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Brief communication

Identification of PSEN1 mutations p.M233L and p.R352C in Han Chinese families with early-onset familial Alzheimer's disease

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ABSTRACT

Early-onset familial Alzheimer's disease (EOFAD) is characterized by the onset of dementia symptoms before 65 years, positive family history, high genetic predisposition, and an autosomal dominant inheritance. We aimed to investigate mutations and to characterize phenotypes in Chinese EOFAD families. Detailed clinical assessments and genetic screening for mutations in the presenilin 1 (PSEN1), presenilin 2, amyloid precursor protein, and APOE genes were carried out in 4 EOFAD families. Two PSEN1 mutations (p.R352C and p.M233L) were identified in 2 EOFAD families, respectively. Mutation p.M233L was associated with prominent very early onset, rapidly progressive dementia, and neurologic symptoms, whereas p.R352C was associated with a progressive dementia, psychiatric syndrome, and chronic disease course. Both mutations are predicted to be pathogenic. Our results showed that mutations in PSEN1 gene might be common in Chinese EOFAD families.

1. Introduction

Early-onset familial Alzheimer's disease (EOFAD) is characterized by the onset of progressive dementia symptoms before 65 years, positive family history, and more aggressive course than late-onset sporadic AD. To date, more than 230 mutations have been identified in the amyloid precursor protein (*APP*), the presenilin 1 (*PSEN1*), and the presenilin 2 (*PSEN2*) genes (Bettens et al., 2010; Wu et al., 2012).

Hitherto, there are a few reports about the *PSEN1*, *PSEN2*, and *APP* gene mutations in Han Chinese families (Jiao et al., 2014; Niu et al., 2014; Peng et al., 2014). Further mutation profiling is needed. In this study, we screened mutations of the 3 AD causal genes in 4 Han Chinese EOAD families. Two *PSEN1* mutations (p.M233L and p.R352C) were identified in 2 of the 4 EOAD families. According to searches with available genetic database, p.R352C is a previously unidentified *PSEN1* mutation, and p.M233L has been reported in European patients. The 2 mutations are associated with cognitive impairment and quite different clinical spectrum.

2. Methods

This study enrolled 4 Han Chinese EOFAD families. Five patients with progressive memory loss and 7 individuals without obvious cognitive dysfunction disorder from these 4 families were clinically evaluated by Mini-Mental State Examination and Montreal Cognitive Assessment (MoCA). All patients and unaffected individuals were recruited from the outpatient psychiatry department of the First Affiliated Hospital of Kunming Medical University, Yunnan Province. Magnetic resonance image scan and

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blood testing were performed to exclude other causes of dementia. The study was approved by the Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Sciences. Written informed consents were obtained from all patients or their guardians.

Pedigrees of the 4 Chinese families with EOFAD are listed in Fig. 1 and Supplementary Fig. 1. The detailed clinical features and sequencing methods are listed in the Supplementary Materials. The primer pairs and polymerase chain reaction conditions are listed in Supplementary Table 1. The APOE status was investigated following the detailed methods described in our previous study (Bi et al., 2014).

3. Results

We evaluate the clinical phenotype and the clinical assessments score for partial individuals of the 4 families (Table 1). Brain magnetic resonance image of the probands from the 4 families showed generalized-global cerebral atrophy (Supplementary Fig. 2).

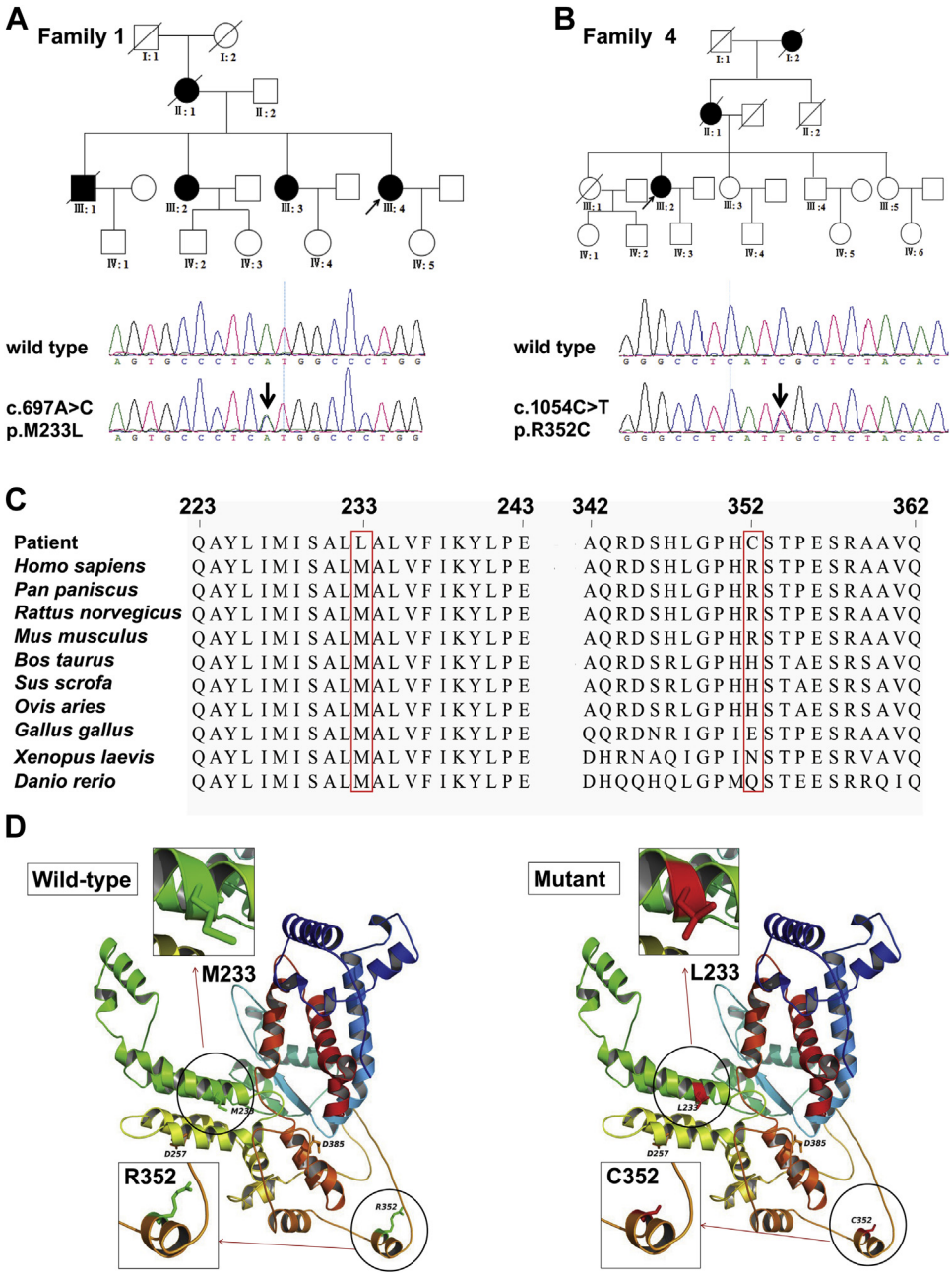

Fig. 1. Pedigrees of EOFAD families with *PSEN1* mutations, sequencing chromatogram, evolutionary conservation analysis, and homology modeling of the PSEN1 protein with and without p.M233L and p.R352C. (A) Family 1 with p.M233L (c.697A>C) mutation. (B) Family 4 with p.R352C (c.1054C>T) mutation. Probands are marked by arrow, black symbols denote affected members, white symbols denote unaffected members, square denotes man, and circle denotes women. (C) Protein sequences of *Homo sapiens* (NP_000012), *Pan paniscus* (XP_003824183), *Mus musculus* (NP_032969), *Rattus norvegicus* (NP_062036), *Bos taurus* (NP_777146), *Ovis aries* (XP_004010819), *Sus scrofa* (NP_001072135), *Gallus gallus* (NP_989494), *Xenopus laevis* (NP_001084023), and *Danio rerio* (NP_571099) were retrieved from GenBank. (D) Secondary structural elements were colored from blue (N-terminus) to red (C-terminus). The residues in codons 233 and 352 were highlighted. The wild-type and mutant residues were colored with green and red, respectively. The active sites D257 and D385 were colored with orange. Mutations p.M233L and p.R352C change the side chain of residues in the positions 233 and 352. Abbreviation: EOFAD, early-onset familial Alzheimer's disease.

Table 1
Clinical characteristics and mutation status of the 4 Chinese families with EOFAD

Sample	Gender	Age (y)	Mutation	APOE genotype	Phenotype and clinical tests score					
					AAO (y)	MMSE	MOCA	Epilepsy	Paralysis	Psychiatric symptom
Family 1										
II: 1	Female	49[a]	NA	NA	44	NA	NA	Yes	Yes	Yes
II: 2	Male	76	ND	ε2/ε3	—	29/30	29/30	—	—	—
III: 1	Male	46[a]	NA	NA	44	NA	NA	Yes	Yes	Yes
III: 2	Female	46	NA	NA	38	NA	NA	Yes	Yes	NA
III: 3	Female	44	PSEN1 (p.M233L)	ε3/ε3	40	1/30	NA	Yes	No	Yes
III: 4	Female	39	PSEN1 (p.M233L)	ε3/ε3	37	19/30	11/30	No	No	No
IV: 4	Female	11	ND	ε3/ε4	—	30/30	30/30	—	—	—
IV: 5	Female	12	PSEN1 (p.M233L)	ε3/ε3	—	30/30	25/30	—	—	—
Family 2										
III:2	Male	58	ND	ε3/ε4	56	0/30	NA	No	No	No
Family 3										
III:1	Female	70	ND	ε4/ε4	60	0/30	NA	No	No	No
IV: 2	Female	45	ND	ε3/ε4	—	30/30	30/30	—	—	—
IV: 6	Male	39	ND	ε3/ε4	—	30/30	30/30	—	—	—
Family 4										
I: 1	Female	74[a]	NA	NA	62	NA	NA	No	No	Yes
II: 1	Female	82[a]	NA	NA	60	NA	NA	No	No	Yes
III: 2	Female	62	PSEN2 (p.R352C)	ε3/ε4	56	4/30	NA	No	No	Yes
III: 3	Female	56	PSEN2 (p.R352C)	ε3/ε3	—	30/30	23/30	—	—	—
IV: 1	Male	35	ND	ε3/ε4	—	30/30	30/30	—	—	—

Key: AAO, age at onset; EOFAD, early-onset familial Alzheimer's disease; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; NA, not available; ND, not detected.
[a] Age at death.

Two missense mutation of *PSEN1* (p.R352C and p.M233L) and a homozygous *APOE* ε4 were respectively identified in 3 of the 4 EOAD families. No mutation of *PSEN2* and *APP* was detected in these families (Table 1 and Fig. 1). Mutation p.M223L in exon 7 maps to the fifth transmembrane domain of PSEN1, on the α-helix surface; mutation p.R352C in exon 10 is located in the cytoplasmic loop of PSEN1 (Fig. 1). An evolutionary conservation analysis revealed that mutation p.M223L led to a highly conserved amino acid change. Mutation p.R352C changed a relative conserved arginine to cysteine. These mutations changed the side chain of residues in the codons 233 and 352 (Fig. 1). For mutation p.M233L and p.R352C, the results of SIFT, Polyphen2, and Mutation Taster predicted a probable-damaging effect and disease-causing effect, respectively.

4. Discussion

PSEN1 is one of the most common and important causative gene of EOFAD, with more than 185 mutations being reported. Mutation p.M233L (c.697A>C, dbSNP ID: rs63751287) of *PSEN1* was previously described in patients with AD (Aldudo et al., 1999; Rogaeva et al., 2001), whereas p.R352C was identified for the first time. Mutation p.M233L was associated with prominent very early onset, rapidly progressive dementia, and neurologic symptoms, whereas p.R352C was associated with a progressive dementia, psychiatric syndrome, and chronic disease course. Our evolutionary conservation analysis and correlation of phenotype-genotype in the pedigrees indicated that these 2 missense mutations might be the cause of the EOFAD in family 1 and family 4. Note that we also observed individuals (IV: 5 of family 1 and III: 3 of family 4) carrying p.M233L and p.R352C have no problems in daily life and their Mini-Mental State Examination score were 30/30, whereas their MoCA score were lower than 26. They were all failed in the clock drawing test and had mild short-term memory loss. Apparently, MoCA may be a sensitive neuropsychological assessment tool to test the cognitive impairment of autosomal dominant EOFAD in their early stage.

The codon 233 of PSEN1 seems to be a hot spot for mutation, as 3 other mutations at codon 233 (p.M233V, p.M233I, and p.M233T) have been reported (Campion et al., 1999; Guerreiro et al., 2010; Houlden et al., 2001; Park et al., 2008; Portet et al., 2003; Raux et al., 2005). All these mutations were described as pathogenic mutations according to the AD and FTDMDB database (Cruts et al., 2012).

Based on these reports and our study, codon 233 mutation carriers have an average age at onset of 37 years (range: 28–45 years) with progressive memory loss and aggressive disease courses. Furthermore, the 233rd codon is highly conserved. Taken together, the 233rd codon is a very important functional amino acid for PSEN1.

The p.R352C (c.1054C>T) is a previously unidentified *PSEN1* mutation. An arginine insertion at codon 352 (insR352) in the *PSEN1* gene was previously identified in a frontotemporal dementia patient with a family history (Tang-Wai et al., 2002). The age at onset of this family was about 60 years, similar to our p.R352C patients. A subsequent research of the insR352 revealed that this mutation did not increase absolute Aβ42 levels, but instead acted as dominant negative presenilin, decreasing Aβ production by inhibiting γ-secretase cleavage of APP (Amtul et al., 2002). The pathogenic mechanism of mutation p.R352C is unknown and needs further investigation.

5. Conclusion

In short, we reported 4 Chinese EOFAD families and detected 2 missense *PSEN1* mutations (p.R352C and p.M233L). The 2 mutations are predicted to be pathogenic. The phenotype of the 2 EOFAD carrying mutation is variable. Future studies are needed to explore the pathogenesis of p.R352C and p.M233L mutations in *PSEN1*, which may illuminate the underpinnings of Alzheimer's disease.

Disclosure statement

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2014.11.009.

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Supplementary Contents

1. Clinical features of the four Chinese families with EOFAD

In Family 1, family history of memory loss was definite and prominent: five affected individuals including four siblings and their mother in this family all affected dementia. Age at onset (AAO) of this family ranged from 37- 44 years old. The proband (III: 4) is a 38 year-old woman who had memory loss for 1 year. Mild memory loss symptom and the decreased work ability was her first symptom. Several neuropsychological assessments further confirmed the defect in cognitive ability: Mini-Mental Status Exam (MMSE) score was 19/30; the score of MoCA was 10/30. Further, this patient showed impairment in multiple impairments in cognitive domains dysfunction, including attention, visual consciousness, visual space structure, executive function and short-term working memory. The proband's mother (II: 1) who suffered obvious progressive cognitive decline at the age of 44 and died at age 49. No dementia was detected in her husband (II: 2), the MMSE score was 29/30 at the age of 76. The proband's brother (III: 1) and two sisters (III: 2, III: 3) were all diagnosed as dementia. The AAO of III: 1 was 44 and he died at age 46 after a disease course of 2 years with rapidly progressing dementia. Subject III: 2 is 46 year-old and AAO was 38. Subject III: 3 is 44 year-old and AAO was 40. From age 42, this patient developed severe dementia, with 1/30 MMSE score. Epilepsy seizure presented frequently. Abnormal behavior, psychiatric symptom and urinary and stool incontinence also affected at age 42. She was reevaluated at age 44, apraxia, aphasia, extrapyramidal signs and ataxia affected.

In Family 2, the proband (III: 2) is a 58 year-old man who had memory loss for 2 years. Five members of Family 2 experienced dementia in their 50s.

The proband (III: 1) of Family 3 is a 70 year-old woman who has suffered memory loss for 10 years. Three members of Family 3 had progressive memory loss.

The proband (III: 2) of Family 4 is a 62 year-old woman who had memory loss for 6 years. At age of 62, she had severe dementia (the MMSE score was 4/30) with serious psychiatric symptom: serious automatic speaking; laughing; behavioral disorders. Her mother and grandmother all experienced memory loss and serious psychiatric symptoms. The AAO of Family 4 patients is in their 56-62 years old and the disease course was about 12-22 years.

2. DNA amplification and sequencing methods:

Blood samples for genetic analysis were obtained from Family 1: II: 2, III: 3, III: 4, IV: 4, IV: 5; Family 2: III: 2; Family 3: III: 1, IV: 2, IV: 6; Family 4: III: 2, III: 3, IV: 3 (Figure 1 and Figure S1). Genomic DNA was extracted from peripheral blood by using the AxyPrepTM Blood Genomic DNA Miniprep Kit (Axygen, USA). We amplified and sequenced the entire exons (except exon 1 of the *PSEN2* and *APP* genes) of common EOFAD causative genes: *PSEN1*, *PSEN2* and *APP*. PCR amplification was performed in a volume of 20μL reaction mixture containing 50–100 ng of DNA, 1mM dNTP, 1 μM of each forward and reverse primer, 1× LA Taq™ PCR buffer, 1 unit of LA Taq polymerase (TaKaRa, Japan). The primer pairs and PCR

conditions are listed in Table S1. PCR products were purified and directly sequenced using PCR primers and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) on a 3730XLDNAsequencer (Applied Biosystems) at the Kunming Biodiversity Large-Apparatus Regional Center, Kunming Institute of Zoology. The DNA sequences were analyzed using the DNASTAR SeqMan software (DNASTAR Inc, Madison, WI, USA).

Table S1. Primers for amplification of exons of *PSEN2*, *PSEN1* and *APP* genes

Primers [a]	Sequence(5'-3')	Region	Annealing temperature (℃)	Product size (bp)
***PSEN1* gene**				
PSEN1-1F	GGACAAAGAGCAGGCAGATG	Exon 01	58	858
PSEN1-1R	TCCTGGTTTCCCACACGG			
PSEN1-2F	TGTGGTTGAAAATGTTTGGTGTC	Exons-02&03[b]	58	749
PSEN1-3R	AAGGCTTCAACTGAGGTGGTG			
PSEN1-4F	GTAAAAGAGAGGACCTGAATGCC	Exon 04	62-58[c]	969
PSEN1-4R	CCTCCCTTGTCCAAATGTGC			
PSEN1-5F	CTGTGTTGGAGGTGGTAATGTGG	Exon 05	58	264
PSEN1-5R	ATAAGAAGAACAGGGTGGAAAGC			
PSEN1-6F	AACAAGAGCGAAACTCCGTC	Exon 06	58	1111
PSEN1-6R	CTGCCTCTTCGAATTTAAGAGCT			
PSEN1-7F	GGCCTGTGCAACTGGTTTCC	Exon 07	58	998
PSEN1-7R	CCCAGCCGAAATCTTCAAATG			
PSEN1-8F	CCACCAGTTCACCTGCCATT	Exon 08	58	607
PSEN1-8R	TCCCCAGTGGTCACCGAATA			
PSEN1-9F	CAGCATTAGGAAGACTGGCGAT	Exon 09	58	856
PSEN1-9R	TAAGTTGCCCAGGGTAATCCG			
PSEN1-10F	CCATAAAGACATTCACTCCCCG	Exon 10	58	1196
PSEN1-10R	TTGCACTCCAACCTGGCGAC			
PSEN1-10S	GAAATAAAGGAGAAAATAGC			
PSEN1-11F	ACCTGACTGGGGCAATGGAC	Exon 11	58	1237
PSEN1-11R	TAGGCTTGAGGCAGCGGTTC			
PSEN1-12F	AGCCTCATCATGCTTCACGG	Exon 12	58	1206
PSEN1-12R	ATTCTGCTGCTGGTGCCTGC			
PSEN1-12S	CTTCCAGATTGAATGAACGTC			
***PSEN2* gene**				
PSEN2-2F	GGGCGTTTTGTTCTTCTT	Exon 02	58	406
PSEN2-2R	CGGAGGGATGGACAGCAGAT			
PSEN2-3F	AGGAAGGTGAGCAGGGAAGC	Exon 03	58	922

PSEN2-3R	GGAAAAATCACCACCCAAACG			
PSEN2-4F	TGTCCAAGTCTCCAGGTCGC	Exons 04&05[b]	62-58[c]	2349
PSEN2-5R	AAGGAAGCTGAGGCATAGGG			
PSEN2-5S	CATTCAAACTTCTCATTTCTGG			
PSEN2-6F	AGCATCAGCCCTTTGCCTTC	Exon 06	58	256
PSEN2-6R	TAAAGGCGGCTGTTTCACGG			
PSEN2-7F	ACTCCTTGGACTTCTGTGCCC	Exons 07&08[b]	58	1299
PSEN2-8R	CCTCTGTTTTACAAAGGCGACT			
PSEN2-9F	TGTAAAACAGAGGGGGGTCCAC	Exon 09	58	1188
PSEN2-9R	ATGCCGCTGGAGGATGGAC			
PSEN2-10F	AAAGCACATTCCAGGCGCAT	Exons 10&11[b]	58	1207
PSEN2-11R	CCGTGTCTCCTTAGCCTGTGG			
PSEN2-12F	AACTGCCCGCTTTTCTCTGC	Exon 12	62-58[c]	538
PSEN2-12R	CCTCCTGTGAGCCTTGGTCT			
PSEN2-13F	GCCTGCCTTCTGGTTCACTC	Exon 13	58	1140
PSEN2-13R	CAGCATCCACAGCCTTACAGC			
***APP* gene**				
APP-2F	GAATGCGGTAGCCTCCACAG	Exon 02	58	991
APP-2R	AGCCCAGGAGTTTCAGACAGC			
APP-3F	CTTGAAAGCACTTCTGGTCCC	Exon 03	58	382
APP-3R	AGTGGCAATGTGCTGAAGAAC			
APP-4F	TTCCTTGATGTCTTCTGCGG	Exon 04	58	277
APP-4R	GCTGTTGCCTCAAAATACCC			
APP-5F	TTAGTTTCCATTCCAGTTGTTCG	Exon 05	58	872
APP-5R	GCCCAAAATCTCAAACCAAAC			
APP-6F	ATGGCATCCCCTTTTAGCAAC	Exon 06	58	701
APP-6R	AGATGACTACCTGGTTGGGCG			
APP-7F	TGCAGATACCTTCCGTCATTTC	Exon 07	62-58[c]	537
APP-7R	AAAGCAGAGTCAGTGGCGAGAG			
APP-8F	AGCAAACAGCCAGAGCCGAG	Exon 08	58	487
APP-8R	GAACCAAGCAGCATCCTCCTC			
APP-9F	TCTGAGAAATAACTGAAAATACGGC	Exon 09	58	303
APP-9R	AGACTGAGGCAGAGGCAAGC			
APP-10F	GTGTGCCTTCCATGAATAGTTG	Exons 10&11[b]	58	1428
APP-11R	ATGGAATGGACAGGGGTTGAA			
APP-12F	AGTGGAACCTCTAACCCATCGC	Exons 12&13[b]	58	1378
APP-13R	CAGTTTCATCGTAAAAGGCAAGC			
APP-14F	TAACTGTGTCAGGAGGAAATGGC	Exon 14	62-58[c]	600

APP-14R	ATACCTCCCAGAACGCCCTTG			
APP-15F	CTGTGGTTCTTTTCTGGCTGC	Exon 15	58	447
APP-15R	TTGGAAGGGCTCTCTTTTAGG			
APP-16F	CAATAAGCCCGTAAGCCAAGC	Exon 16	58	809
APP-16R	CAAGTCTCCTGCGTCAGCCTC			
APP-17F	GCTATCTTCCCACCACTCACC	Exon 17	58	853
APP-17R	GTACTGGTTTTTGTTGCTTGTG			
APP-18F	CACTGGACACGTTGCAAGCG	Exon 18	58	1704
APP-18R	CTTGAGGGCCAAAATATGGC			
APP-18S	CTGTAACACAAGTAGATGCC			

[a] F-Forward; R-Reverse; S-Sequencing primer.

[b] Two neighboring exons were amplified together by one pair of primers.

[C] Amplification reaction was performed with a first amplification cycle at a higher (62 ℃) and the remaining cycles at a lower (58 ℃) annealing temperature.

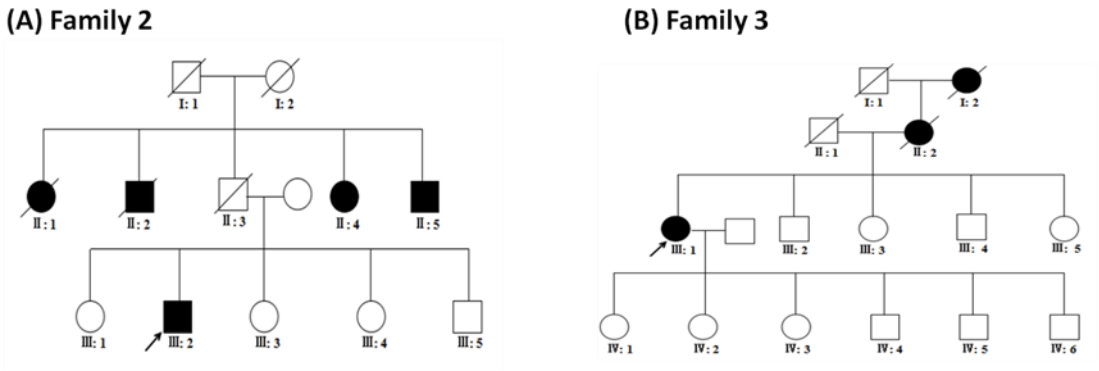

Figure S1. Pedigrees of Family 2 and Family 3.

Probands are marked by arrow, black symbols denote affected members, white symbols denote unaffected members, square denotes man, circle denotes women.

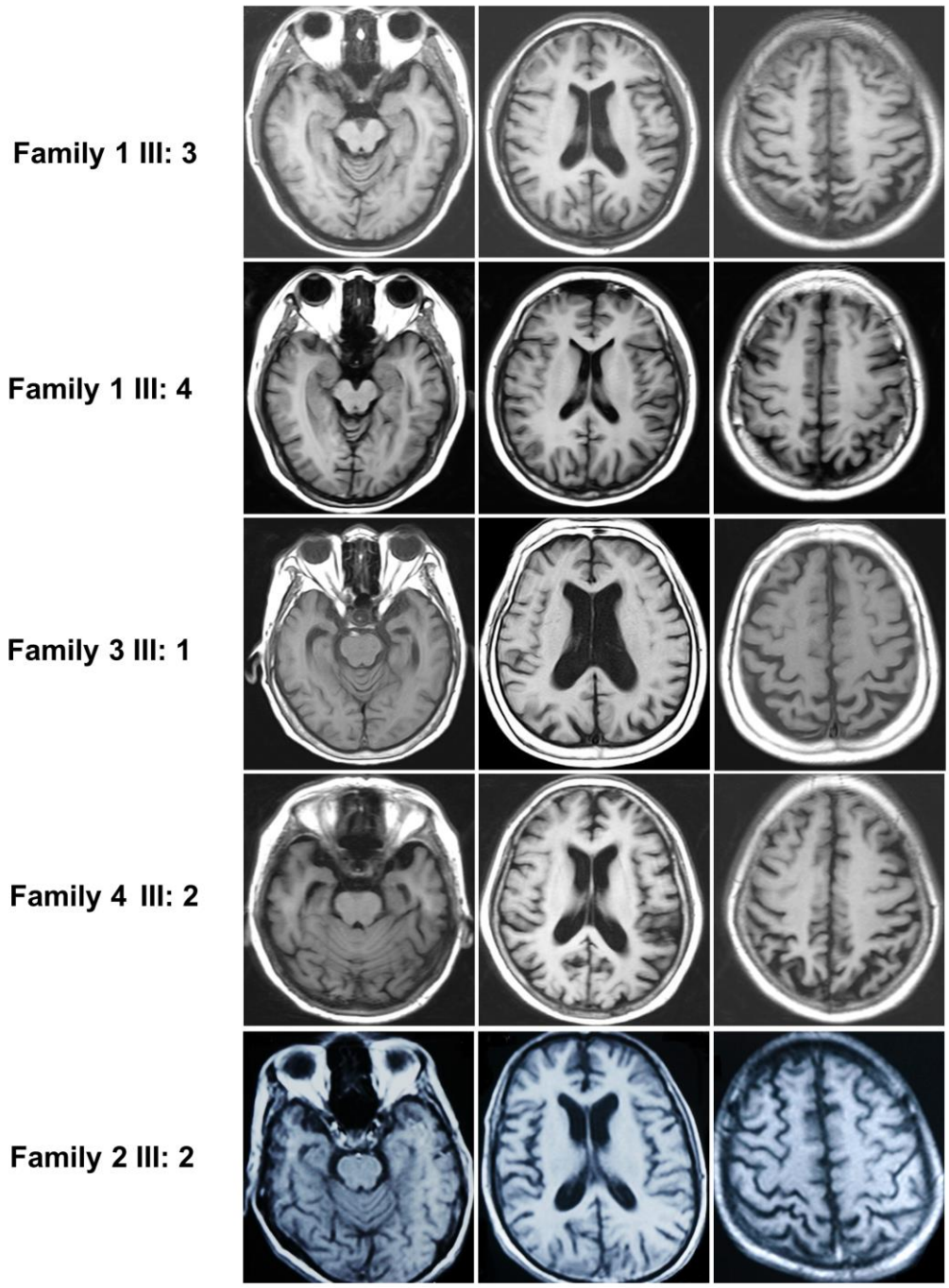

Figure S2. Axial T1-weighted brain MRI of the probands from the four EOFAD Families.

Brain MRI of the probands of Family 1 (III: 3), Family2 (III: 2), Family 3 (III: 1) and Family 4 (III: 2) showed generalized global cerebral atrophy, especially in the frontotemporal regions, hippocampal areas and lateral ventricle dilation. The atrophy of III: 4 of Family 1 was not obvious.