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Mutation screening in Chinese patients with familial Alzheimer's disease by whole-exome sequencing

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ABSTRACT

Familial Alzheimer's disease (FAD) is characterized by a positive family history of dementia and typically occurs at an early age with an autosomal dominant pattern of inheritance. Amyloid precursor protein (*APP*), presenilin1 (*PSEN1*), and presenilin2 (*PSEN2*) are the major causative genes of FAD. The spectrum of mutations in patients with FAD has been investigated extensively in the Caucasian population but rarely in the Chinese population. Here, we performed whole-exome sequencing in a total of 15 unrelated Chinese patients with FAD. Among them, 12 were found to carry missense variants in *APP, PSEN1,* and *PSEN2*. Two novel variants (*APP*: p.D244G, p.K687Q), 3 variants not previously associated with FAD (*APP*: p.T297M, p.D332G; *PSEN1*: p.R157S), and 7 previously reported pathogenic variants (*APP*: p.V717I; *PSEN1*: p.M139I, p.T147I, p.L173W, p.F177S, p.R269H; *PSEN2*: p.V139M) were identified. The novel variant *APP* p.K687Q was classified as likely pathogenic, and the other 4 variants (*APP*: p.D244G, p.T297M, p.D332G; *PSEN1*: p.R157S) were classified as uncertain significance. Therefore, *APP, PSEN1,* and *PSEN2* mutations account for 2 (25.0%), 5 (62.5%), and 1 (12.5%) of the genotyped cases positive for mutations, respectively. Furthermore, the genotype–phenotype correlations were described. Our findings broaden the genetic spectrum of FAD with *APP, PSEN1,* and *PSEN2* variants.

1. Introduction

Alzheimer's disease (AD), the most common form of neurodegenerative disorder, is characterized by irreversible memory loss and cognitive deterioration. Most cases are sporadic AD and typically have disease onsets after 65 years of age. Cases with an age at onset before 65 years are defined as early onset AD, which accounts for approximately 1%–2% of all cases (Bekris et al., 2010; Efthymiou and Goate, 2017). Patients with familial AD (FAD) have a largely autosomal dominant pattern of inheritance and commonly aggregate in patients with early onset AD (Hardy, 2006). Mutations in 3 genes, including amyloid precursor protein gene (*APP*), presenilin 1 gene (*PSEN1*), and presenilin 2 gene (*PSEN2*), have been identified as the main genetic causes of FAD (Kennedy et al., 2003).

To date, studies have been performed in different populations to screen the mutations in *APP, PSEN1,* and *PSEN2* in patients with FAD (Ikeuchi et al., 2008; Janssen et al., 2003; Jiao et al., 2014; Lanoiselee et al., 2017; Park et al., 2008; Sassi et al., 2014; Zekanowski et al., 2003). The *APP* gene is located on chromosome 21, which contains 18 exons for encoding the APP protein and mutations in *APP* account for a minority of FAD, but it may differ in different populations (Hardy, 2017). *PSEN1* gene is located on chromosome 14 with 12 exons and *PSEN2* gene is located on chromosome 1 with 13 exons. They have similar structures, with a homology of 67% (Cacace et al., 2016). Both are critical in gamma secretase activity. Mutations in *PSEN1* account for 30%–70% of all cases of FAD, and mutations in *PSEN2* have been predominantly reported in European population with relatively rare frequency (Cai et al., 2015; Giri et al., 2017). One study has been performed to screen for these 3 genes in patients with FAD in Mainland China by Sanger sequencing (Jiao et al., 2014). The frequency of *APP* and *PSEN1* mutations in FAD families was 6.1% and 15.2%, respectively. Only 18.75% (6/32) of the

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considered families were found to have the causative mutations, and the genetic causes are still unknown for the remaining 26 families (Jiao et al., 2014), which suggests that other potential genes may be involved in the pathogenesis of FAD. Recently, another study performed targeted next-generation sequencing in 3 genes (*PSEN1*, *PSEN2,* and *APP*) in 61 AD and 35 Chinese patients with FTD (24% has a positive family history) (Shi et al., 2015). The frequency of positive mutations in the genotyped patients with AD was 8.2% (5/61). According to the above 2 studies, the genetic spectrum of FAD in Chinese population seems quite different from that in studies performed in the United Kingdom (21/31, 68%) (Janssen et al., 2003) and France (24/34, 71%) (Campion et al., 1999).

Compared with direct Sanger sequencing and targeted next-generation sequencing, the whole-exome sequencing (WES) is more effective for investigating all causative genes of FAD and detecting the potential causative genes associated with FAD. In previous studies, the WES has been used to examine many inherited neurological disorders, including paroxysmal kinesigenic dyskinesia disease (Chen et al., 2011), cerebellar ataxia disease (Fogel et al., 2014), and primary familial brain calcification disease (Yao et al., 2018). Here, we performed mutation screening in 15 unrelated patients from Chinese FAD families using WES. Furthermore, the genotype−phenotype correlations in patients carrying mutations or possible pathogenic variants with FAD were determined.

2. Materials and methods

2.1. Subjects

Fifteen FAD probands and 7 family members, include the 2 affected members (Family 2 II-2, Family 9 II-2) and 5 unaffected members (Family 2 III-1, Family 3 III-2, Family 6 II-4, Family 8 II-2, Family 10 II-2), were recruited for the study from Second Affiliated Hospital in Zhejiang University School of Medicine from March 2015 to January 2017. They were evaluated by at least 2 senior neurologists and were diagnosed with FAD based on the DSM-IV-TR (American Psychiatric Association, 2000) and NINCDS-ADRDA criteria (McKhann et al., 1984), along with a family history of autosomal dominant inheritance. The onset age of these probands was under 65 years of age (mean age 45.92 $\pm$ 11.09 years; range 30−63). The mini-mental state examination (MMSE) and apolipoprotein E (*APOE*) genotypes were evaluated as described previously (Tao et al., 2017). Five hundred controls with similar ages, genders, and origins to those of the patients from the community epidemiologic investigations were included in this study. Written informed consents were obtained from all participants. This study was approved by the Ethics Committee of Second Affiliated Hospital in Zhejiang University School of Medicine.

2.2. Whole-exome sequencing and bioinformatic analysis

Genomic DNA was extracted from a peripheral ethylenediaminetetraacetic acid-treated blood sample of each patient by using QIAamp blood genomic extraction kits (Qiagen, Hilden, Germany). The prepared samples of probands were captured by the Agilent Sure Select Human All Exon V6 products and sequenced on the Illumina HiSeq X Ten platform (XY Biotechnology Co Ltd, Hangzhou, China). Sequence reads were aligned against the human reference genome (UCSC hg19). The ANNOVAR software was performed to annotate the variants. The single-nucleotide polymorphism database (https://www.ncbi.nlm.nih.gov/snp/), the 1000 Genomes Project (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/), the ExAC database (https://exac.broad.institute.org/), and genome aggregation database (http://gnomad.Broad.institute.org/) were used to check the frequency of the identified variants in the general population. Moreover, subsequent filtering was restricted to the genes linked to FAD. The list for causative genes of FAD, potential genes associated with FAD, and TOP10 risk genes with AD (http://www.alzgene.org/) are shown in Supplementary Table 1. The possible protein functional changes caused by the variants were predicted by 3 software programs, CADD (http://cadd.gs.washington.edu/), SIFT (http://sift.jcvi.org/), and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/).

2.3. Sanger sequencing

All potential variants were verified by Sanger sequencing, which was performed on an ABI 3500xL Dx Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using a procedure described previously (Dong et al., 2016). All the available familial members and the 500 controls were sequenced to confirm the identified mutations. The results were analyzed according to the standard *APP* (NM_000484.3), *PSEN1* (NM_000021.3), and *PSEN2* (NM_000447.2) reference sequence.

3. Results

3.1. Identification of variants

WES was carried out in 15 unrelated patients with FAD. The coverage of the fraction of target bases indicated that 94.17% target bases had >30x coverage. On average, 39,729 variants including single-nucleotide variants and indels were identified in each FAD proband. All the variants detected in the genes described in Supplementary Table 1 were listed in Supplementary Table 2. The filtering criteria include: (1) the frequency of variants in the general population must be less than 1%, (2) synonymous variants were excluded, (3) Variants disrupted gene function via frameshift or generation of a stop codon in the previously reported FAD causative genes were included, (4) Variants predicted to have functional impact by 3 software programs (SIFT, PolyPhen-2, and CADD) were included. After being filtered and confirmed by Sanger sequencing, 12 possible pathogenic variants, including 7 previously reported pathogenic variants (*APP*: p.V717I; *PSEN1*: p.M139I, p.T147I, p.L173W, p.F177S and p.R269H; *PSEN2*: p.V139M), 3 variants not previously associated with FAD (*APP*: p.T297M, p.D332G; *PSEN1*: p.R157S), and 2 novel variants (*APP*: p.D244G, p.K687Q), were identified in 12 FAD probands. None of the variants were detected in the 500 elderly, healthy Chinese subjects. According to the American College of Medical Genetics and Genomics (ACMG) standards (Richards et al., 2015), the novel variant p.K687Q in *APP* was classified as a likely pathogenic variant (3 pieces of moderate pathogenic evidence and 2 pieces of supporting pathogenic evidence), whereas the other 4 variants (*APP*: p.D244G, p.T297M, p.D332G; *PSEN1*: p.R157S) were classified as uncertain significance. Therefore, *APP, PSEN1,* and *PSEN2* mutations account for 2 (25.0%), 5 (62.5%), and 1 (12.5%) of the genotyped cases positive for mutations, respectively. All the potential pathogenic variants are shown in Table 1. No variant in the genes linked to FAD was found in the remaining 3 FAD probands.

3.2. Clinical features of patients carrying APP variants

Five variants in *APP* were identified in 6 probands. Two of them were novel variants. The clinical phenotypes of all the probands with different *APP* variants were summarized in Table 2.

The novel variant p.K687Q (c.2059 A>C) in *APP* was detected in the probands from Family 1 (II-2, Case 1) and Family 2 (II-4, Case 2) (Figs. 1A, B, G, and M). This variant affected the same amino acid as a

Table 1
The identified potential pathogenic variants in the present study

Gene	Case no.	Refseq NM	Nucleotide change	Amino acid change	Variant type	1000G (All*)	ExAC (All*)	gnomAD (All*)	Polyphen2	SIFT	CADD	Control	dbSNP	First report
APP	1, 2	NM_000484.3	c. 2059A>C	#p.K687Q	Heterozygous	0	0	0	D	T	25.3	0/500	-	Present study
APP	3	NM_000484.3	c. 731A>G	#p.D244G	Heterozygous	0	0	4.12E-06	P	T	20.1	0/500	-	Present study
APP	4	NM_000484.3	c. 890C>T	p.T297M	Heterozygous	0	2E-04	2.11E-04	D	D	32	0/500	rs557227002	Present study
APP	5	NM_000484.3	c. 995A>G	p.D332G	Heterozygous	0	1.66E-05	8.13E-06	D	T	20.3	0/500	rs773998162	Present study
APP	7	NM_000484.3	c. 2149G>A	p.V717I	Heterozygous	0	0	0	D	T	34	-	rs63750264	Goate A et al. (1991)
PSEN1	6	NM_000021.3	c. 471G>T	p.R157S	Heterozygous	2E-04	6.59E-05	6.09E-05	D	D	31	0/500	rs201617677	Present study
PSEN1	5	NM_000021.3	c. 417G>A	p.M139I	Heterozygous	0	0	0	D	T	24.9	0/500	rs63750522	Boteva et al. (1996)
PSEN1	8	NM_000021.3	c. 530T>C	p.F177S	Heterozygous	0	0	0	D	D	29.7	0/500	rs63749806	Rogaeva et al. (2001)
PSEN1	9	NM_000021.3	c. 806G>A	p.R269H	Heterozygous	0	8.70E-06	4.07E-06	D	D	34	0/500	rs63750900	Gomez-Isla et al. (1997)
PSEN1	10	NM_000021.3	c. 518T>G	p.L173W	Heterozygous	0	0	0	D	D	25	0/500	rs63750299	Campion et al. (1999)
PSEN1	11	NM_000021.3	c. 440C>T	p.T147I	Heterozygous	0	0	0	D	D	31	0/500	rs63750907	Campion et al. (1999)
PSEN2	12	NM_000447.2	c. 415G>A	p.V139M	Heterozygous	0	0.0001	1.18E-04	P	T	23.2	0/500	rs202178897	Bernardi et al. (2008)

Key: 1000G, 1000 Genomes Project; ExAC, Exome Aggregation Consortium; D (Polyphen2), probably damaging; P (Polyphen2), possibly damaging; T(SIFT), tolerated; D (SIFT), deleterious; #, novel variants; -, not present; *, global population.

Table 2
Clinical data of FAD probands

Case NO.	1	2	3	4	5		6	7	8	9	10	11	12
Gene	*APP*	*APP*	*APP*	*APP*	*APP*	*PSEN1*	*PSEN1*	*APP*	*PSEN1*	*PSEN1*	*PSEN1*	*PSEN1*	*PSEN2*
Nucleotide	c.2059A>C	c.2059A>C	c.731A>G	c.890C>T	c.995A>G	c.417G> A	c.471G>T	c.2149G> A	c.530T>C	c.806G>A	c.518T>G	c.440C>T	c.415G>A
Amino acid	p.K687Q	p.K687Q	p.D244G	p.T297M	p.D332G	p.M139I	p.R157S	p.V717I	p.F177S	p.R269H	p.L173W	p.T147I	p.V139M
Age at study, (y)	65	54	55	54	47		62	55	36	57	32	36	38
Sex	Male	Male	Female	Male	Male		Male	Female	Male	Male	Male	Female	Male
Age at onset, (y)	63	52	54	50	44		60	46	30	51	31	35	35
Inheritance	AD	AD	AD	AD	AD		AD	AD	AD	AD	AD	AD	AD
MMSE	10	24	23	24	17		20	21	NA	3	4	8	23
APOE	ε2/ε3	ε3/ε3	ε2/ε3	ε3/ε3	ε3/ε4		ε3/ε4	ε2/ε3	ε3/ε3	ε3/ε3	ε3/ε3	ε3/ε3	ε3/ε4
Onset symptom	amnesia	amnesia	amnesia	amnesia	amnesia		amnesia	amnesia	amnesia	amnesia	amnesia	amnesia	amnesia

Key: AD, autosomal dominant; MMSE, mini-mental state examination; +, present; -, not present; NA, not available.

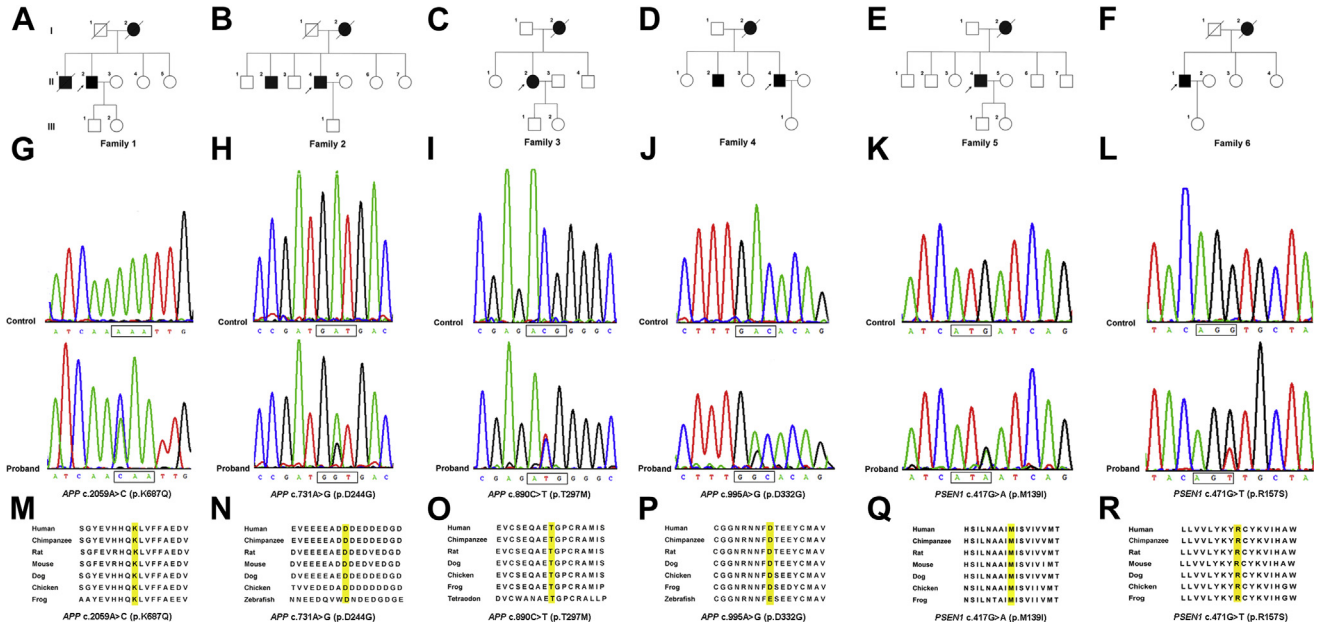

Fig. 1. Pedigrees, novel variants, and variants not previously associated with FAD. (A-F) Pedigrees carrying the novel variants and the variants not previously associated with FAD in *APP* and *PSEN1*. (G-L) Sequencing chromatograms of the *APP* variants (p.K687Q, p.D244G, p.T297M, and p.D322G) and *PSEN1* variants (p.M139I, p.R157S). (M-R) Conservation analysis of the *APP* variants (p.K687Q, p.D244G, p.T297M and p.D322G) and *PSEN1* variants (p.M139I, p.R157S).

previously reported pathogenic mutation (c.2061 A>T, p.K687N) (Kaden et al., 2012). The clinical phenotypes of the patients carrying this variant in our study were typical amnestic syndromes with no other atypical phenotypes. The proband in Family 1 (II-2, Case 1) is a 65-year-old male who developed progressive memory impairment at the age of 63 years. He presented with severe cognitive deficits, with a decline in short-term memory, visuospatial tasks, and calculation abilities and an MMSE score of 10/30. The MRI analysis revealed mild global brain atrophy without vascular lesions. His mother (Family 1, I-2) and elder brother (Family 1, II-1) had similar clinical presentations. However, both of them died and were unavailable for genetic evaluation. The proband in Family 2 (II-4, Case 2) was presented with a slow, insidious progressive decline in immediate memory as the first symptom at age 52 years. His elder brother (Family 2, II-2) was clinically diagnosed as probable AD with the same mutation. He displayed progressive decline of memory at the age of 54 years. The proband and his elder brother presented with similar clinical characteristics and both were without noncognitive neurological symptoms. This variant completely segregated with the disease phenotype within Family 2.

Another novel variant p.D244G (c.731 A>G) in *APP* was identified in a 55-year-old female who presented progressive memory deficits at the age of 54 years (Family 3, II-2, Case 3) (Figs. 1C, H, and N). Her MMSE score was 23/30, and her *APOE* genotype was ε2/ε3. Similar clinical characteristics were reported for her mother who died unfortunately (Family 3, I-2). No family members were available for genetic evaluation.

The *APP* variant p.T297M (c.890 C>T) was detected in a 54-year-old male (Family 4, II-4, Case 4) (Figs. 1D, I, and O). His initial symptom was a progressive decline of learning abilities and concentration deficit at age 50 years. Over the next 4 years, these symptoms worsened progressively and he became mildly depressed. He had no remarkable past medical history. His mother (Family 4, I-2) and elder brother (Family 4, II-2) had similar age of onset and clinical performances. However, neither was accessible for genetic evaluation.

The *APP* variant p.D332G (c.995 A>G) was identified in a 47-year-old male (Family 5, II-4, Case 5) (Figs. 1E, J, and P). He displayed cognitive deficits of various modalities, including short-term memory impairment, decline of visual construction abilities, deficits of calculation abilities, and mild personality change. His MMSE score was 17/30. Brian MRI indicated atrophy of the bilateral temporal lobe, parietal lobe, and hippocampus without vascular lesions. His mother (Family 5 I-2) presented with similar symptoms but lack of other clinical information.

The recognized pathogenic *APP* variant p.V717I (c.2149 G>A) has been reported in the Chinese population previously (Jiao et al., 2014). Here, we identified this mutation in the proband of Family 7 (II-2, Case 7) (Supplementary Figs. 1A and G) who reported gradual memory deficits starting at the age of 46 years. The phenotypes of patients carrying this mutation have been reported previously with heterogeneous clinical manifestations, including dementia, language impairment, neuropsychiatric symptoms, cerebellar ataxia, spastic paraparesis, and schizophrenic-like syndromes (Zhang et al., 2017). In our study, the proband demonstrated memory deficits, occasional seizures, and swallowing problems. Over the next 7 years, the distresses worsened progressively and severely affecting her quality of life. She was bedridden at approximately 54 years of age. Myoclonus, spastic paraparesis, cerebellar ataxia, and extrapyramidal signs were not observed. Her MMSE score was 21/30 and her *APOE* genotype was ε2/ε3. A similar phenotype was also reported for her mother.

3.3. *Clinical features of patients carrying* PSEN1 *and* PSEN2 *variants*

Six variants in *PSEN1* and 1 variant in *PSEN2* were identified in the present study. The variant p.R157S (c.471 G>T) in *PSEN1* has never been reported in patients with FAD previously. It was detected in a 62-year-old male who presented with memory deficits at the age of 60 years (Family 6, II-1, Case 6) (Figs. 1F, L, and R). The memory disturbance progressed slowly and irritability was noticed by his relatives 2 years later. He was still able to perform activities of daily living. No other noncognitive neurological symptoms were noted. His mother (I-2) died at approximately 90 years of age with cognitive impairment.

The recognized pathogenic variant p.F177S (c.530 T>C) in *PSEN1* was identified in the proband of Family 8 (III-1, Case 8) (Supplementary Figs. 1B and H) who was affected by memory loss when he was 30 years old. In contrast to the other patients with *PSEN1* variants, involuntary movement, cerebellar ataxia, and difficulties with speaking and swallowing were observed in this patient. Brain MRI showed signs of global brain atrophy, especially in the frontal regions, temporal regions, and hippocampus. His grandmother, father (died at 37 years old), and father's younger brother (died in his 30s) had been affected by dementia.

The recognized pathogenic variant p.M139I (c.417 G>A) in *PSEN1* (Figs. 1E, K, and Q) was identified in case 5 from family 5, who also carrying an *APP* variant p.D332G (c.995 A>G) (Figs. 1E, J, and P). The clinical phenotype of case 5 had been described previously. The clinical phenotypes of probands with other 3 reported pathogenic *PSEN1* variants, including p.R269H (c.806 G>A) (Supplementary Figs. 1C and I), p.L173W (c.518 T>G) (Supplementary Figs. 1D and J), and p.T147I (c.440 C>T) (Supplementary Figs. 1E and K), displayed typical cognitive deficits and were summarized in Table 2.

For *PSEN2*, we reported the known pathogenic variant p.V139M (c.415 G>A) for the first time in a Chinese patient (Family 12, II-1, Case 12) (Supplementary Figs. 1F and L). It was first reported in an Italian late-onset FAD family with memory and language deficits (Bernardi et al., 2008). However, in our study, this variant presented with different phenotypes including early-onset predominant memory loss and a slow progression. His father (Family 12, I-1) presented with similar symptoms in his 40s but was unavailable for genetic evaluation.

4. Discussion

AD is considered a multifactorial disease, and its pathogenesis remains elusive. Discoveries in FAD patients have provided critical insights into the cellular mechanisms of the disease (Selkoe and Hardy, 2016). Although tremendous efforts have been made to illustrate the pathogenesis of AD in recent decades, the mechanisms of neuron degeneration in the disease remain unknown, and there still lack sufficient knowledge for the early diagnosis and effective treatment of the disease (Scheltens et al., 2016).

APP, *PSEN1,* and *PSEN2* are the leading 3 causative genes in autosomal dominant FAD. All 3 genes are involved in the amyloid-β peptide (Aβ) metabolism. According to the Alzforum databases (https://www.alzforum.org/mutations), 52 mutations within *APP*, 241 mutations within *PSEN1,* and 45 mutations within *PSEN2* that linked with AD have been identified. According to the previous studies performed to screen for the causative variants in Chinese patients with FAD, mutations in *PSEN1* are the main cause of Chinese FAD. The first novel *PSEN1* mutation (p.V97L) was reported in a FAD family from northern China in 2005 (Jia et al., 2005). Another novel *PSEN1* mutation (p.S169del) was identified in a FAD family from southern China in 2010 (Guo et al., 2010). Recently, 4 novel *PSEN1* mutations (p.A434T, p.I167del, p.F105C, and p.L248P) were identified in 4 respective families, and 1 previously recognized pathogenic mutation in *APP* (p.V717I) was detected in 2 families in a study containing a total of 32 FAD families, but no *PSEN2* mutation was detected (Jiao et al., 2014). More recently, another novel *PSEN1* mutation (p.F388L) was reported in a Chinese FAD family (Zhan et al., 2017). The frequency of *APP* and *PSEN2* mutations was relatively lower than that of *PSEN1*. Only 2 novel *APP* mutations (p.D678H and p.K724M) (Lan et al., 2014; Peng et al., 2014) and 4 novel *PSEN2* mutations (p.P123L, p.N141Y, p.H169N, and p.V214L) (Niu et al., 2014; Shi et al., 2015; Xia et al., 2015) have been reported in the Chinese FAD families.

In our study, of the 15 index patients from the clinically diagnosed FAD pedigrees, 12 (80%) were found to carry missense variants in *APP*, *PSEN1,* and *PSEN2*. Seven of them were previously recognized as pathogenic variants, and 1 novel variant was considered to be likely pathogenic. The proportion of causative mutations (8 of 15, 53.3%) in our study was close to that of a previous mutation analysis of patients with FAD in the United Kingdom (21 of 31, 68%) (Janssen et al., 2003) and France (24 of 34, 71%) (Campion et al., 1999). However, compared with the previous study performed in Chinese patients with FAD (18.75%) (Jiao et al., 2014), the frequency of mutation was higher in our study (53.3%). Several reasons should be considered. First, in our study, WES combined with Sanger sequencing was used, whereas Sanger sequencing was used in the previous study (Jiao et al., 2014). Compared with WES, Sanger sequencing has more false negative results. The most common reason for false negative results obtained by Sanger sequencing is "human error" (primarily misread sequence and sample switch) (Jennings and Kirschmann, 2016). In addition, WES is more powerful than Sanger sequencing for detecting mosaicism. These 2 technologies complement each other; the accuracy and effectiveness will be improved when they are used together. Second, the enrollment criteria may affect the results. In addition, the sample size was comparatively small in both of the 2 studies which may also have effects on the results. Although the p. K687Q variant in *APP* was classified as likely pathogenic, the p.D244G, p.T297M, and p.D332G variants in *APP* and the p.R157S variant in *PSEN1* were all classified as uncertain significance because of limited access to family members' data for genetic evaluation. Functional analyses are warranted to further determine the consequences of the amino acid changes. In addition, there is no known pathogenic variant in the remaining 3 cases. WES for the family members and the copy number variations detection are needed to further determine the unknown disease-causing genes and other types of variants.

As described in previous reports, different mutations or even mutations at the same codon site in FAD causative genes may result in different clinical phenotypes (Ryan and Rossor, 2010). We summarize the disease-phenotype of 7 recognized mutations found in the present study in Supplementary Table 3. In our study, the proband carrying the *APP* mutation (p.V717I) demonstrated memory deficits, occasional seizures, and swallowing problems, which have been described in Chinese patients with FAD with the p.V717I mutation (Zhang et al., 2017). The variant p.V717I in *APP* is located near the γ-secretase cleavage site. It may increase the hydrophobicity of the APP TM domain and elevate the Aβ42 over Aβ40 ratio (De Jonghe et al., 2001; Murrell et al., 2000). The remaining *APP* mutations identified in our study resulted in relatively typical FAD clinical phenotypes.

Many *PSEN1* mutations have been reported to be related to specific clinical phenotypes. The *PSEN1* mutation p.T147I was first reported in a French FAD family. The phenotypes of this mutation were characterized by young age of onset, limb spasticity, and expressive aphasia (Denvir et al., 2015). However, in our study, the patient carrying the same variant presented with typical amnestic symptoms. According to a previous report, the p.T147I variant increased the amount of Aβ42 and ratio of Aβ42 over Aβ40 (Sun et al., 2017). Another *PSEN1* mutation p.F177S has been reported in German and U.S. FAD families (Hausner et al., 2014; Rogaeva et al., 2001). It was reported to be associated with a young age of onset and rapid progression. In addition to these characteristics, the proband carrying p.F177S variant in our study presented with involuntary movement, cerebellar ataxia, and cerebellar atrophy in brain MRI. Two reports previously described the clinical features of the p.R269H mutation (Gomez-Isla et al., 1997; Janssen et al., 2003). In addition to the progressive memory impairment, some noncognitive neurological features, such as myoclonus, auditory hallucinations, and myoclonus, were reported. However, we did not observe any atypical symptoms in our patient with p.R269H mutation.

The case 5 (Family 5 II-4) carried the variant p.M139I in *PSEN1* gene and the variant p.D332G in *APP* gene. The *PSEN1* variant p.M139I has been reported in a South Korean FAD family. The proband of this family was a 38-year-old female with early memory impairment. No noncognitive neurological feature was noticed (Kim et al., 2010). In our study, the proband carrying the p.M139I variant presented the similar clinical characteristics to the previously reported patient (Kim et al., 2010). The variant p.D332G in *APP* was never reported in patients with FAD previously and was classified as uncertain significance. Thus, the *PSEN1* variant (p.M139I) was responsible for the clinical phenotypes. However, the role of the variant p.D332G in *APP* gene needs to be further determined.

In conclusion, we first reported the mutation screening in a Chinese cohort of patients with FAD by WES. In addition, we identified a novel likely pathogenic variant p.K687Q in *APP* gene.

5. Disclosure

The authors report no actual or potential conflicts of interest. This study was approved by the Ethics Committee of Second Affiliated Hospital, Zhejiang University School of Medicine, and written informed consents were obtained from all participants.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2018.11.024.

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