Alzheimer's
Research & Therapy

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Identification and characterization of variants in *PSEN1*, *PSEN2*, and *APP* genes in Chinese patients with early-onset Alzheimer's disease

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Abstract

Variants in *PSEN1*, *PSEN2*, and *APP* are major genetic causes of early-onset Alzheimer's disease (EOAD). Our study aimed to identify the genotypic and phenotypic spectrums in a Chinese EOAD cohort and confirm their pathogenicity by functional analysis. This study included 304 unrelated clinically diagnosed EOAD participants of Chinese Han ancestry. Whole-exome sequencing revealed that 26 out of 304 individuals (8.6%) carried rare variants in *PSEN1*, *PSEN2*, and *APP*, including 16 in *PSEN1* (5.3%), 6 in *PSEN2* (2.0%), and 4 in *APP* (1.3%). Eight variants were novel, including *PSEN1* p.Q56R, *PSEN1* p.L174P, *PSEN1* p.S289P, *PSEN1* p.Y466C, *PSEN2* p.R17W, *PSEN2* p.F331Y, *APP* p.D197N, and *APP* p.D252V. Functional study revealed that the PS1 L174P, S289P, R377M, Y466C, PS2 V214L, and M239T mutants increased Aβ42 levels and Aβ42/Aβ40 ratios. The PS1 L174P, R377M, and Y466C mutants decreased the maturation of presenilin-1. Our findings highlight the prevalence and pathogenic significance of *APP*/*PSENs* variants in a Chinese EOAD cohort and expand the phenotypic and genotypic spectrum of EOAD.

Keywords Alzheimer's disease, EOAD, Amyloid β, *APP*, *PSEN1*, *PSEN2*

Introduction

Alzheimer's disease (AD) is a common neurodegenerative disease that is clinically characterized by progressive memory decline and cognitive dysfunction [1]. A common cutoff point for separating AD patients into early-onset (EOAD) and late-onset groups is 65 years old [2]. Familial EOAD represents approximately 35% to 60% of all EOAD cases [3–5], and sporadic individuals make up the other half of EOAD patients. For EOAD three major genes have been identified: *PSEN1*, *PSEN2*, and *APP* [6]. The amyloid protein precursor protein (APP) encoded by the *APP* gene is the precursor of the Amyloid β (Aβ) peptides, which is the major component of the extracellular amyloid plaques and one of the pathological hallmarks of AD [7]. The presenilin-1 (PS1) and presenilin-2 (PS2) proteins encoded by *PSEN1* and *PSEN2*, respectively, are multi-transmembrane domain proteins and affect the γ-secretase-dependent generation of Aβ peptides.

Profiling the variant spectrum of specific ethnic groups of EOAD and determining the functional impact of the identified variants will provide valuable insights into the

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pathogenesis of AD [4, 5, 8]. Currently, there has been no genetic investigation performed in large cohorts comprised of both sporadic and familial EOAD cases in Chinese populations. We performed a genetic study for 304 Chinese EOAD patients consecutively recruited at the Xuanwu Hospital and detailed the genotype–phenotype correlations. We focused on genetic screening and functional analysis of *APP*, *PSEN1*, and *PSEN2* variants in our cohort and aim to better understand their involvement in the pathogenesis of AD. The identification of novel AD variants and the determination of their pathogenicity could be important when mechanism-based therapies become available.

Material and methods

Participants

Diagnosis of AD was clinically established according to the 2011 NIA-AA recommendations [9]. A database was established at the Department of Neurology of Xuanwu Hospital, China, which included EOAD patients consecutively recruited between July 1, 2014, and April 31, 2024. This study included 304 unrelated EOAD patients of Chinese Han ancestry. Family history was investigated for up to 3 sequential generations for each patient. We defined 'sporadic' as patients with no known family history of neuropsychiatric disorders, including dementia, amyotrophic lateral sclerosis (ALS), Parkinson's syndromes, psychosis, depression, and suicide. Patients underwent detailed clinical interviews, physical examinations, neuropsychological assessments, genetic testing, and neuroimaging studies including cerebral ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET), ^{18}F-florbetapir positron emission tomography (AV45 PET), or magnetic resonance imaging (MRI) examinations within one month of recruitment. For all patients, careful clinical, neurological examination, and blood tests for vitamin status, thyroid function, HIV, and Treponema pallidum infection were conducted to avoid the possibility of reversible dementia. Meanwhile, 292 age-matched normal control participants were recruited from the general community of older adults. Selection criteria included education-adjusted cutoff values for the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA), as well as a score of 0 on the Clinical Dementia Rating (CDR) sum of boxes [10–12].

The study was approved by the Ethics Committees of the Xuanwu Hospital of Capital Medical University (Approval number: 2020026), and it was carried out in compliance with the Declaration of Helsinki's principles. Written informed consent was obtained from each patient or their guardian.

DNA isolation, PRNP octapeptide repeat analysis, and C9orf72 genotyping

Genomic DNA was extracted from peripheral blood lymphocytes following a standard protocol. All DNA samples were normalized to 50–100 ng/μl. The presence of the insertion or deletion of octapeptide repeats in *PRNP* was verified by nested polymerase chain reaction (PCR) and agarose electrophoresis as previously described [13]. The duplications in *APP* were assessed using multiplex ligation-dependent probe amplification (MLPA) (MRC Holland, Amsterdam, Holland). The hexanucleotide repeat expansions in *C9orf72* were also detected by adopting the methods previously described [14].

Whole-exome sequencing (WES) study

To comprehensively investigate the potential genetic cause of these patients, we first performed WES of genomic DNA from the patients. We summarized AD, FTD, and other dementia-related genes using Online Mendelian Inheritance in Man (OMIM) and PubMed database (Supplementary Table 1). Exome capture was performed with a SureSelect Human All Exon V6 + UTR (89Mb) Kit (Agilent Technologies, Santa Clara, CA, USA). Paired-end sequencing was carried out on a HiSeq2500 (Illumina, San Diego, CA, USA) using a HiSeq SBS Kit V4 (Illumina), which generated 100-bp reads. The average and minimum sequencing depths were 205× and 10×, respectively. The reference databases utilized included GRCh38/hg38 (http://genome.ucsc.edu), HGMD (https://portal.biobase-international.com), ExAC (https://exac.broadinstitute.org/), 1000 Genome (https://www.internationalgenome.org/), gnomAD (http://gnomad.broadinstitute.org), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and dbSNP (https://www.ncbi.nlm.nih.gov/SNP). WES data were analyzed for single-nucleotide variants (SNVs) and insertion-deletions (InDels) in dementia-related causing and susceptible genes. The significant results were comprehensively evaluated in aspects including minor allele frequency, conservation, predicted pathogenicity, disease association, and confirmation with Sanger sequencing. All heterozygous variants with an allele frequency < 0.1% and homozygous and potentially compound heterozygous variants were considered. MutationTaster (http://www.mutationtaster.org), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), PROVEAN (Protein Variation Effect Analyzer), and SIFT (https://provean.jcvi.org/) were used for bioinformatics analyses to predict the pathogenicity of the variants.

Cases were considered to have a definite genetic diagnosis if a variant was classified as pathogenic or likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines [15]. For

assessment of the *ApoE* status, the three alleles *ApoE2*, *ApoE3*, and *ApoE4* were determined according to the presence of variants of rs7412 and rs429358 in the WES data.

Construction of expression plasmids

cDNA coding for wild-type (WT) human PS1 (NP_000012.1), PS2 (NP_000438.2), APP695 (NP_958817.1), or the human APP Swedish KM-NL variant (APP695Swe) was cloned into pcDNA4-Myc.His A vector, respectively. Variants were introduced into PSEN1, PSEN2, APP695, and APP695Swe cDNA using PCR-based site-directed mutagenesis. The high-purity, endotoxin-free plasmids were prepared by Escherichia coli. The complete nucleotide sequences of the expression plasmids were verified by Sanger sequencing.

Cell culture and transfection

HEK293 cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) at 37 °C in a humidified incubator with 5% CO2. Plasmids were transfected into cells using lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. HEK293 cells containing APP695Swe were co-transfected with plasmids harboring WT and candidate *PSEN1* and *PSEN2* variants, respectively. The *APP* p.D197N and p.D252V variants were engineered into the APP695 and APP695Swe cDNA constructs and expressed in HEK293 cells, which were harvested 48 h post-transfection.

ELISA assay

Human Aβ40 and Aβ42 ELISA kits (Aβ40/Aβ42 ELISA kits, IBL, Hamburg, Germany) were used to Determine Aβ levels in the cell media according to the manufacturer's instructions. Briefly, cell media were added into the wells of a 96-well plate for incubation at 4 °C overnight. Plate wells were then sequentially incubated with the secondary antibody for 2 h at room temperature. The reaction substrate was then added into plate wells, followed by a stop solution. Within 10 min, color intensity was measured at 450 nm. The concentration of Aβ40 and Aβ42 in the samples was determined by comparing the O.D. of the samples to the O.D. of a standard curve in the same ELISA plate.

Western blot

Cells were lysed in RIPA buffer with 1 × protease inhibitors cocktail (Applygen, China) and 1 phosphatase inhibitors cocktail (Applygen, China) on ice for 30 min. The lysate was centrifuged at 12,000 rpm for 30 min at 4 °C and then the supernatant was transferred to a fresh tube and stored at −80 °C. Protein concentrations were determined using the BCA assay (Applygen, China). Protein lysates were separated in 8%−12% SDS-PAGE and transferred onto the PVDF membrane. After blocking nonspecific sites with 5% skim milk, the membranes were incubated with primary and secondary antibodies sequentially. Immunodetection was performed using enhanced chemiluminescent (ECL) substrates for HRP following the manufacturer's instructions (Millipore, German). Antibodies used in this study are listed in Supplementary Table 2.

Statistical analysis

Aβ levels and quantitative data of western blots were presented as mean ± standard error. Statistical significance was tested by using SPSS23 (IBM, Armonk, NY, US) or GraphPad Prism 7.0 software (Graphpad Software Inc., La Jolla, CA, US). Multiple comparisons were tested with ANOVA followed by Turkey's post hoc test. Two groups of data were compared by Student's *t*-test. $p < 0.05$ was considered to be statistically significant.

Results

Demographic feature and variant spectrum of AD cohort

The baseline characteristics of patients and healthy controls are shown in Table 1. Of the 304 EOAD patients, the age at onset ranged between 28 and 65, with an average of 55.5 ± 7.7 years. 19.7% (60/304) of subjects, who had at least one first-degree or second-degree relative affected by dementia or related disorders as described in the Methods section, were classified as having a positive family history of dementia. The remaining 244 (80.3%) patients were classified as sporadic patients because they reported no family members with dementia (Fig. 1 A Left panel).

Table 1 Demographic data of early-onset AD patients and elderly healthy controls in our cohort

	Total AD	Sporadic AD	Familial AD	Control
Cases, n	304	244	60	292
Female, n (%)	170 (55.9%)	140 (57.4%)	30 (50.0%)	155 (53.1%)
Age (years)	57.5 ± 8.1	56.9 ± 7.7	62.5 ± 8.3	55.2 ± 15.4
AAO (years)	55.5 ± 7.7	54.9 ± 7.1	58.9 ± 7.2	-
Disease duration (years)	2.4 ± 2.7	2.2 ± 2.6	3.3 ± 3.0	-
MMSE score	17.0 ± 8.7	16.9 ± 8.5	17.5 ± 9.7	27.4 ± 2.6
MoCA score	12.4 ± 8.4	12.0 ± 8.3	13.7 ± 8.9	26.0 ± 3.1
Genetically diagnosed	26 (8.6%)	16 (6.6%)	10 (16.7%)	-

AAO Age at onset, *MMSE* Mini-Mental State Examination, *MoCA* Montreal Cognitive Assessment, *AD* Alzheimer's disease

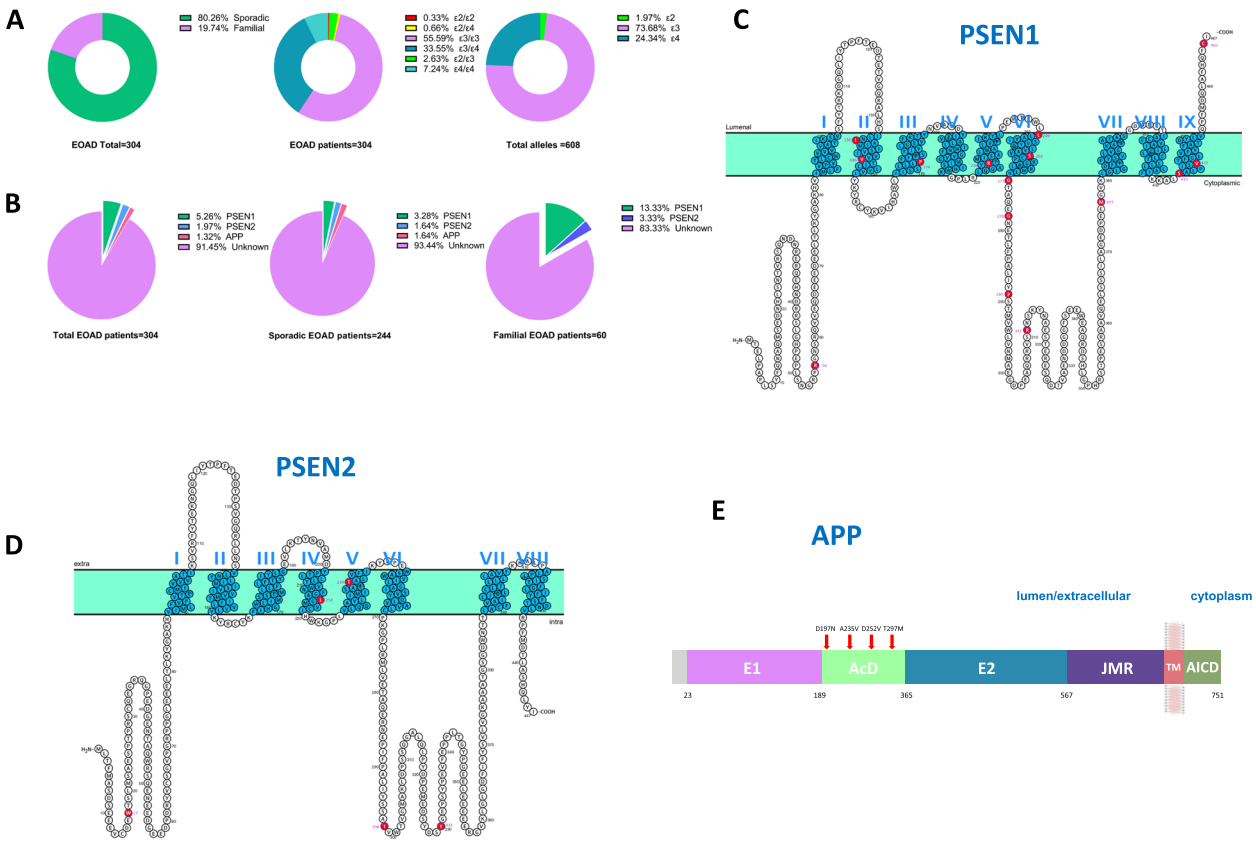

Fig. 1 Schematic representation of the frequencies and locations of *PSEN1*, *PSEN2*, and *APP* variants. **A** Left panel: Pie chart of the percentage of familial and sporadic EOAD patients. Right panel: Schematic diagram of the distribution of *APOE* allele frequencies in our cohort. **B** Pie charts representing the percentage of *PSEN1*, *PSEN2*, and *APP* variants represented in our cohort. **C** This diagram shows the amino acid sequence of PS1 and the distribution of the variants reported in this study. Presenilin 1 contains 467 amino acids with nine potential transmembrane domains. Red circles represent the variants identified in this study. **D** Distribution of amino acid sequence in presenilin 2. PS2 has a similar structure but contains 448 amino acids. Red circles represent the variants identified in this study. **E** The structural domain arrangement of the amyloid precursor protein expressed in the APP695 isoform, which contains many functional domains as illustrated. SP: Signal peptide; E1: Ectodomain 1; E2: Ectodomain 2; TM: Transmembrane domain; AcD: acidic domain; JMR: juxtamembrane region. AICD: APP intracellular domain. The APP D197N, p.A235V, D252V, and p.T297M variants were indicated by red arrows

To evaluate the correlation between *APOE* genotype and susceptibility to EOAD in mainland China, we examined the genotype and allele frequencies of these polymorphisms in 304 Chinese EOAD patients and 292 healthy controls. The *APOE* genotype in EOAD patients was shown in Fig. 1 A Right panel. The *ApoE ε4* allele frequency was significantly increased among EOAD patients compared with controls (OR: 4.0479, $p < 0.001$, Supplementary Table 3).

We identified rare variants in the probands from 10 EOAD families and in 16 sporadic EOAD cases, including 16 *PSEN1*, 6 *PSEN2*, and 4 *APP* variant carriers (Fig. 1 B). In this study, we define rare variants as non-synonymous variants with a Minor Allele Frequency (MAF) of less than 0.001, predicted to be deleterious or to affect protein structure or function, warranting further analysis. According to the ACMG criteria, 4 pathogenic variants, 12 likely pathogenic variants, and 8 variants of uncertain significance (VUS) in *PSEN1*, *PSEN2*, and *APP* were identified (Table 2). Moreover, exome sequencing identified 158 VUS in dementia-related genes that may act as risk factors, including the rare variants p.Ser2121Ser in *SORL1*, p.Thr218Ile in *TREM2*, and 18 variants in *ABCA7* (Supplementary Table 4). The *APP* duplications were not identified in the 304 EOAD patients using MLPA. This paper focused on the *PSEN1*, *PSEN2*, and *APP* rare variants.

Variant interpretation

Fifteen *PSEN1*, five *PSEN2*, and four *APP* rare variants were found in the cohort. The *PSEN1* p.M146V [16], p.L226R [17], p.L262S [18], p.E273G [19], I249L [20], p.K311R [21], R377M [22], P433S [23], p.I437V [24], *PSEN2* V214L [25], M239T [26], p.M298T [18], *APP*

Table 2 Interpretation of identified variants and their pathogenicity

Gene	Mutation	Function	Novel/known	SNP-ID	Mutation Tasting/SIFT/ Provean/PolyPhen-2	CADD score	Clinvar	GnomAD, ExAC, 1000 Genomes Frequency	ACMG	Publication (PMID)
PSEN1	NM_000021 p.Gln56Arg/c.167A > G	missense	Novel	rs754392688	D/T/D/B	16.57	NA	4/251420, 2/121286,0	VUS: PM2 + PP3 + PP4	NA
PSEN1	NM_000021 p.Ala136Val/c.407C > T	missense	Known	NA	D/D/D/P	26.1	NA	0,0,0	LP: PM1 + PM2 + PM5 + PP3 + PP4	33,973,882
PSEN1	NM_000021 p.Met146Val/c.436A > G	missense	Known	rs63750306	D/D/D/D	24.4	P	0,0,0	LP: PM1 + PM2 + PP3 + PP4 + PP5	9,712,537
PSEN1	NM_000021 p.Leu174Pro /c.521 T > C	missense	Novel	NA	D/D/D/D	25.3	NA	0,0,0	LP: PM1 + PM2 + PM5 + PP3 + PP4	NA
PSEN1	NM_000021 p.Leu226Arg/c.677 T > G	missense	Known	rs63749961	D/D/D/D	29.1	NA	0,0,0	LP: PM1 + PM2 + PP3 + PP4 + PP5	15,196,662
PSEN1	NM_000021 p.Ile249Leu/c.745A > C	missense	Known	rs1362575880	D/D/N/D	23.9	LP	1/251452,0,0	P: PS1 + PM1 + PM2 + PP3 + PP4	31,914,229
PSEN1	NM_000021 p.Leu262Ser/c.785 T > C	missense	Known	NA	D/D/D/D	29.6	NA	0,0,0	LP: PM1 + PM2 + PP3 + PP4 + PP5	30,954,774
PSEN1	NM_000021 p.Glu273Gly/c.818A > G	missense	Known	NA	D/D/D/D	29.8	NA	0,0,0	LP: PM1 + PM2 + PP3 + PP4 + PP5	22,475,797
PSEN1	NM_000021 p.Arg278Gly/c.832A > G	missense	Known	NA	D/D/D/D	29.1	NA	0,0,0	LP: PM1 + PM2 + PM5 + PP3 + PP4	37,712,079
PSEN1	NM_000021 p.Ser289Pro/c.865 T > C	missense	Novel	NA	D/D/D/D	32	NA	0,0,0	LP: PM1 + PM2 + PP3 + PP4	NA
PSEN1	NM_000021 p.Lys311Arg/ c.932A > G	missense	Known	rs115865530	D/T/N/P	14.04	VUS	35/251356, 16/118970,0	VUS: PM2 + PP3 + PP4 + PP5	28,269,784
PSEN1	NM_000021 p.Arg377Met/c.1130G > T	missense	Known	rs63751051	D/D/D/D	33	NA	0,0,0	P: PS1 + PM1 + PM2 + PP3 + PP4	36,306,459
PSEN1	NM_000021 p.Pro433Ser/c.1297C > T	missense	Known	rs1566657804	D/D/D/D	29.1	LP	0,0,0	P: PS1 + PM1 + PM2 + PP3 + PP4	30,279,455
PSEN1	NM_000021 p.Ile437Val/ c.1309A > G	missense	Known	rs764971634	D/D/N/P	22.2	LP	3/140276,1/121400,0	LP: PM1 + PM2 + PP3 + PP4 + PP5	27,930,341
PSEN1	NM_000021 p.Tyr466Cys/c.1397A > G	missense	Novel	NA	D/D/D/D	26.8	NA	0,0,0	LP: PM1 + PM2 + PP3 + PP4	NA
PSEN2	NM_000447 p.Arg17Trp/c.49C > T	missense	Novel	rs199644116	D/D/N/P	24.8	VUS	1/140236, 4/121314,0	LP: PM1 + PM2 + PP3 + PP4	NA
PSEN2	NM_000447 p.Val214Leu/c.640G > T	missense	Known	rs574125890	D/D/N/D	24.3	VUS	15/140286,0,6/5008	VUS: PM1 + PP3 + PP4	28,008,242
PSEN2	NM_000447 p.Met239Thr/c.716 T > C	missense	Known	NA	D/D/D/D	23.8	NA	0,0,0	P: PS1 + PM1 + PM2 + PP3 + PP4	35,491,795
PSEN2	NM_000447 p.Met298Thr/c.893 T > C	missense	Known	NA	D/D/N/D	25.7	NA	0,0,0	LP: PM1 + PM2 + PP3 + PP4 + PP5	30,954,774
PSEN2	NM_000447 p.Phe331Tyr/c.992 T > A	missense	Novel	NA	D/T/N/D	18.82	NA	0,0,0	VUS: PM1 + PM2 + PP4	NA
APP	NM_000484 p.Asp197Asn/c.589G > A	missense	Novel	NA	D/D/N/D	23.4	NA	0,0,0	VUS: PM2 + PP3 + PP4	NA

Table 2 (continued)

Gene	Mutation	Function	Novel/known	SNP-ID	Mutation Tasting/SIFT/Provean/PolyPhen-2	CADD score	Clinvar	GnomAD, ExAC, 1000 Genomes Frequency	ACMG	Publication (PMID)
APP	NM_000484 p.Ala235Val/c.704C>T	missense	Known	rs139819006	D/T/D/B	18.78	VUS	34/248594, 13/120884,0	VUS: PM2 + PP3 + PP4 + PP5	26,242,991
APP	NM_000484 p.Asp252Val/c.755A>T	missense	Novel	NA	D/D/N/B	22.2	NA	0,0,0	VUS: PM2 + PP3 + PP4	NA
APP	NM_000484 p.Thr297Met/c.890C>T	missense	Known	rs557227002	D/D/D/D	28.7	B	51/251178, 25/121250,0	VUS: PM2 + PP3 + PP4 + PP5	30,598,257

Mutationtaster: D = Disease-causing, N = Polymorphism; SIFT: D = Damaging, T = Tolerated; Provean: D = Deleterious, N = Neutral; Polyphen-2: D = Probably damaging, P = Possibly damaging, B = Benign; Clinvar/ACMG: P = pathogenic, LP = likely pathogenic, VUS = variants of uncertain significance

NA Not available

p.A235V [27], and p.T297M [28] were reported by other groups and the *PSEN1* variants of A136V [29], I249L [30], P433S [30], and *PSEN2* M239T [29] were previously reported by our group. The *PSEN1* R278G variant was identified in an African family with hereditary spastic paraplegia, followed by progressive aphasia [31]. The other eight variants including *PSEN1* p.Q56R, L174P, S289P, Y466C, *PSEN2* p.R17W, p.F331Y, *APP* D197N, and D252V were newly identified. These variants were rare or not found in ExAC, 1000 Genome, or GnomAD databases. They were predicted to be damaging by the Mutationtaster, SIFT, PROVEAN, or Polyphen2 software. The genetic characteristics of the variants and their pathogenicity are summarized in Table 2.

Structurally, most PS1 and PS2 substitutions were located in predicted transmembrane regions within the presenilin domain (Fig. 1 C and D). The PS1 R377M substitution was on the edge of the transmembrane (TM7) region, and the PS1 Y466C substitution was in the extracellular domain adjacent to the C-terminus. The APP D197N, A235V, D252V, and T297M substitutions were located in the acidic domain of the APP protein (Fig. 1 E).

Clinical characteristics of variant carriers

All 26 patients with the variants in *PSEN1*, *PSEN2,* and *APP* met the clinical diagnosis of probable AD [9]. The detailed information is shown in Table 3, the pedigrees of the EOAD patients with positive family histories are shown in Fig. 2, and the neuroimaging studies of the patients functionally analyzed are presented in Fig. 3.

The clinical characteristics of Patient 2 with *PSEN1* p.A136V, Patient 7 with *PSEN1* p.I249L, Patient 14 with *PSEN1* p.P433S, and Patient 20 with *PSEN2* p.M239T variants were described in our previous reports [29, 30]. The symptoms of Patient 14 and Patient 20 gradually progressed and the follow-up neuroimaging study was performed for them at the ages of 44 and 62, respectively (Fig. 3).

Patient 4 with the *PSEN1* p.L174P variant had a very early age of onset but no family history. Her father who is currently 55 years old has no symptoms of dementia or a family history of dementia. Her mother died of a cerebellar tumor at the age of 32. The elder siblings of her mother are healthy, and their parents, who passed away in their 70 s, showed no signs of dementia. The patient's father, younger brother, and daughter were genetically tested and no variants in genes related to dementia were found. Therefore, the *PSEN1* p.L174P variant in Patient 4 may be a de novo variant, inherited from her mother, or a result of non-paternity. Patient 4 had been suffering from memory loss for one and a half years. She also presented with slow reactions and difficulty in communication. The AV45 PET was positive (Fig. 3). The cerebrospinal fluid (CSF) Aβ−42 level was decreased and the p-Tau181 level was elevated.

Patient 11 with *PSEN1* p.S289P variant presented with a cognitive decline for 2 years. She had diminished verbal expression and comprehension. She also became unable to calculate numbers and apathetic. CSF Aβ42 was decreased and the Aβ−42/Aβ−40 ratio was decreased. She was diagnosed with probable AD. Patient 13 with the *PSEN1* p.R377M variant had a family history. Her father developed dementia in his forties and passed away at age 58 (Fig. 2). Her younger brother and sister are healthy and a genetic test revealed no variants in dementia-related genes. Patient 13 presented memory decline three years ago. She became depressed, apathetic, and frequently disoriented in a strange location. AV45 PET was positive (Fig. 3).

Segregation analysis

Segregation analysis was performed for Patient 5 with *PSEN1* p.L226R, Patient 9 with *PSEN1* p.E273G, and Patient 22 with *PSEN2* p.F331Y. The *PSEN1* p.L226R variant was also detected in Patient 5's older sister, who presented with memory loss and delusions at the age of 54. The *PSEN1* p.E273G variant was identified in Patient 9's older sister, who exhibited language impairment and memory loss at the age of 49. However, the *PSEN2* p.F331Y variant was not detected in Patient 22's younger sister, who presented with psychiatric symptoms at the age of 44. The affected siblings and unaffected family members in other families declined to undergo genetic testing.

The analysis of PSEN1, PSEN2, and APP variants for Aβ production

Functional analysis of the PSEN1 I249L and P433S mutants showed increased Aβ42 levels and Aβ42/Aβ40 ratios in our previous study [30]. Therefore, we performed functional analysis for the newly identified *PSEN1* and *APP* variants. Moreover, the *PSEN2* V214L and M239T variants, which were frequently reported in Asian populations, were functionally validated.

To examine the effect of the *PSEN1* and *PSEN2* variants on APP processing, PS1 and PS2 WT and their mutants were co-expressed with the APP Swedish mutant (APPswe) in HEK293 cells (Fig. 4A).

Compared with PS1 WT and the Q56R mutant, the Ab40 level was marginally lowered by the PS1 L174P, S289P, R377M, and Y466C substitutions. However, Aβ42 levels and the Aβ42/ Aβ40 ratios were significantly increased in all cells expressing these mutants. Among these PS1 mutants, the PS1 R377M mutant produced the least amount of Aβ 40 (87.04 ± 9. 65%, compared to PS1 WT) and the highest amount of Aβ 42 (236.5 ± 30.76%,

Table 3 The clinical characteristics of 26 mutation carriers

ID	Gene	Mutation	Family history	Sex	Age (years)	AAO (years)	Disease Duration (years)	MMSE	MoCA	CDR	Symptoms	MRI	FDG-PET	*APOE*
Patient 1	*PSEN1*	p.Q56R	No	Female	37	36	1	20	14	1	Memory decline	Bilateral hippocampus atrophy	NA	*ε3/ε4*
Patient 2	*PSEN1*	p.A136V	Yes	Male	56	50	6	6	4	3	Progressive memory decline, disorientation, executive dysfunction	Global brain atrophy, particularly pronounced in the bilateral hippocampi	Hypo-metabolism in the bilateral parietal, temporal, and occipital lobes	*ε2/ε4*
Patient 3	*PSEN1*	p.M146V	Yes	Female	42	40	2	18	12	2	Progressive memory decline, disorientation, executive dysfunction	Atrophy in the bilateral temporal and parietal lobes	NA	*ε3/ε3*
Patient 4	*PSEN1*	p.L174P	No	Female	30	28	2	3	3	3	Memory loss, slow reactions and difficulty in communication	Bilateral hippocampus atrophy	Hypo-metabolism in the bilateral hippocampal, temporal, and frontal lobes	*ε3/ε3*
Patient 5	*PSEN1*	p.L226R	Yes	Male	50	46	4	19	14	2	Progressive memory decline, language disability	Atrophy in the bilateral temporal and parietal lobes	NA	*ε3/ε4*
Patient 6	*PSEN1*	p.I249L	No	Female	64	60	4	19	14	2	Memory disturbance	Atrophy in the bilateral temporal, parietal, and occipital lobes	Hypo-metabolism in bilateral parietal lobes, right temporal lobe, middle left temporal lobe, and right posterior frontal lobe	*ε3/ε3*
Patient 7	*PSEN1*	p.I249L	Yes	Female	62	54	8	12	7	2	Memory loss	Atrophy in hippocampal and cortex	Hypo-metabolism in the bilateral hippocampal and temporal lobes	*ε3/ε3*
Patient 8	*PSEN1*	p.L262S	Yes	Male	59	57	2	5	2	3	Language impairment, memory loss	Atrophy in the bilateral parietal, temporal lobes, and hippocampal	NA	*ε3/ε3*
Patient 9	*PSEN1*	p.E273G	Yes	Female	47	44	3	20	15	1	Memory loss	Atrophy in the bilateral temporal and parietal lobes	Hypo-metabolism in the bilateral hippocampal, temporal, and frontal lobes	*ε3/ε4*
Patient 10	*PSEN1*	p.R278G	No	Female	34	33	1	23	17	1	Memory loss, get lost in familiar places	Bilateral hippocampus atrophy	NA	*ε3/ε3*

Table 3 (continued)

ID	Gene	Mutation	Family history	Sex	Age (years)	AAO (years)	Disease Duration (years)	MMSE	MoCA	CDR	Symptoms	MRI	FDG-PET	*APOE*
Patient 11	*PSEN1*	p.S289P	No	Female	55	53	2	11	5	2	Memory loss, unable to calculate numbers, apathy, diminished verbal expression and comprehension	Atrophy in the bilateral hippocampal, parietal, and temporal lobes	Hypo-metabolism in the bilateral hippocampal, parietal, and temporal lobes	*ε3/ε3*
Patient 12	*PSEN1*	p.K311R	No	Female	67	63	4	21	15	1		Atrophy in the bilateral parietal, temporal lobes, and hippocampal	NA	*ε2/ε3*
Patient 13	*PSEN1*	p.R377M	Yes	Female	64	61	3	4	0	3	Memory decline, depression, apathy, and disorientation	Atrophy in the bilateral hippocampus	Hypo-metabolism in the bilateral temporal, parietal lobes, and hippocampus	*ε3/ε3*
Patient 14	*PSEN1*	p.P433S	Yes	Male	44	34	10	23	18	1	Memory decline, apathetic, social disinhibition	Atrophy in the bilateral parietal, temporal lobes, and hippocampal	Hypo-metabolism in bilateral parietal and temporal lobes	*ε3/ε3*
Patient 15	*PSEN1*	p.I437V	No	Male	74	69	5	7	4	3	Memory loss, delusions	Atrophy in the bilateral temporal lobes and hippocampus	NA	*ε3/ε4*
Patient 16	*PSEN1*	p.Y466C	No	Male	63	57	6	16	12	1	Memory loss	Atrophy in bilateral temporal, parietal, and occipital lobes	Hypo-metabolism in bilateral temporal, parietal, and occipital lobes	*ε3/ε3*
Patient 17	*PSEN2*	p.R17W	No	Female	67	61	6	12	6	2	Memory decline, disorientation, executive dysfunction	Atrophy in the bilateral parietal, temporal lobes, and hippocampus	NA	*ε3/ε3*
Patient 18	*PSEN2*	p.V214L	No	Female	59	57	2	15	12	1	Memory loss	Atrophy in bilateral temporal lobes and hippocampus	Hypo-metabolism in bilateral temporal lobes and hippocampus	*ε2/ε3*
Patient 19	*PSEN2*	p.V214L	No	Female	59	57	2	8	4	3	Memory loss, confusion and disorientation	Atrophy in the bilateral parietal, temporal lobes, and hippocampal	Hypo-metabolism in the bilateral temporal, parietal, and frontal lobes	*ε3/ε3*
Patient 20	*PSEN2*	p.M239T	No	Male	62	58	4	14	7	2	Memory loss, repetitive and impulsive behavior	Atrophy in bilateral parietal and occipital lobes	Hypo-metabolism in bilateral parietal and occipital lobes	*ε3/ε4*

Table 3 (continued)

ID	Gene	Mutation	Family history	Sex	Age (years)	AAO (years)	Disease Duration (years)	MMSE	MoCA	CDR	Symptoms	MRI	FDG-PET	*APOE*
Patient 21	*PSEN2*	p.M298T	Yes	Male	65	60	5	9	5	3	Memory loss, paranoid, apathetic	Atrophy in the bilateral parietal, temporal lobes, and hippocampus	Hypo-metabolism in the bilateral hippocampal, parietal, and temporal lobes	*ε3/ε3*
Patient 22	*PSEN2*	p.F331Y	Yes	Male	50	47	3	17	14	1	Memory loss	Atrophy in the bilateral temporal and parietal lobes	NA	*ε3/ε4*
Patient 23	*APP*	p.D197N	No	Male	66	64	2	18	15	1	Memory loss, loss of inhibitions	Atrophy in the bilateral parietal, temporal lobes, and hippocampus	Hypo-metabolism in the bilateral parietal, temporal lobes, and hippocampus	*ε3/ε4*
Patient 24	*APP*	p.A235V	No	Male	59	54	5	21	15	1	Memory loss, social withdrawal	Bilateral hippocampus atrophy	NA	*ε3/ε3*
Patient 25	*APP*	p.D252V	No	Male	68	64	4	22	16	1	Memory loss, changes in sleeping habits	Atrophy in the bilateral temporal, parietal, and frontal lobes	Hypo-metabolism in the bilateral temporal, parietal, and frontal lobes	*ε3/ε3*
Patient 26	*APP*	p.T297M	No	Female	59	53	6	17	13	2	Memory loss, apathetic and irritable	Atrophy in the bilateral temporal and parietal lobes	Hypo-metabolism in the bilateral parietal, temporal lobes, and hippocampus	*ε3/ε4*

AAO Age at onset, *MMSE* Mini-Mental State Examination, *MoCA* Montreal Cognitive Assessment, *CDR* Clinical Dementia Rating, *ApoE* Apolipoprotein E, *MRI* Magnetic resonance imaging, *FDG-PET* ^{18}F-fluorodeoxyglucose positron emission tomography, *AV45-PET* ^{18}F-florbetapir positron emission tomography, *CSF* cerebrospinal fluid, *AD* Alzheimer's disease, *NA* Not available

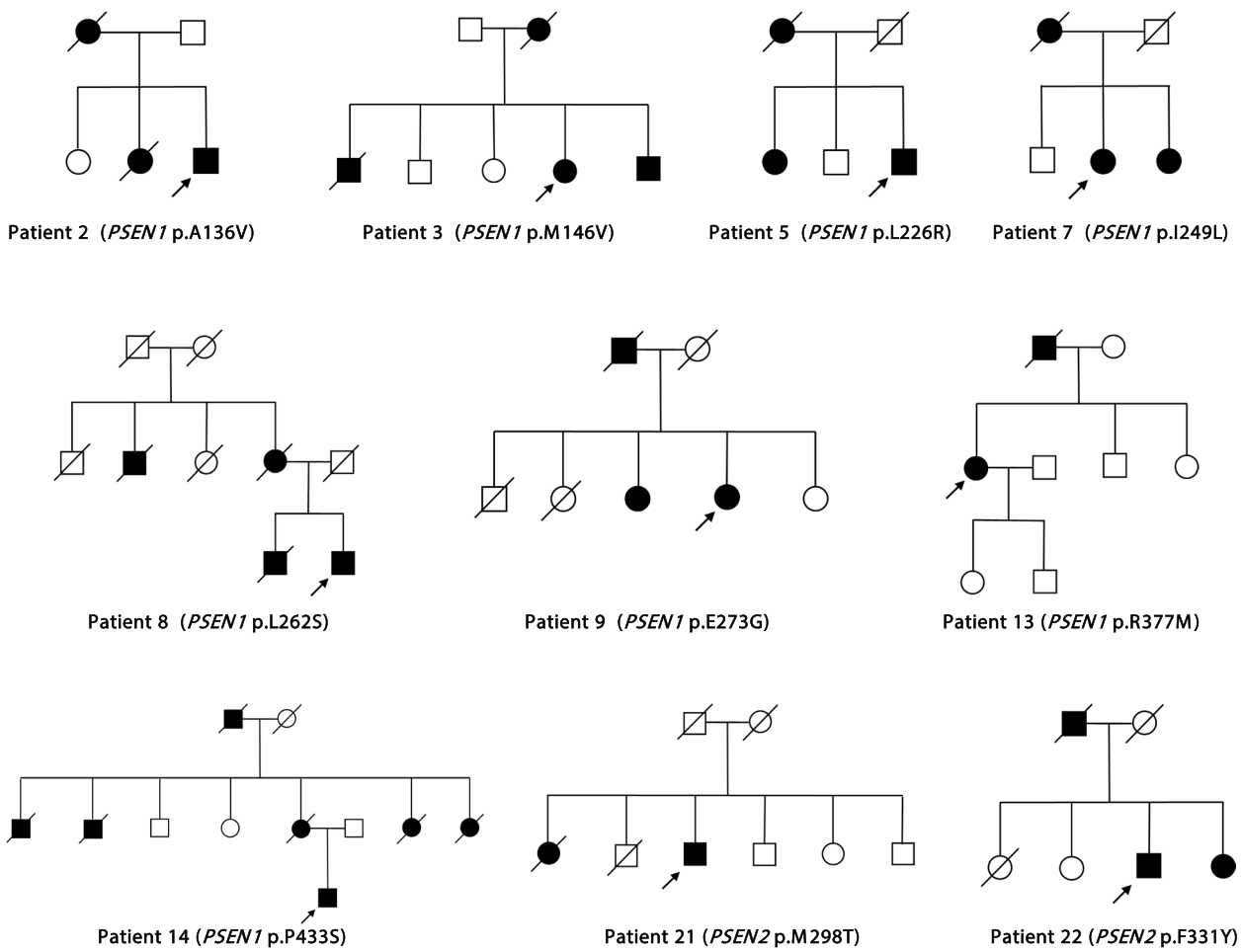

Fig. 2 Pedigrees of the EOAD families with variants identified in this study

compared to PS1 WT, $p < 0.001$), which resulted in the highest Aβ42: Aβ40 ratio. The PS1 L174P, S289P, R377M, and Y466C mutants significantly increased the Aβ42: Aβ40 ratio compared to PS1 WT mostly due to higher Aβ42 production (Fig. 4A).

To characterize the effects of the variants in the *PSEN2* gene, PS2 WT and mutants were co-expressed with APPswe in HEK293 cells. Compared to PS2 WT, both the PS2 V214L and M239T mutants increased Aβ42 levels (V214L: 139.5 ± 24.02% compared to PS2 WT, $p < 0.05$; M239T: 146.5 ± 18.04% compared to PS2 WT, $p < 0.05$) and Aβ42/Aβ40 ratios (V214L: 142.3 ± 22.04% compared to PS2 WT, $p < 0.05$; M239T: 154.3 ± 18.02% compared to PS2 WT, $p < 0.01$). Aβ40 levels did not differ between the PS2 WT and the PS2 mutants (Fig. 4B).

To examine the impact of the *APP* variants on Aβ generation, Aβ in conditioned media of HEK293 cells transiently transfected with APPSwe, APPSwe with the D197N variant (APPSwe/D197N), and APPSwe with the D252V variant (APPSwe/D252V) were analyzed using ELISA. Neither the D197N nor the D252V mutant affected Aβ40 or Aβ42 production. While there was a slight increase in the Aβ40 level by the D197N mutant, it was statistically insignificant (Fig. 4C). The plasmid quantities and concentrations of Aβ40 and Aβ42 levels, as determined by ELISA, are provided in Supplementary Table 5.

PSEN1 variants affect maturation

To explore the molecular mechanism underlying the altered Aβ production by the PS mutants, we first examined the maturation of PS1. After synthesis, PS1 undergoes posttranslational modifications including proteolysis maturation. PS1 holoprotein is cleaved into an N-terminal fragment and a C-terminal fragment (CTF). While this maturation appears to be non-essential for PS1 functions, some AD-associated variants in *PSEN1* may suppress PS1 maturation and as such increase the Aβ42: Aβ40 ratio [32]. The result showed that PS1 L174P, R377M, and Y466C mutants, but not the Q56R mutant

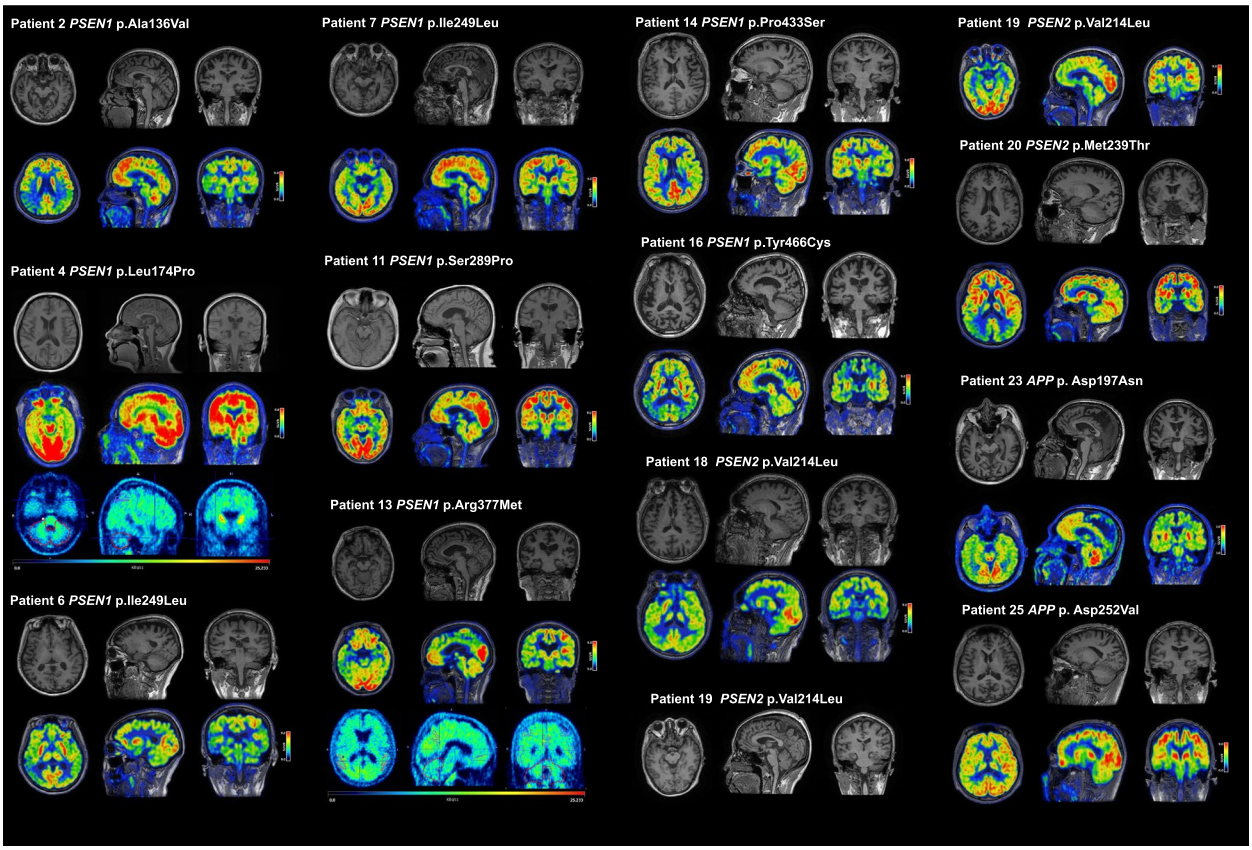

Fig. 3 A neuroimaging study of EOAD patients with functionally analyzed variants identified in this cohort. The AV45 PET images for Patients 2 and 6 were displayed. The MRI/FDG PET images for the patients with functionally analyzed variants in this study were displayed

decreased the amount of CTF and the CTF/holo-PS1 ratio. PS1 S289P mutant also suppressed PS1 maturation, but this effect did not reach statistical significance. Hence, the *PSEN1* L174P, R377M, and Y466C are canonic AD pathogenic variants (Fig. 5 A and B).

Discussion

In this study, we found 15 *PSEN1*, 5 *PSEN2*, and 4 *APP* rare variants in a Chinese cohort mostly comprised of sporadic EOAD patients, including 8 novel variants *PSEN1* p.Q56R, *PSEN1* p.L174P, *PSEN1* p.S289P, *PSEN1* p.Y466C, *PSEN2* p.R17W, *PSEN2* p.F331Y, *APP* p.D197N, and *APP* p.D252V. Functional analysis revealed that the PS1 L174P, S289P, R377M, Y466C, PS2 V214L, and M239T mutants increased Aβ42 levels and Aβ42/Aβ40 ratios, suggesting that they may be pathogenic for AD.

De novo variants [33], incomplete penetrance, somatic mosaicism, non-paternity, insufficient clinical assessment of parents, non-genetic factors, and multifactorial (genetic) causes are potential mechanisms responsible for sporadic EOAD cases, which make up 80.3% of patients in our cohort. The variant frequencies for the three genes in our cohort were 5.3% for *PSEN1*, 2.0% for *PSEN2*, and 1.3% for *APP*, and 91.4% of the patients remain genetically unexplained. Furthermore, according to ACMG criteria, 17 patients (5.6%) were identified as harboring a likely pathogenic or pathogenic variant considered to be the cause of the disease, with 14 in *PSEN1* and 3 in *PSEN2*. To date, there has been no genetic investigation performed in large EOAD cohorts focusing on the prevalence of the three genes in Chinese populations. Previous studies conducted in large Chinese cohorts without separating EOAD and late-onset AD showed a relatively lower frequency of variants identified in the three genes [34, 35]. In EOAD patient cohorts of European descent, the estimated variant frequencies for the three genes were 4.3%–13.2% for *PSEN1*, 1%–13% for *PSEN2* [4, 36–44], and 1%–4.9% for *APP* [4, 6]. Recently, a large study on European cohorts reported an overall detection rate of likely pathogenic/pathogenic variants in the *APP*, *PSEN1*, and *PSEN2* genes at 12.3% [45]. Similarly, another study on Asian patients found that 16% of EOAD cases carried pathogenic variants in the *APP*, *PSEN1*, or *PSEN2* genes [46]. In both studies, the majority of EOAD patients had a positive family history. The relatively low

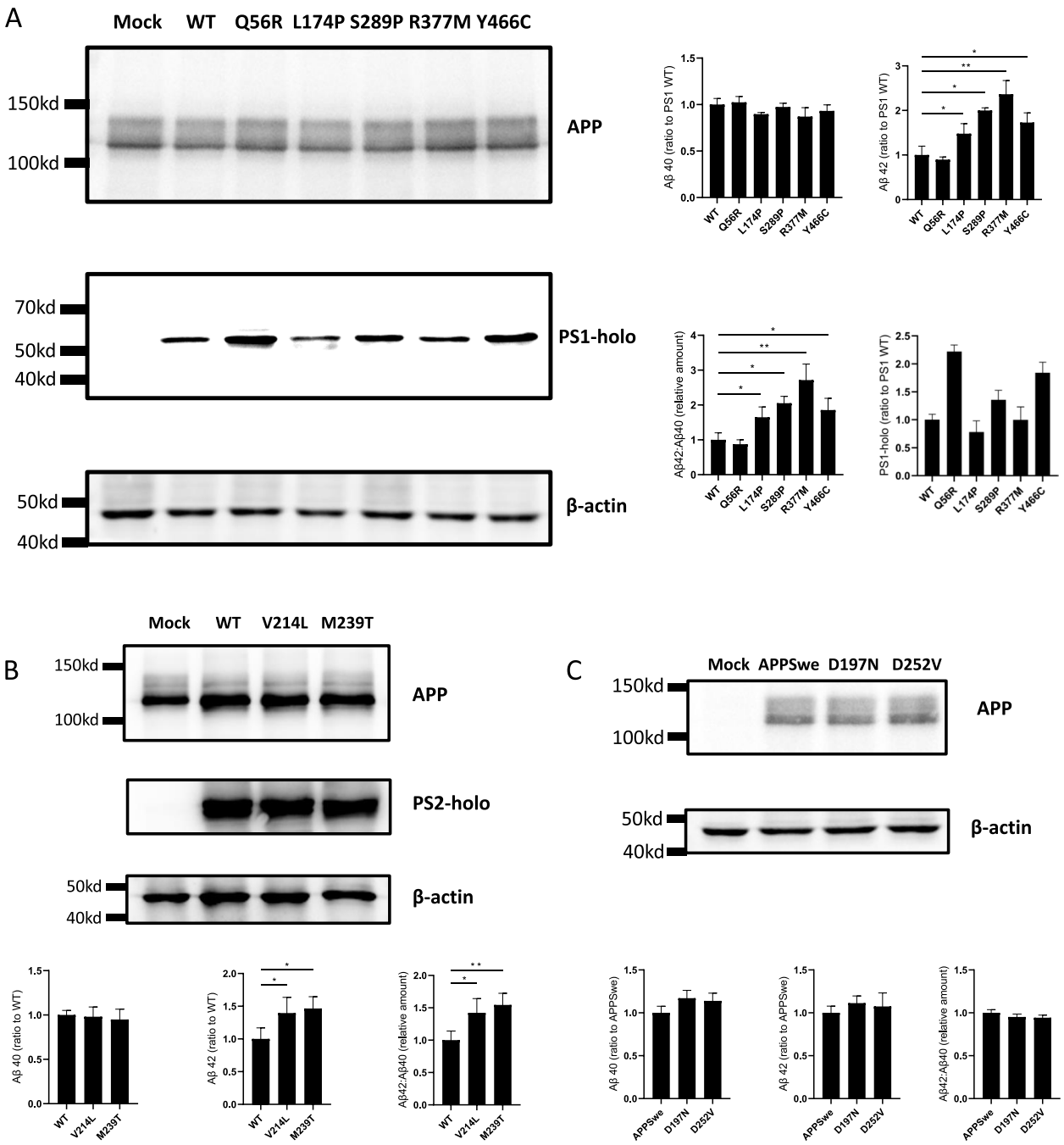

Fig. 4 Aβ−40 and Aβ−42 protein expression study. Aβ−40 and Aβ−42 protein expression levels in cell media of each group. WT and indicated mutants were co-expressed with the APP Swedish mutant in HEK239 cells and the conditioned media were harvested 48 h post-transfection for ELISA-determination of Aβ−40 and Aβ−42. Cell lysates were subjected to Western blot for APP, PS1, PS2, and β-actin as an internal standard. **A** Western blotting of cell lysates and quantification of Aβ42, Aβ40, and ratios of Aβ42 to Aβ40 relative to PS1 WT in conditioned medium of cells expressing PS1 WT and PS1 Q56R, L174P, S289P, R377M, and Y466C mutants. The Aβ levels were normalized to the total protein levels in PSEN1 WT-expressing cells. Quantifications of the full-length PS1 in the corresponding cell lysates relative to PS1 WT were also provided. The PS1 levels were normalized to the β-actin levels. **B** Western blotting of cell lysates and quantification of Aβ42, Aβ40, and ratios of Aβ42 to Aβ40 relative to PS2 WT in conditioned medium of cells expressing PS2 WT and PS2 V214L, M239T mutants. The Aβ levels were normalized to the total protein levels in PSEN2 WT-expressing cells. **C** Western blotting of cell lysates and quantification of Aβ42, Aβ40, and ratios of Aβ42 to Aβ40 relative to APPSwe in conditioned medium of cells expressing Mock (empty vector transfected), APPSwe, D197N (APPSwe combined with D197N variant), and D252V (APPSwe combined with D252V variant) mutants. The Aβ levels were normalized to the total protein levels in APPSwe-expressing cells. All Aβ level normalizations were performed relative to the total protein levels, which may reflect the number of cells. This experiment was performed three times with reproducible similar results

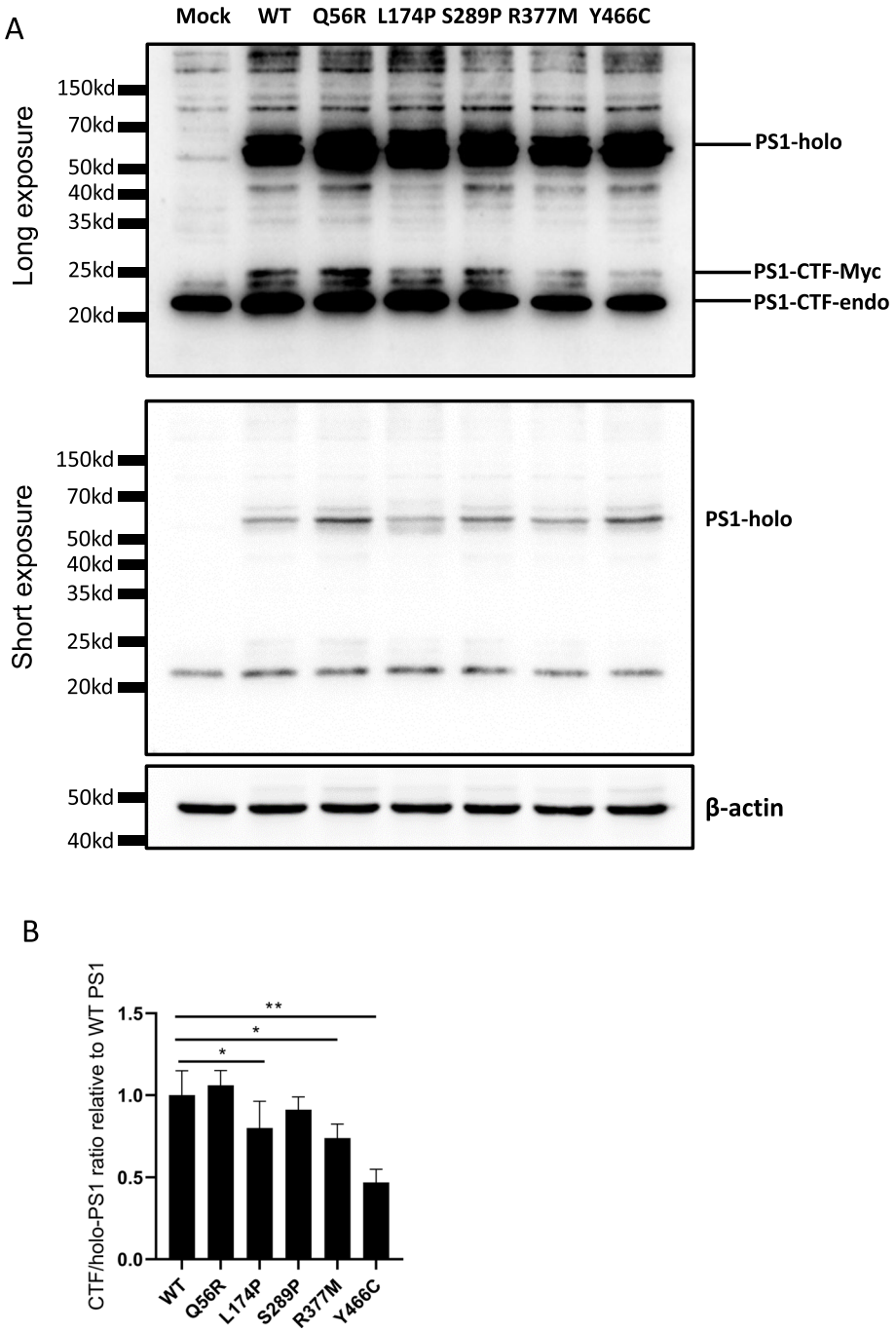

Fig. 5 The PS1 maturation analysis. **A** The PS1 maturation in vitro. Indicated PS1mutants carrying a C-terminal Myc-his tag were expressed in HEK293 cells, and the lysates were blotted for PS1 using a PS1 antibody that recognizes the C terminus of PS1. The endogenous PS1 C-terminal fragment (endo-CTF) and the CTFs derived from the overexpressed PS1 carrying a C-terminally fused Myc-His tag (Myc-CTF) were seen. The Myc-CTFs were detected after relatively long exposure. **B** The protein bands were quantified using Quantity One (Bio-Rad), and the ratios of CTF/holo-PS1 were plotted. *: $p < 0.05$, **: $p < 0.01$

frequency of the three genes identified in our cohort may be attributable to the high proportion of sporadic cases.

We also confirmed that the *ApoE ε4* allele is a risk factor for EOAD in Chinese patients and the frequencies of *ApoE ε2, ε3,* and *ε4* in both EOAD patients and older

healthy adult controls were similar to previous studies performed in Chinese populations [47].

PSEN1 p.I249L was formerly reported by our group in a pedigree with two EOAD patients who presented subsequent psychotic symptoms [30]. This time it was identified in another unrelated sporadic patient diagnosed with AD. Interestingly, it was also found to be associated with sporadic ALS [48]. Our team previously reported the *PSEN1* p. P433S variant in a pedigree with homogeneously early age of onset; all of the affected family members displayed significant memory deficits in their 30 s [30]. p. P433S was later reported in another patient with an onset of age of 43.5 years [23]. Additional studies may be required to confirm the association of this variant with a relatively early age of onset. *PSEN1* p.R377M variant was previously reported in a family with early onset age (onset age 38–41) [49] and it was recently reported in a Chinese EOAD patient [22]. However, neither of these studies had performed functional analysis for this variant. The clinical features of the p.R377M carrier in our cohort were similar to previous cases, with early-onset short-term memory impairment being the most prominent symptom.

The *PSEN1* p.L174P, p.S289P and p.Y466C variants identified in our cohort were novel. Notably, the *PSEN1* p.L174M and p.L174R variants have been reported in the literature. The *PSEN1* p.L174M was found in a familial AD patient, who manifested early-onset memory disturbances, insomnia, and nocturnal myoclonic jerks [50]. L174M was also reported in a large Cuban family with early-onset memory impairment as the main symptom in all affected patients [51]. The *PSEN1* p.L174R variant was described in two members of a Bavarian family [52]. Leucine at position 174 is highly conserved among species and is identical in presenilin 1 and presenilin 2 proteins, which suggests that Leu174 is important for the functional activity of the protein. We presented a very early onset AD patient with the *PSEN1* p.L174P variant and demonstrated in vitro that the L174P mutant significantly increased the Aβ42 level and the Aβ42: Aβ40 ratio.

The p.S289P and p.Y466C variants in *PSEN1*, where the amino acids of both substitutions were highly conserved, were absent in gnomAD or controls and were predicted to be deleterious. The Y466C substitution occurs at the penultimate amino acid in the C-terminal region of the PS1 protein, potentially impacting its stability. In our functional analysis, both mutants increased Aβ42 levels and Aβ42/Aβ40 ratios. Notably, the Y466C mutant decreased the amount of CTF and suppressed PS1 maturation to the highest extent compared to other mutants.

Although the variant *PSEN1* p.Q56R is the only variant found in patient 1, there is no additional evidence to confirm its pathogenicity. This patient may be a sporadic AD case without a known genetic cause, given the absence of family history. Consistently, this mutant did not affect Aβ generation. However, neither can we rule out the possibility that this variant is AD-pathogenic. Despite its limited effect on APP processing, PS1 mutant might contribute to AD through other mechanisms independent of Aβ generation, such as interference with autophagy-lysosomal functions [53]. Not all AD-associated *PSEN1* variants contribute to AD by upregulating Aβ or the Aβ42/Aβ40 ratio [24].

The causative variants p.V214L and p.M239T of *PSEN2* had been previously confirmed in Asian patients, but they never have been reported in Caucasians before. To date, seven cases with *PSEN2* p.V214L variant have been found, and all of them were Asian [5, 25, 54–56]. Including the two cases we present here, all nine cases reported memory impairment as the initial main complaint, but the age at onset (from 33 to 69 years), sex, family history, comorbidities, and neuroimaging displayed heterogeneity. One case had extrapyramidal symptoms [56], and the other presented migraine, subarachnoid hemorrhage, and patent foramen ovale [54]. However, none of these investigations included a functional analysis of PS2 V214L, and the pathogenesis of this variant was therefore unknown. This variant was considered as VUS in a study due to incomplete disease penetrance in a pedigree and its allele frequency (gnomAD: 0.000151, ExAC East Asian: 0.002543) [55]. Moreover, a recent systematic screen conducted in HEK293 PSEN1/2 dKO cells transduced with a lentivirus expressing human APP-695, reported that the *PSEN2* p.V214L variant has no effect on Aβ levels [57]. In our study, we co-transfected PSEN2 plasmids and APP695Swe plasmids into HEK293 cells, establishing a cell line that expressed endogenous PSEN proteins alongside overexpressed PSEN2 mutant proteins and APP695Swe. The presence of endogenous PS1 and PS2 may have influenced the impact of the PS2 mutant on the Aβ42/40 ratio. We report for the first time that the PS2 V214L mutant increases the Aβ42 level and the Aβ42/Aβ40 ratio, suggesting that *PSEN2* p.V214L may either modify the risk for AD or represent a pathogenic AD variant with potentially incomplete penetrance.

PSEN2 p.M239T was identified in a 48-year-old female with memory loss and a deficit in visuospatial and executive domains [26]. The patient we reported also showed early-onset progressive visual disturbance. Further studies may be needed to confirm the genotype–phenotype correlation between severe deficit in the visuospatial domain and the *PSEN2* p.M239T variant. In our study, functional analysis demonstrated that the M239T mutant increased the Aβ42:Aβ40 ratio in vitro, confirming its pathogenicity.

In our functional analysis, Both APP D197N and APP D252V mutants showed no significant effect on Aβ40 or Aβ42 production. To date, all functionally confirmed variants in the *APP* gene are located in exons 16 and 17, which occur either within the Aβ-coding region or immediately proximal [58]. However, *APP* p.D197N and p.D252V are two rare variants located in a highly conserved region. Both are absent in gnomAD or controls and are predicted to be damaging by in silico algorithms. Recently, it was discovered that APP Ser198Pro amino acid substitution, which is adjacent to APP D197, increased Aβ production in cultured cells and a transgenic mouse model of amyloidosis [59]. Ser198Pro was thus considered to be a partially penetrant AD-linked variant in *APP* present outside of exons 16 and 17. In addition to Aβ shedding, which is believed to contribute to AD, APP and its N-terminal fragment generated by cleavage by the α-secretases could also have neuroprotective properties [60, 61]. These mutants might influence these functions of APP, given their location within the N-terminal region of the APP protein. To ascertain the pathogenicity of the *APP* p.D197N and p.D252V variants, more comprehensive functional analyses may be warranted.

This study has some limitations. First, although the patients in our cohort were consecutively recruited at the outpatient department, the epidemiology of EOAD in this region may not be accurately reflected due to the limited sample size. Second, the *PSEN1* p.Q56R, *APP* p.D197N, and *APP* p.D252V variants did not show pathogenicity in our functional confirmation study. Nevertheless, these variants cannot be necessarily determined as non-pathogenic based on these results alone. Further family screening and functional analyses in vivo may be necessary to confirm their penetrance and pathogenicity. Third, the rare variants identified in *SORL1*, *TREM2*, and *ABCA7* require further investigation in future studies.

Conclusions

In this study, we found 15 *PSEN1*, 5 *PSEN2*, and 4 *APP* rare variants in a Chinese cohort comprised of 304 EOAD patients, including 8 novel variants. We performed a functional analysis for the variants *PSEN1* p.L174P, *PSEN1* p.S289P, *PSEN1* p.R377M, *PSEN1* p.Y466C, *PSEN2* p.V214L, and *PSEN2* p.M239T, for which no functional analysis has yet been performed, and suggested that they may be pathogenic for AD. Our results highlight the prevalence and pathogenic significance of *APP* /*PSENs* variants in a Chinese EOAD cohort and expand the phenotypic and genotypic spectrum of EOAD.

Abbreviations

AD	Alzheimer's disease
EOAD	Early-onset Alzheimer's disease
APP	Amyloid protein precursor protein
Aβ	Amyloid β
PS1	Presenilin-1
PS2	Presenilin-2
ALS	Amyotrophic lateral sclerosis
^{18}F-FDG PET	^{18}F-fluorodeoxyglucose positron emission tomography
AV45 PET	^{18}F-florbetapir positron emission tomography
MRI	Magnetic resonance imaging
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
CDR	Clinical Dementia Rating
PCR	Polymerase chain reaction
WES	Whole-exome sequencing
OMIM	Online Mendelian Inheritance in Man
SNV	Single-nucleotide variant
InDels	Insertion-deletions
ACMG	American College of Medical Genetics and Genomics
WT	Wild-type
APPSwe	APP Swedish KM-NL variant
ECL	Enhanced chemiluminescent
VUS	Variants of uncertain significance
TM	Transmembrane
CSF	Cerebrospinal fluid
CTF	C-terminal fragment

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-025-01702-0.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5

Acknowledgements
The authors appreciate all cohort individuals and their families for their participation in this study.

Authors' contributions
HTN, ZW, and LYW designed and conceptualized the study. MC and DMJ provided the patients of the study. HTN performed the functional study. WPL, YL, and YMW analyzed and interpreted the data. HTN, ZW, and LYW drafted and revised the manuscript. The authors have read and approved the final manuscript.

Funding
This work was sponsored by Beijing Nova Program and grants from National Natural Science Foundation of China [no.82201573].

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committees of the Xuanwu Hospital of Capital Medical University (approval number: 2020026), and it was carried out in compliance with the Declaration of Helsinki's principles. Written informed consent was obtained from each patient or their guardian.

Consent for publication
Written informed consent for publication was obtained from the guardian of each patient.

Competing interests
The authors declare no competing interests.

Received: 13 September 2024 Accepted: 19 February 2025
Published online: 27 February 2025

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