute. The missense mutations were predicted for pathogenicity using Combined Annotation Dependent Depletion (CADD) and five predictive algorithms: PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2>), PANTHER (<http://www.pantherdb.org/tools/csnpscoreForm.jsp>), Mutpred2 (<http://mutpred.mutdb.org>), PROVEAN (<http://provean.jcvi.org>), and Mutation Taster (<http://www.mutationtaster.org>). Human Splicing Finder version 3.1 (<http://www.umd.be/HSF3/>) was used to predict whether the *PSENs/APP* synonymous variants would lead to splicing defects. Mutations in *PSENs* were considered to be in a non-hydrophobic or hydrophobic region according to the UniProt database (www.uniprot.org/). The allele frequency of missense mutations and synonymous/UTR variants in *PSENs/APP* was searched on the genome aggregation database (gnomAD database, <http://gnomad-old.broadinstitute.org/>) and Chinese millionome database (CMDB, <https://db.cngb.org/cmdb/>). Variants with an allele frequency of <0.01 were classified as rare variants.

2.3 | Statistical analysis

Statistical analysis was performed using SPSS software (ver. 19.0; IBM Corp., Armonk, NY, USA). A *P*-value of <0.05 was considered statistically significant. Quantitative variables such as affected generations, affected family members, age, AAO, years of education, and cognitive assessment score are expressed as the mean $\pm$ SD and the range. All data were tested for normality and homogeneity of variance using the Shapiro-Wilk test and Levene variance equality test. We used the Mann-Whitney *U* test to analyze differences among pedigrees with *PSENs/APP* missense mutations, pedigrees with *PSENs/APP* synonymous/UTR variants, and pedigrees without *PSENs/APP* gene

mutations. We applied one-way analysis of variance and Bonferroni correction to the AAO data, and the Kruskal-Wallis test to the remaining data when comparing characteristics among pedigrees with *PSEN1*, *APP*, and *PSEN2* missense mutations. The Mann-Whitney *U* test was used to compare affected generations and family members, and the Student *t* test was used to evaluate the mean AAO in pedigrees with *PSENs* mutations between hydrophobic and non-hydrophobic regions. The chi-square test, continuity correction test, and Fisher exact test were used to analyze categorical data, such as the proportion of female patients, *APOE* ϵ 4 allele frequency, and proportion of EO cases.

3 | RESULTS

3.1 | Demographics of the pedigrees in the CFAN cohort

A total of 404 pedigrees collected nationwide (Figure A1, Supplementary materials 2 and 3), including 1330 patients with either AD or MCI, were enrolled (Figure 1), among which 53 pedigrees (13.12%) had *PSENs/APP* missense mutations (35 *PSEN1*, 11 *APP*, and 7 *PSEN2* mutation families), 15 (3.71%) had *PSENs/APP* synonymous/UTR variants, and 336 (83.17%) had no *PSENs/APP* gene mutations. The mean AAO of the pedigrees was 66.21 years, and 44.60% patients were defined as EO (Table 1). Among the 1330 patients, 644 passed away, 242 were unavailable for clinical samples due to severe disease stage or refusal to undergo a laboratory examination, and 444 visited clinics and underwent comprehensive testing (Figure 1). For these 444 patients, the mean AAO was 62.65 years (range of 23 to 84 years), and the mean scores were 19.24 for the Mini-Mental State Examination (MMSE), 15.07 for the Montreal Cognitive Assessment (MoCA), and 1.14 for the Clinical Dementia Rating Global (CDR global). In total, 48.63% of patients were *APOE* ϵ 4-negative, 37.16% had one *APOE* ϵ 4 allele, and 14.21% had two *APOE* ϵ 4 alleles.

We classified the pedigrees according to the proportion of EO patients in the family. The results indicated that the detection rate of *PSENs/APP* missense mutations increased significantly when the proportion of EO patients in the family was >75% (*P* < 0.001 vs the 0%–25% group; *P* < 0.001 vs the 25%–50% group; *P* = 0.001 vs the 50%–75% group) (Figure 2A). In addition, we analyzed the distribution of the *APOE* ϵ 4 allele carried by patients in pedigrees with and without *PSENs/APP* missense mutations, and found a higher proportion of patients with *APOE* ϵ 4 and a lower proportion of patients without *APOE* ϵ 4 in pedigrees without *PSENs/APP* missense mutations than in pedigrees with *PSENs/APP* missense mutations (*P* < 0.001; *P* < 0.001) (Figure 2B).

3.2 | Characteristics of pedigrees with *PSENs/APP* missense mutations

Forty *PSENs/APP* missense mutations were identified in the CFAN, which are summarized in Table 2. Thirty-five pedigrees carried

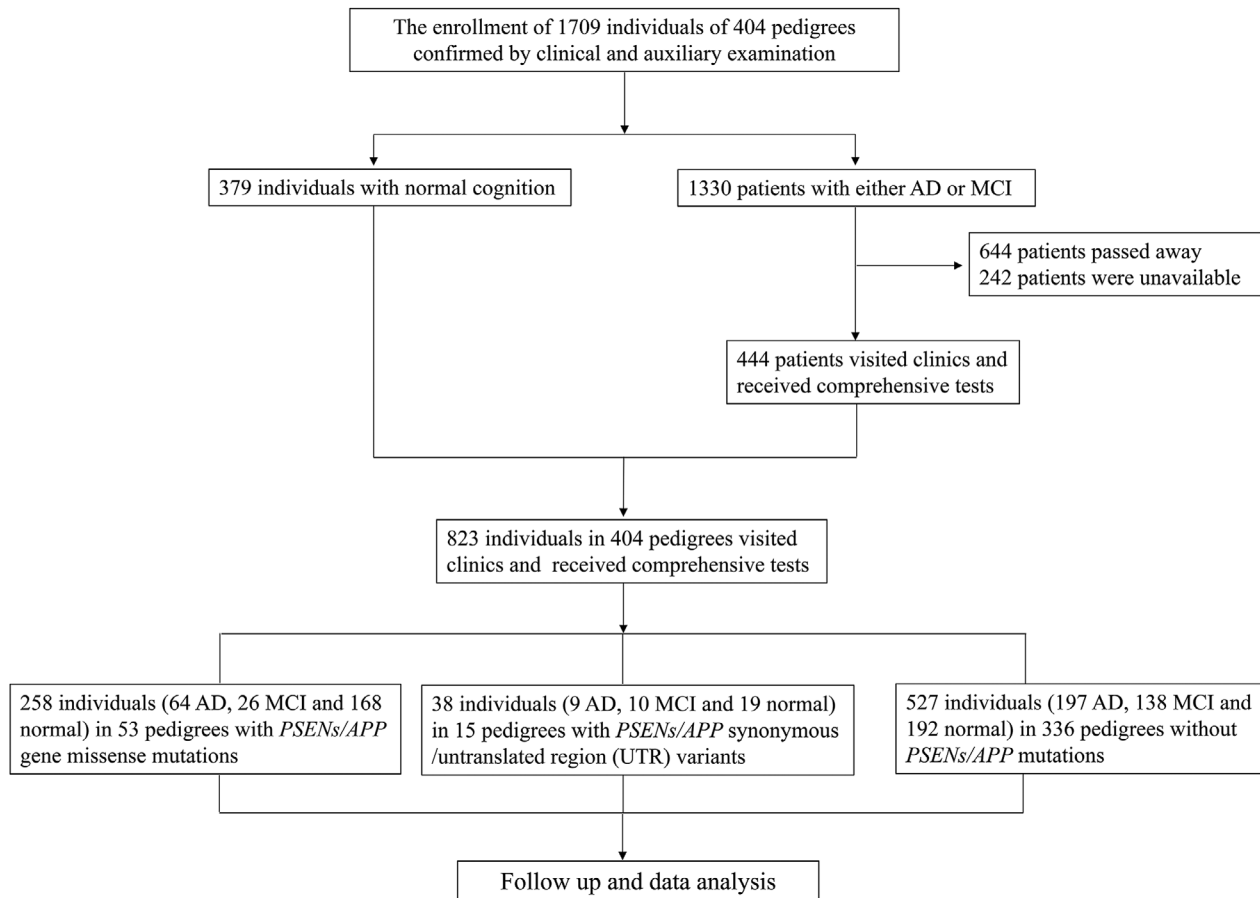


FIGURE 1 Flowchart of the enrolled pedigrees

a total of 31 missense mutations in *PSEN1*, among which 10 were first identified by our team: NM_000021.3:c.71A>G N24S, NM_000021.3:c.289G>T V97L, NM_000021.3:c.298A>T I100F, NM_000021.3:c.332G>T G111V, NM_000021.3:c.349C>A P117T, NM_000021.3:c.393C>G H131Q, NM_000021.3:c.415A>T M139L, NM_000021.3:c.515T>G L172W, NM_000021.3:c.745A>C I249L, NM_000021.3:c.932A>G K311R. Eleven pedigrees had five missense mutations in *APP* (NM_000484.3:c.2165T>A M722K, first identified by our team) and seven pedigrees had four missense mutations in *PSEN2* (Supplementary Table A.3). Among all of the first identified mutations, nine were predicted to have a “damaging effect” by at least two algorithms, with the exception of NM_000021.3:c.71A>G N24S and NM_000021.3:c.393C>G H131Q in *PSEN1* (Table 2). Three (*PSEN1*: NM_000021.3:c.71A>G N24S, NM_000021.3:c.745A>C I249L and NM_000021.3:c.932A>G K311R) of 11 missense mutations were found with global allele frequencies of <0.001 and the remaining eight were not found (Supplementary Table A.4). We also described the distribution of the changed amino acids due to *PSENs/APP* missense mutations (Figure 3).

The mean $\pm$ SD AAO for pedigrees with *PSEN1*, *APP*, and *PSEN2* missense mutations were 50.59 ± 11.41 , 47.92 ± 7.14 , and 61.47 ± 8.71 years, respectively. The mean AAO of the pedigrees with *PSEN2* mutations was significantly older than that of the pedigrees with *PSEN1* mutations ($P = 0.049$). Similarly, patients’ AAO in the *PSEN2* mutations

pedigree was also later than that in the *APP* ($P = 0.000$) and *PSEN1* ($P = 0.000$) mutations pedigrees. The *APP* and *PSEN1* pedigrees had more affected generations than the *PSEN2* pedigrees, and the frequency of the *APOE* ϵ 4 genotype in patients in the *APP* pedigrees was significantly lower than that in the *PSEN1* ($P = 0.033$) and *PSEN2* pedigrees ($P = 0.015$). Apart from these cases, we did not find any significant difference among the *PSEN1*, *APP*, and *PSEN2* missense mutation groups in affected family members; the proportion of affected female members; scores on the MMSE, MoCA, CDR global, or CDR sum of box (CDR SOB) (Supplementary Table A.5). Of the *PSEN1* and *PSEN2* missense mutations, 20 mutations in 21 pedigrees and 2 mutations in 3 pedigrees were located in hydrophobic regions, respectively (Table 2). Pedigrees carrying *PSEN1* missense mutations in hydrophobic regions had a significantly earlier mean AAO than those carrying mutations in non-hydrophobic regions (46.39 ± 8.05 vs 56.96 ± 12.93 years, $P = 0.010$) (Supplementary Table A.6). However, no significant difference was observed in *PSEN2* missense mutations between hydrophobic regions and non-hydrophobic regions (Supplementary Table A.7).

We also identified 11 synonymous and UTR variants in pedigrees (Supplementary Table A.8), including 4 *PSEN1*, one *APP*, and 3 *PSEN2* synonymous variants; one *PSEN1* 3’UTR variant; one *PSEN2* 5’UTR; and one *PSEN2* 3’UTR variant. In total, 55.88% (19/34) of patients with these synonymous or UTR variants were *APOE* ϵ 4 carriers (Table 3). Six of eight synonymous mutations were predicted to have

TABLE 1 Demographic profile of CFAN

	Characteristics	Data (mean $\pm$ SD or %)
Pedigrees (n = 404)	Affected generations	2.06 $\pm$ 0.52 (range: 1–4)
	Affected family members	3.35 $\pm$ 2.14 (range: 2–23)
	Early onset family members	1.31 $\pm$ 1.80 (range: 0–14)
	Affected females	55.86% (743/1330)
	Pedigree mean AAO	66.21 $\pm$ 9.83 (range: 31.86–86.50)
	Pedigrees without <i>PSENs/APP</i> mutations	83.17% (336/404)
	Pedigrees with <i>PSENs/APP</i> mutations	13.12% (53/404)
	Pedigrees with <i>PSEN1</i> missense mutations	8.66% (35/404)
	Pedigrees with <i>APP</i> missense mutations	2.72% (11/404)
	Pedigrees with <i>PSEN2</i> missense mutations	1.73% (7/404)
	Pedigrees with <i>PSENs/APP</i> synonymous/UTR variants	3.71% (15/404)
	Early onset patients	44.60% (516/1157 ^a)
	Affected subjects	53.95% (444/823 ^b)
Affected subjects (n = 444)	Females	55.35% (243/439)
	Early onset patients	52.70% (234/444)
	Age	65.78 $\pm$ 11.20 (range: 32–90)
	Education (y)	10.07 $\pm$ 4.56 (range: 0–21)
	AAO	62.65 $\pm$ 10.88 (range: 23–84)
	MMSE	19.24 $\pm$ 7.64 (range: 0–30)
	MoCA	15.07 $\pm$ 7.27 (range: 0–28)
	CDR global	1.14 $\pm$ 0.79 (range: 0–3)
	CDR SOB	5.57 $\pm$ 4.80 (range: 0–18)
	APOE NA	9.68% (43/444)
	APOE ϵ 4+/+	14.21% (57/401)
	APOE ϵ 4+/-	37.16% (149/401)
	APOE ϵ 4-/-	48.63% (195/401)

AAO, age at onset; CDR, Clinical Dementia Rating; CDR SOB, CDR sum of box; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; NA, not available; SD, standard deviation.

^aPatients based on family history.

^bParticipants who visited clinics.

“potential alteration of splicing” according to the Human Splicing Finder (Supplementary Table A.9). Ten of 11 synonymous/UTR variants were found in the gnom AD database, in which seven (*PSEN1*: NM_000021.3:c.234C>T G78G, NM_000021.3:c.654A>G P218P, NM_000021.3:c.843G>C T281T, NM_000021.3:c.*9C>T 3'UTR; *APP*: NM_000484.3:c.2124G>A G708G; *PSEN2*: NM_000447.2:c.639A>G A213A, NM_000447.2:c.*71C>A 3'UTR) had global allele frequencies of <0.01. In the CMDB database, 4 of 11 synonymous/UTR variants were found, in which 2 (*PSEN1*: NM_000021.3:c.654A>G P218P; *APP*: NM_000484.3:c.2124G>A G708G) had allele frequencies of <0.01 in Chinese patients (Supplementary Table A.10).

3.3 | Comparison of pedigrees with different mutations or variants

Pedigrees with *PSENs/APP* missense mutations had a significantly higher proportion of EO cases (67.85% vs 30.25%, $P < 0.001$; 67.85%

vs 38.60%, $P < 0.001$), more affected generations (2.50 $\pm$ 0.67 vs 1.99 $\pm$ 0.46, $P < 0.001$; 2.50 $\pm$ 0.67 vs 2.07 $\pm$ 0.25, $P < 0.001$), more affected family members (6.35 $\pm$ 3.61 vs 2.89 $\pm$ 1.40, $P < 0.001$; 6.35 $\pm$ 3.61 vs 3.80 $\pm$ 1.11, $P < 0.001$), and earlier pedigree mean AAO (51.68 $\pm$ 10.94 vs 68.38 $\pm$ 7.66 years, $P < 0.001$; 51.68 $\pm$ 10.94 vs 65.22 $\pm$ 5.45 years, $P < 0.001$) than pedigrees without *PSENs/APP* mutations and with *PSENs/APP* synonymous/UTR variants (Table 3). In addition, the MMSE, MoCA, CDR global, and CDR SOB scores of patients from pedigrees with *PSENs/APP* missense mutations were poorer than those from the other two types of pedigrees (Table 3). Patients from pedigrees without *PSENs/APP* mutations had a significantly higher percentage of APOE ϵ 4/ ϵ 4 than did those from pedigrees with *PSENs/APP* missense mutations (14.85% vs 1.60%, $P < 0.001$). Furthermore, the APOE ϵ 4 allele frequency was also lower in pedigrees with *PSENs/APP* missense mutations than in those without *PSENs/APP* mutations (12.57% vs 37.00%, $P < 0.001$) or with *PSENs/APP* synonymous/UTR variants (12.57% vs 35.29%, $P < 0.001$) (Table 3).

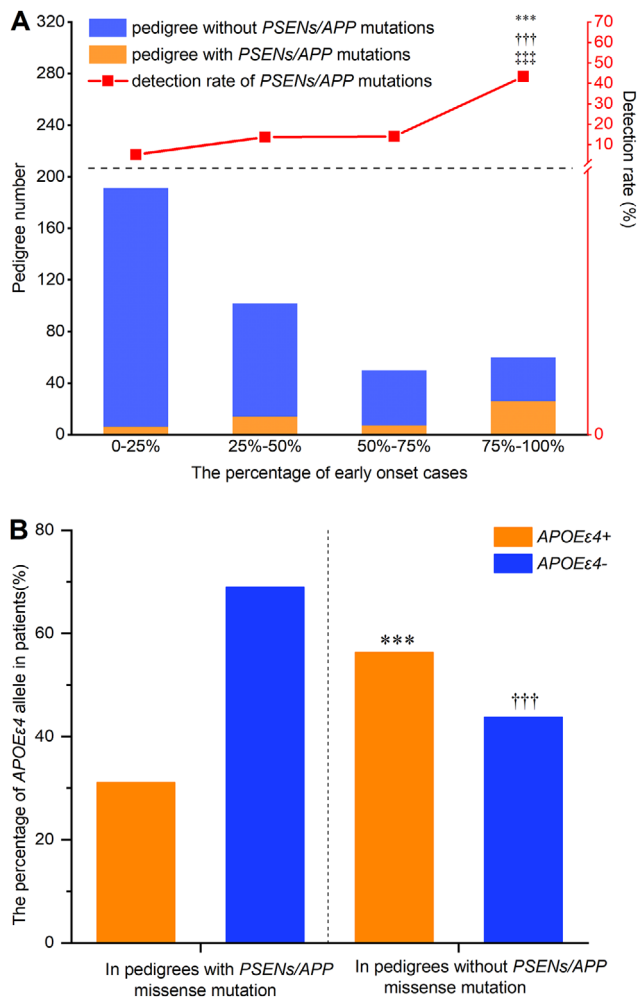


FIGURE 2 Detection rate of PSENs/APP mutations and the APOEε4 allele distribution in familial Alzheimer's disease. (A) The number of pedigrees with a different proportion of early onset (EO) cases to total patient number (EO/total number of patients) is indicated in the blue and orange columns, and the detection rate of PSENs/APP mutations is indicated by the upper red line. Note that the missense detection rate increased following the increased EO/total number of patients (** $P < 0.001$ vs the 0%–25% group; ††† $P < 0.001$ vs the 25%–50% group; ††† $P = 0.001$ vs the 50%–75% group). (B) The distribution of the APOEε4 allele carried by patients in pedigrees with and without PSENs/APP missense mutation is indicated (blue, APOEε4–; orange, APOEε4+). A higher proportion of APOEε4+ patients and a lower proportion of APOEε4– patients was found in pedigrees without PSENs/APP missense mutation than in pedigrees with PSENs/APP missense mutation (*** $P < 0.001$; ††† $P < 0.001$)

3.4 | APOEε4 genotypes in EOAD and LOAD pedigrees

A total of 33.25% (270/812) of the patients were patients with EO who lacked the PSENs/APP mutations. However, in the EO pedigrees without PSENs/APP mutations, 73.45% (202/275) of the patients exhibited EO. Frequency of the APOEε4 allele was significantly higher in patients with late-onset Alzheimer's disease (LOAD) pedigrees (mean AAO ≥ 65 years) than in those with early-onset Alzheimer's disease (EOAD)

pedigrees (mean AAO < 65 years) (34.81% vs 25.89%; $P = 0.001$). No significant difference in APOEε4 frequency was found between EOAD pedigrees without PSENs/APP mutations and LOAD pedigrees without PSENs/APP mutations. However, it was higher in the LOAD pedigrees with PSENs/APP mutations than in the EOAD pedigrees with PSENs/APP mutations (24.32% vs 14.59%; $P = 0.038$) (Supplementary Table A.11).

4 | DISCUSSION

The CFAN is the first, large, multicenter nationwide cohort of Chinese patients with FAD. In this study, 404 families with 1330 patients were included, among which 40 missense mutations in PSENs/APP were identified. The percentage of patients with APOE ε4/ε4 in the pedigrees without PSENs/APP mutations was significantly higher than that in the pedigrees with PSENs/APP mutations (14.85% and 1.60%, respectively). The major clinical features included an earlier mean AAO, more severe cognitive impairment, and fewer APOEε4 alleles in pedigrees with PSENs/APP missense mutations, and vice versa for pedigrees without PSENs/APP mutations. The detection rate of PSENs/APP mutations in our study was 13.12%, which is similar to that of previous studies in Asian populations (11.11% in Japanese²³ and 16.70% in Korean²⁴), but is lower than that in Caucasians (17.50% to 67.74%).^{25–27}

In the present study, 11 missense mutations were first identified by the CFAN, including 10 in PSEN1 and one in APP. Among the 11 missense mutations, 5 were reported previously by our team,^{28–32} and were confirmed to increase the levels of Aβ42 and/or phosphorylated tau in vitro or in vivo. However, no indications regarding the remaining six mutations in PSEN1 were found to be related to AD in the literature or the AlzForum website and AD Mutation Database. According to Guerreiro's algorithm,³³ two mutations (NM_000021.3:c.71A>G N24S and NM_000021.3:c.349C>A P117T) were classified as definite pathogenicity, and four mutations (NM_000021.3:c.298A>T I100F, NM_000021.3:c.393C>G H131Q, NM_000021.3:c.515T>G L172W, and NM_000021.3:c.745A>C I249L) were classified as probable pathogenicity. In addition, these mutations were predicted to be damaging by at least one predictive algorithm, and their CADD scores were all > 15 , indicating the pathogenicity of these unreported mutations. Further studies are needed to verify the pathogenicity and mechanisms of these unexplored mutations, both in vitro and in vivo. To summarize the features of the mutations we identified, two results should be discussed. First, 20 PSEN1 missense mutations (64.52%) were located in hydrophobic regions, and patients carrying these mutations had an earlier AAO than those with mutations located in non-hydrophobic regions, suggesting that these two types of regions had different impacts on the pathological process. Of interest, the PSEN1 K311R mutation in the non-hydrophobic region was found in three pedigrees with relatively late mean AAOs, ranging from 67 to 73.75 years, further supporting the conclusion that the mutations have a weak effect in the non-hydrophobic region. A possible explanation for this phenomenon is that mutations in hydrophobic regions³⁴ affect the structure of the PS1 protein or the γ-secretase active site, resulting in an increase in the Aβ42/Aβ40 ratio, promoting the aggregation

TABLE 2 Characteristics of PSENs/APP missense mutations

Family	Pedigree mutations	Chromosome position	Ref/Alt	Damaging ^c	PHRED CADD	Pedigree mean AAO	Affected generations	Affected family members	Proband's diagnosis	Proband's CDR global	Proband's age	Proband's AAO	Proband's APOE
1	PSEN1 N245 ^a	14:73614798	A/G	1	15.04	65.8	1	6	AD	1	74	69	3/3
2	PSEN1 M84V ^b	14:73637667	A/G	5	23.8	46.5	2	4	AD	NA	64	48	3/3
3	PSEN1 V97L ^{a,b}	14:73637706	G/T	5	26.7	51.4	3	5	AD	NA	68	58	3/3
4	PSEN1 I100F ^{a,b}	14:73637715	A/T	5	26.8	49	1	4	AD	3	60	51	3/2
5	PSEN1 F105I	14:73637730	T/A	5	27.7	48	4	6	AD	NA	NA	NA	4/3
6	PSEN1 F105L	14:73637730	T/C	4	27	63.67	4	6	AD	1	65	63	3/3
7	PSEN1 G111V ^a	14:73637749	G/T	5	23.8	63.6	2	5	MCI	0.5	55	53	3/3
8	PSEN1 P117T ^a	14:73640284	C/A	5	26.5	32.13	3	8	AD	2	33	29	3/3
9	PSEN1 P117S	14:73640284	C/T	5	27.3	36	2	3	AD	3	40	34	3/2
10	PSEN1 H131Q ^a	14:73640328	C/G	1	22	58.25	2	5	AD	3	59	58	4/2
11	PSEN1 M139L ^{a,b}	14:73640350	A/T	3	23.4	49.71	3	10	AD	2	49	45	4/3
12	PSEN1 M139V ^b	14:73640350	A/G	4	23.3	44.67	3	12	AD	1	54	51	3/3
13	PSEN1 M146V ^b	14:73640371	A/G	5	24.6	37	3	6	AD	2	40	36	4/3
14	PSEN1 R157S	14:73640406	G/T	5	26.1	67.60	3	6	AD	NA	80	72	3/3
15	PSEN1 H163R	14:73653568	A/G	4	23.9	49.89	3	13	AD	NA	64	58	3/3
16	PSEN1 L172W ^{a,b}	14:73653595	T/G	4	22.8	47	2	5	AD	2	51	49	4/3
17	PSEN1 L173S ^b	14:73653598	T/C	4	24.1	37.6	3	6	AD	1	39	36	3/3
18	PSEN1 L173P ^b	14:73653599	G/C	5	24.3	41.67	2	6	AD	2	44	41	3/3

(Continues)

TABLE 2 (Continued)

Family	Pedigree mutations	Chromosome position	Ref/Alt	Damaging ^c	PHRED CADD	Pedigree mean AAO	Affected generations	Affected family members	Proband's diagnosis	Proband's CDR global	Proband's age	Proband's AAO	Proband's APOE
19	PSEN1 L173F ^b	14:73653599	G/C	5	24.3	39	2	4	AD	1	44	38	4/4
20	PSEN1 G206S ^b	14:73659419	G/A	5	30	40	2	4	AD	2	40	37	3/3
21	PSEN1 G206A ^b	14:73659420	G/C	5	27.2	64.5	3	3	AD	2	74	69	3/3
22	PSEN1 I213T ^b	14:73659441	T/C	5	27.6	43.25	3	6	AD	2	44	42	4/3
23	PSEN1 M233L ^b	14:73659500	A/C	5	26.8	42.17	3	6	AD	1	38	37	4/3
24	PSEN1 I249L ^{a,b}	14:73659548	A/C	4	23.9	55.83	2	6	AD	1	56	54	3/3
25	PSEN1 P264L ^b	14:73664760	C/T	5	32	47	2	4	MD	2	52	50	4/3
26	PSEN1 L271V	14:73664780	C/G	5	23.6	43	4	23	AD	1	50	45	4/3
27	PSEN1 L282V ^b	14:73664813	C/G	1	24.4	44.5	3	6	AD	2	45	44	3/3
28	PSEN1 A285V ^b	14:73664823	C/T	5	32	62.75	2	4	AD	2	69	65	3/2
29	PSEN1 K311R ^a	14:73673157	A/G	2	14.34	67	2	3	MCI	0.5	73	69	3/2
30	PSEN1 K311R ^a	14:73673157	A/G	2	14.34	61.4	3	7	MCI	0.5	74	66	3/3
31	PSEN1 K311R ^a	14:73673157	A/G	2	14.34	73.75	2	5	MCI	0.5	79	77	4/3
32	PSEN1 K311R ^a	14:73673157	A/G	2	14.34	67.33	2	3	AD	NA	85	73	3/3
33	PSEN1 G378E ^b	14:73683837	G/A	5	32	34.13	3	8	MCI	0.5	40	23	4/3
34	PSEN1 L392V ^b	14:73683878	C/G	5	23.5	45.6	3	6	AD	2	49	42	3/3
35	PSEN1 P433S ^b	14:73685890	C/T	5	28.4	42.8	3	7	MCI	0.5	40	NA	3/3
36	APP V715M ^b	21:27264102	G/A	4	27.6	46	3	4	AD	1	46	42	3/3

(Continues)

TABLE 2 (Continued)

Family	Pedigree mutations	Chromosome position	Ref/Alt	Damaging ^c	PHRED CADD	Pedigree mean AAO	Affected generations	Affected family members	Proband's diagnosis	Proband's CDR global	Proband's age	Proband's AAO	Proband's APOE
37	APP V715M ^b	21:27264102	G/A	4	27.6	46	1	3	AD	NA	47	45	3/2
38	APP I716F ^b	21:27264099	A/T	5	28.6	31.86	3	8	AD	1	34	33	3/2
39	APP I716T ^b	21:27264098	T/C	5	27.8	41	2	6	MCI	0.5	48	44	3/3
40	APP V717I ^b	21:27264096	G/A	4	25.3	58.5	3	10	AD	3	73	61	3/3
41	APP V717I ^b	21:27264096	G/A	4	25.3	51.8	3	12	AD	3	54	47	3/3
42	APP V717I ^b	21:27264096	G/A	4	25.3	49.33	3	5	AD	2	59	58	3/3
43	APP V717I ^b	21:27264096	G/A	4	25.3	50.75	2	5	AD	2	53	50	3/3
44	APP V717I ^b	21:27264096	G/A	4	25.3	45	3	7	AD	3	56	54	4/3
45	APP V717I ^b	21:27264096	G/A	4	25.3	50.47	3	15	MCI	0.5	54	52	3/3
46	APP M722K ^{a,b}	21:27264080	T/A	5	29	46.4	3	6	AD	1	46	42	4/3
47	PSEN2 G34S	1:227069708	G/A	2	21.1	57	2	4	AD	2	76	69	4/3
48	PSEN2 G34S	1:227069708	G/A	2	21.1	56.2	2	5	AD	3	60	52	3/3
49	PSEN2 G34S	1:227069708	G/A	2	21.1	71.67	2	2	MCI	0.5	77	75	4/3
50	PSEN2 R62H	1:227071449	G/A	0	13.19	64.5	2	5	MCI	0.5	NA	45	3/3
51	PSEN2 V214L ^b	1:227076603	G/T	3	24.7	62	2	4	AD	2	68	58	3/3
52	PSEN2 V214L ^b	1:227076603	G/T	3	24.7	47.5	2	7	MCI	NA	47	42	3/3
53	PSEN2 M298T ^b	1:227078985	T/C	4	26	71.40	2	7	MCI	0	57	NA	3/3

AAO, age at onset; CDR, Clinical Dementia Rating; NA, not available; PHRED CADD, PHRED-scaled Combined Annotation Dependent Depletion score; Ref/Alt, reference allele and altered allele according to Genome Reference Consortium Human Build 37 (GRCh37/hg19).

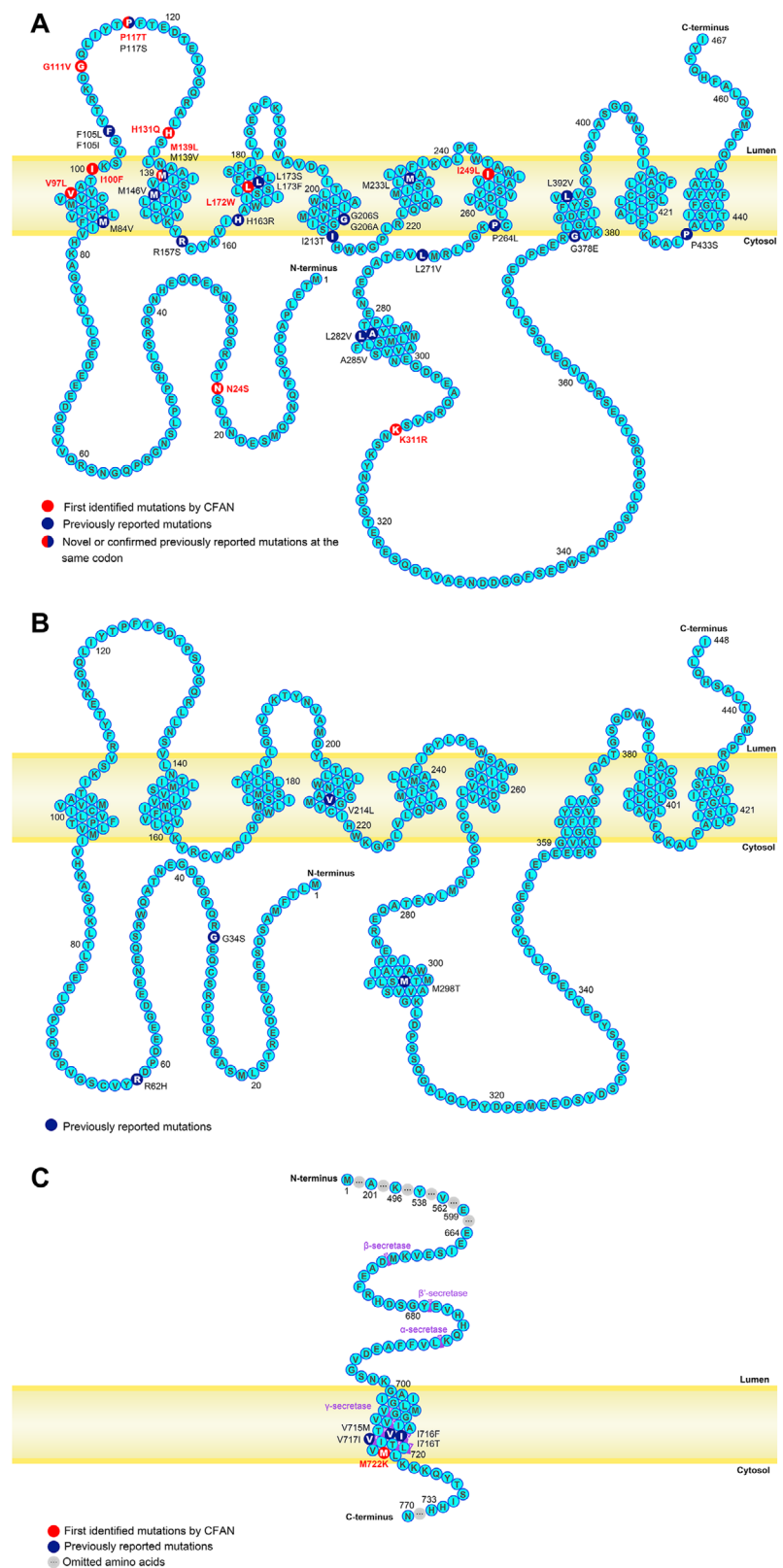
A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

^aMutations first identified in CFAN.

^bMutations that located at the hydrophobic region.

^cNumber of algorithms with a prediction of damaging effect ("probably damaging" or "pathogenicity" or "deleterious" or "disease causing") for each variant, the prediction algorithms include PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2>), PANTHER (<http://www.pantherdb.org/tools/csnpscoreForm.jsp>), Mutpred2 (<http://mutpred.mutdb.org>), PROVEAN (<http://provean.jcvi.org>), and Mutation Taster (<http://www.mutationtaster.org>).

FIGURE 3 Distribution of amino acid substitutions in the PS1, PS2, and APP proteins. The full-length amino acid sequence of PS1 (A) and PS2 (B), and a portion of the amino acid sequence of APP (C). Red circles: Mutation first identified in the Chinese Familial Alzheimer's Disease Network (CFAN). Blue circles: Mutation previously reported by other studies, and also confirmed by the CFAN. Blue-red circles: Two amino acid mutations exist at the same location, for which the red color in the circle indicates that it was first identified in the CFAN and blue color in the same circle indicates that it was reported by other studies. Note: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr



of $A\beta$, and eventually inducing the onset of AD. Second, dividing missense mutations based on mean AAOs revealed five mutations in LOAD pedigrees—N24S, R157S, and K311R on *PSEN1* and G34S and M298T on *PSEN2*—suggesting relatively weak pathogenicity of these mutations. The mutations with late AAOs in FAD have been also recorded

according to the AlzForum website, indicating that, although there were fewer of these mutations than mutations in EO, they certainly exist.

Moreover, eight *PSENs/APP* synonymous variants and three UTR variants were also identified, and six of the synonymous variants (75%)

TABLE 3 Comparison of pedigrees in CFAN

	Pedigrees with <i>PSENs/APP</i> missense mutations (n = 53) (mean ± SD or %)	Pedigrees without <i>PSENs/APP</i> mutations (n = 336) (mean ± SD or %)	Pedigree with <i>PSENs/APP</i> synonymous/UTR variants (n = 15) (mean ± SD or %)	P value
Early onset cases within the family (%)	67.85% (211/311)	30.25% (291/962)	38.60% (22/57)	<0.000*, <0.001 [†] , <0.001 [‡]
Affected generations	2.50 ± 0.67 (range: 1–4)	1.99 ± 0.46 (range: 1–4)	2.07 ± 0.25 (range: 2–3)	<0.001*, <0.001 [†] , <0.001 [‡]
Affected family members	6.35 ± 3.61 (range: 2–23)	2.89 ± 1.40 (range: 2–20)	3.8 ± 1.11 (range: 2–6)	<0.001*, <0.001 [†] , <0.001 [‡] , 0.001 [§]
Affected females (%)	52.73% (164/311)	56.76% (546/962)	57.89% (33/57)	0.440*
Pedigree mean AAO	51.68 ± 10.94 (range: 31.86–73.75)	68.38 ± 7.66 (range: 42.00–86.50)	65.22 ± 5.45 (range: 55–77)	<0.001*, <0.001 [†] , <0.001 [‡]
Patients' MMSE	15.41 ± 9.16 (range: 0–30)	19.26 ± 7.81 (range: 0–30)	20.35 ± 8.27 (range: 5–29)	0.004*, 0.004 [†]
Patients' MoCA	11.84 ± 8.42 (range: 0–28)	15.06 ± 7.35 (range: 0–28)	16.24 ± 8.43 (range: 2–27)	0.006*, 0.006 [†]
Patients' CDR global	1.44 ± 0.92 (range: 0.0–3.0)	1.09 ± 0.75 (range: 0.0–3.0)	0.94 ± 0.73 (range: 0.5–3)	0.015*, 0.025 [†]
Patients' CDR SOB	7.56 ± 5.60 (range: 0.0–18.0)	5.18 ± 4.50 (range: 0.0–18.0)	4.50 ± 4.75 (range: 0.5–18)	0.024*, 0.031 [†]
Patients with <i>APOE</i> ε4+/+	1.60% (3/187)	14.85% (60/404)	14.71% (5/34)	<0.001*, <0.001 [†] , <0.001 [‡]
Patients with <i>APOE</i> ε4+/-	21.93% (41/187)	44.31% (179/404)	41.18% (14/34)	<0.001*, <0.001 [†] , 0.017 [‡]
Patients with <i>APOE</i> ε4-/-	76.47% (143/187)	40.84% (165/404)	44.12% (15/34)	<0.001*, <0.001 [†] , <0.001 [‡]
Patients with at least one <i>APOE</i> ε4 allele	23.53% (44/187)	59.16% (239/404)	55.88% (19/34)	<0.001*, <0.001 [†] , <0.001 [‡]
Frequency of <i>APOE</i> ε4 allele	12.57% (47/374)	37.00% (299/808)	35.29% (24/68)	<0.001*, <0.001 [†] , <0.001 [‡]

AAO, age at onset; CDR, Clinical Dementia Rating; CDR SOB, CDR sum of box; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; SD, standard deviation.

*Total P value.

[†]P value between pedigrees with *PSENs/APP* missense mutations and pedigrees without *PSENs/APP* missense mutations.

[‡]P value between pedigrees with *PSENs/APP* missense mutations and pedigree with *PSENs/APP* synonymous/UTR variants.

[§]P value between pedigrees without *PSENs/APP* missense mutations and pedigree with *PSENs/APP* synonymous/UTR variants.

were predicted to affect messenger RNA (mRNA) splicing according to the Human Splicing Finder. 44.12% (15/34) of the patients in pedigrees with *PSENs/APP* synonymous/UTR variants were *APOE*ε4-negative, which led us to explore the possible effect of these variants. As indicated by Perez-Tur,³⁵ mutations that destroyed the splicing sites of *PSEN1* could cause AD through a change, or gain, of function rather than complete loss of function of the protein. Several studies^{36–38} have reported synonymous variants, but few have focused on their functions. The synonymous variant NM_000447.2:c.708T>C S236S on *PSEN2*, which was found in the present study,³⁹ was reported to be related to AD cases, indicating that it may be pathogenic. In addition, the number of studies focusing on UTR variants has increased. The 5' UTR variant on *PSEN2* identified in the CFAN, NM_000447.2:c.-275C>T, was found to be associated with an increased risk of schizophrenia.⁴⁰ However, the proband (AD, *APOE*ε4/ε3) of the pedigree with this variant in the current study complained of memory impairment but not psychiatric symptoms, indicating that UTR variants may have different clinical phenotypes. Further study might be needed.

In our study, the number of pedigrees without *PSENs/APP* mutations was markedly higher than that of pedigrees with *PSENs/APP* mutations, which accounted for 83.17% (336/404) of all enrolled pedigrees. This suggests that additional genetic factors may increase the risk of FAD. Some previous studies reported that the *APOE*ε4 allele is a

major genetic risk factor for late onset FAD^{41,42} and early-onset FAD (EOFAD).⁴³ In addition, it has been demonstrated that the effect of the *APOE*ε4 allele on AD risk is comparable to the effect of a major genetic factor with semi-dominant inheritance.⁴⁴ However, the effect of the *APOE*ε4 allele on familial aggregation of AD remains controversial. One study reported that the *APOE*ε4 allele does not represent a risk factor for EOFAD.⁴⁵ In the present study, 59.16% of patients in these pedigrees without *PSENs/APP* mutations presented with one or two *APOE*ε4 alleles, which was a significantly higher proportion than that in the pedigrees with *PSENs/APP* mutations (59.16% vs 23.53%). The percentage of patients with *APOE*ε4/ε4 in the pedigrees without *PSENs/APP* mutations was significantly higher than that in the pedigrees with *PSENs/APP* mutations (14.85% and 1.60%, respectively). Thus, it is highly possible that *APOE*ε4 could be a promoter of the clustering of FAD without *PSENs/APP* mutations. Nevertheless, this raises the question of why 40.84% of the patients from pedigrees without *PSENs/APP* mutations did not carry *APOE*ε4. It is reasonable to hypothesize that other unidentified genetic mutations or environmental factors,⁴⁶ acting independently or in concert, are involved in the development of AD. This hypothesis needs to be confirmed by further study.

Our study has several limitations. First, the AAO and family history data were collected based on the recall of some of the participants in this study. To reduce recall inaccuracy, the AAO was confirmed by at

least two close informants with normal cognition. Second, we collected CRFs, brain structural MRI data, and peripheral blood samples only from participants who visited the hospital. Family members who passed away and patients who refused laboratory examinations may have affected the results. We will increase the participation rate to reduce bias in a future study. Third, we could not completely avoid missing other dementia-associated genes, such as triggering receptor expressed on myeloid cells 2⁴⁷ and sortilin related receptor 1,⁴⁸ or other potential causal genes.

In conclusion, the 11 missense mutations in *PSENs/APP* pedigrees first identified by the CFAN are indicative of heterogeneity in the pathogenesis of AD between those of Chinese ethnicity and other ethnic groups. The *APOEε4* allele may play an important role in the clustering of affected members in pedigrees without *PSENs/APP* mutations. However, we cannot exclude the potential impact of other pathogenic genes that may be involved in the development of FAD. The present study highlights that the FAD population in China needs extensive investigation, and a concerted effort is necessary to identify the crucial pathological genes underlying the pathogenesis of AD.

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AUTHOR CONTRIBUTIONS

Longfei Jia, Jianping Jia, Luxi Shen, Qiongqiong Qiu, and Yue Fu contributed to the conception and research design; Yi Tang, Cuibai Wei, Fen Wang, Dongmei Guo, Aihong Zhou, Xiumei Zuo, Yueyi Yu, Dan Li, Lina Zhao, and Hongmei Jin recruited the participants and did clinical assessment; Wei Qin, Qi Wang, and Ying Li extracted genomic DNA and analyzed the phenotype data; Yue Fu, Luxi Shen, Min Zhu, and Longfei Jia performed the data analysis; Yue Fu, Luxi Shen, Heng Zhang, Min Zhu, Qiongqiong Qiu, Xin Yan, Chaojun Kong, Jing Hao, Jianping Jia, and Longfei Jia explained the results; Yue Fu, Luxi Shen, Heng Zhang, and Min Zhu generated the tables and figures. All authors are responsible for the drafting of the article. All authors contributed to revision and editing of the manuscript.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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SUPPORTING INFORMATION

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

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