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RESEARCH ARTICLE

Clinical Phenotype and Mutation Spectrum of Alzheimer's Disease with Causative Genetic Mutation in a Chinese Cohort

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Abstract: ***Background:*** Alzheimer's disease with a causative genetic mutation (AD-CGM) is an uncommon form, characterized by a heterogeneous clinical phenotype and variations in the genotype of racial groups affected.

Objective: We aimed to systemically describe the phenotype variance and mutation spectrum in the large sample size of the Peking Union Medical College Hospital (PUMCH) cohort, Beijing, China.

Methods: Next-generation sequencing (NGS) was carried out in 1108 patients diagnosed with dementia. A total of 40 Han Chinese patients with three AD gene mutations were enrolled. A systemic review of all the patients was performed, including clinical history, neurocognitive assessment, brain magnetic resonance imaging, and cerebrospinal fluid (CSF) biomarkers.

Results: We studied the following gene mutation variants: 12 AβPP, 13 PSEN1, and 9 PSEN2, and 23 among them were novel. Most of them were early-onset, but PSEN1 mutation carriers had the youngest onset age. The commonest symptoms were similar to those of AD, including an amnestic syndrome, followed by psychiatric symptoms and movement disorder. On MRI, parietal and posterior temporal atrophy was prominent in PSEN1 and PSEN2 mutation carriers, while AβPP mutation carriers had more vascular changes. The CSF biomarkers profile was indistinguishable from sporadic AD.

Conclusion: We identified a small group of AD-CGM subjects representing 3.6% among more than 1000 demented patients in the PUMCH cohort. These subjects usually presented with early-onset dementia and exhibited significant clinical and genetic heterogeneity. Identification required complete screening of genetic mutations using NGS. Although family history was usually present, we found non-familial cases of all three genetic mutations.

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1. INTRODUCTION

Alzheimer's disease with a causative genetic mutation (AD-CGM) represents a rare group of patients with dementia of Alzheimer's type. Three pathogenic genes have been found to cause AD-CGM, presenilin 1 (PSEN1), presenilin 2 (PSEN2), and amyloid β protein precursor (AβPP) genes [1-3]. The clinical phenotype is heterogeneous and usually differs from typical sporadic late-onset Alzheimer's disease (LOAD) [4]. Most cases of AD-CGM have an early age of onset with rapid cognitive deterioration. Besides memory and cognition impairment, they may have other neurological symptoms, such as movement disorders [4]. Most cases have autosomal dominant Alzheimer's disease (ADAD), but there are some reports of patients without a family history. To date, over 200 mutations have been identified in AD-CGM, and most are ADAD [5]. The mutations affect a common pathogenic pathway in amyloid precursor protein synthesis and proteolysis, leading to excessive production of beta-amyloid (Aβ) [5].

PSEN1 mutation is the most common genetic cause of AD-CGM. Typical features of patients with PSEN1 mutations include the youngest age at onset and the shorter duration of the disease [6] compared with other AD-CGM cases. Language impairment, psychiatric and behavior problems may be present in addition to the amnestic syndrome [6]. Other neurological symptoms found in patients with PSEN1

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mutations include seizures, spastic paraparesis, extrapyramidal signs, and ataxia [6]. PSEN2 and AβPP mutations are less common than PSEN1 mutations. However, the mutation spectrum and clinical phenotypes are different among different ethnic groups. For instance, Asian patients with PSEN1 mutations present more frequently with disorientation and personality change but less frequently with the atypical clinical features mentioned above [7]. Several mutations have been reported previously among Han Chinese with AD-CGM based on pedigrees [8]. However, we have observed that some cases of AD-CGM presented without a family history, and we aimed to systemically describe the phenotype variance and mutation spectrum in the large sample size of the Peking Union Medical College Hospital cohort, Beijing, China.

2. MATERIALS AND METHODS

2.1. Participants

Next-generation sequencing was carried out in 1108 patients diagnosed with dementia in dementia and leukoencephalopathy clinic of Peking Union Medical College Hospital (PUMCH-cohort), Beijing, China, between Jan 2016 and Jan. 2019. The diagnosis of dementia was defined according to DSM-V [9]. In total, 40 patients (40/1108=3.6%) with Alzheimer's gene mutation were enrolled. They were from different families and unrelated. They all fulfilled the definition of probable AD dementia in a carrier of a causative AD genetic mutation (AD-CGM) according to 2011 NIA-AA guidelines [10]. The study was done in accord with the ethical standards of the Helsinki Declaration of 1975. All patients or their caregivers were informed of the purpose of the study and the future publication of the article. Written consent was obtained from each patient or their caregiver(s). The ethics committee of Peking Union Medical College Hospital approved the study (No. JS1836). The ethnicity of all these patients was Han Chinese. Detailed clinical information and family histories were provided by reliable informants (27/40=67.5% with family history reports). All patients underwent complete physical and neurological examination, laboratory blood tests, and detail cognitive assessment. The cognitive battery included the following screening tests: Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment-PUMCH edition (MoCA-PUMCH edition) [11], Activities of Daily Living (ADLs), Clinical dementia rating scale (CDR), and Hospital Anxiety and Depression Scale (HAD). After screening, a detailed domain-specific neuropsychological battery test was performed, including story memory, category fluency, Trail Making A and B, digit symbol, digit span, auditory verbal learning test, block design test, and modified Rey figure, among others. All these chosen scales had been validated in the Mandarin language with available normative data for the Chinese population.

2.2. Neuroimaging and Cerebrospinal Fluid Analysis

Magnetic resonance imaging (MRI) examinations were performed in participants using a 3T MRI scanner (Avanto, Siemens, Erlangen, Germany). Axial T1-weighted, T2-weighted, T2-Flair sagittal, and/or coronal T1-weighted imaging was performed. Brain atrophy involving the temporal lobe and parietal lobe as well as white matter lesions, were evaluated visually. Cerebral vascular changes, including infarction and lacunae, were recorded. Electroencephalography (EEG) (Nihon Kohden, Japan) studies were performed in a subset of the participants (20 cases). Brain ^{18}F-FDG positron emission tomography, computed tomography (PET/CT) (Biograph, Siemens, Erlangen, Germany) were conducted in some participants (4 cases).

In 8 of the patients, lumbar puncture was performed after informed consent, and cerebrospinal fluid (CSF) biomarkers for AD were measured. The collection of CSF was gravity drip, and low protein absorption tubes were used. All CSF samples were sub-packed and stored at -80°C. Commercial accessible ELISA kits were utilized for measurement of CSF t-tau, p-tau, Amyloid β_{42} ($A\beta_{42}$) respectively by INNOTEST Tau ELISA, Phospho-tau, and $A\beta_{42}$ (Fujirebio, Ghent, Belgium).

2.3. Genome Sequencing and Variants Identification

Written informed consent for genetic analysis was obtained from all patients. Genomic DNA was extracted from fresh peripheral blood leukocytes. Whole exome sequencing was carried out in 281 cases, and the rest 827 cases underwent targeted panel sequencing for selected loci, including *AβPP*, *PSEN1*, and *PSEN2*. Next-Generation Sequencing (NGS) technology was performed on Illumina HiSeq (Illumina, USA), which was verified by Sanger sequencing. The results were analyzed according to the standard *AβPP* (NM_000484.3), *PSEN1* (NM_000021.3), and *PSEN2* (NM_000447.2) reference sequence. Besides, we analyzed the genotype of the *Apolipoprotein E*(*ApoE*) gene.

All the variants detected are listed in Supplementary Table **1**. All variants were searched in ALZFORUM, Mutations Database (https://www.alzforum.org/mutations) and PubMed (https://pubmed.ncbi.nlm.nih.gov), then classified as a novel or reported. They were all classified as pathogenic, likely pathogenic, or uncertain significance according to American College of Medical Genetics and Genomics (ACMG) standards [12]. The including criteria in the analysis were based on bioinformatic analysis and were detailed as follows: the filtering was restricted to causative AD genetic mutations; variants previously reported to be causative AD mutations; non-synonymous variants causing missense, frameshift, or splicing mutation; the frequency of variants in the general population (1000 Genomes project, ExAC database, 1000 healthy Chinese controls) must be less than 0.5%; the variants were predicted to be functional protein damage by three function prediction software analysis (SIFT, PolyPhen, and LRT); benign and like benign variants on ACMG classification were discarded.

2.4. Statistical Analysis

Statistical analysis was performed on SPSS 22.0. Measurement data was described as average±standard deviation, and enumeration data was described as a ratio. Measurement data were statistically analyzed with *t*-test and ANOVA, while enumeration data (frequencies) was analyzed with the χ^2 test. Statistical graphs were produced with GraphPad Prism.

3. RESULTS

3.1. Demographic and Basic Information of the Participants (Table 1)

We reported the results of 40 AD-CGM patients among a total of 1108 subjects with dementia in the PUMCH-cohort (40/1108=3.6%). Participants included the following mutations: 17 *AβPP* (17/1108=1.5%), 14 *PSEN1* (14/1108=1.2%), and 9 *PSEN2* (9/1108=0.8%). Variants included were 12 *AβPP*, 13 *PSEN1,* and 9 *PSEN2*. In all the 34 variants detected, 23 were novel, and 11 were reported in the literature (Supplementary Table **1**). The demographic and basic clinical information of the three genetic groups are summarized in Table **1**. Detailed information of the participants are listed in Supplementary Table **2**.

3.2. Phenotype Distribution of the Participants (Table 2)

An amnestic syndrome at onset was the initial manifestation ranging in frequency from 50% to 66.6%, similar to LOAD. Psychiatric symptoms and behavioral problems occurred in 21/40 patients (52.5%). In all 3 mutations, there were patients with aphasia at onset (7 patients, 16.5%) and movement disorders (9/40=22%). In the *AβPP* and *PSEN1* mutation, group 3 patients presented with episodes of cognitive disturbance followed by progressive memory loss, which was uncommon in AD. In the process of disease progression, dementia was characterized by the involvement of all cognitive domains. Psychiatric symptoms and behavioral complications (irritability, delusions, agitation, and depression) were more commonly observed than movement disorder manifestations such as parkinsonism, tremor, spasticity, or pyramidal sign, while no seizures or ataxia were reported. Irritability was significantly more found in *PSEN1* mutation carriers, and agitation was more found in *PSEN2* mutation carriers. As for movement disorders, spasticity/pyramidal sign was most common in *PSEN1* mutation carriers. A total of 39 patients underwent brain MRI evaluation, except for one patient with a metal implant. The MRI of the brain revealed bilateral symmetrical cortical atrophy involving predominantly temporal and parietal lobes in 67% to 70% of the cases. Parietal lobe atrophy was more common in our patients than in sporadic LOAD. White matter hyperintensities were evaluated using the Fazekas scale [13]. Fazekas stages 2 and 3 were used to define severe white matter lesions that were noted in 12/39 (30.8%) patients in our cohort. On the other hand, vascular changes including lacunae and infarction were found in 29.4% of *AβPP* mutation carriers, while only 1/13 (8%) of *PSEN1* and no *AβPP* mutation carriers had vascular changes. Electroencephalography (EEG) was obtained in 50% of the patients. Abnormal results included an increase of θ or σ slow waves in the bilateral frontal and temporal lobe or diffuse slow waves. No epileptic discharges were found. In 8 patients with CSF biomarkers analysis, the results were in concordance with typical AD manifestations, that included an increase in t-tau (Average 901.8pg/ml, cut-off >286.8pg/ml) and p-tau (Average 79.8pg/ml, cut-off >55.3pg/ml) levels, ratio of t-tau/ $A\beta_{1\text{-}42}$ (Average 1.94, cut-off>0.67), with a decrease in $A\beta_{1\text{-}42}$ (Average 546.9pg/ml, cut-off <614.6pg/ml). FDG-PET was obtained in 4 patients and PiB-PET in a single patient; all of them revealed typical AD changes. The bilateral temporal lobe, parietal lobe, and posterior cingulate gyrus hypometabolism were found. These results supported the diagnosis of AD and were indistinguishable from those described in sporadic LOAD.

Table 1. Demographic and basic information of *APP, PSEN1,* and *PSEN2* mutation carriers.

-	*APP*	*PSEN1*	*PSEN2*	*p*
No. of patients	17	14	9	-
No. of variants	12	13	9	-
Sex (male: female)	9:8	7:7	4:5	0.919
Age of onset ($\bar{x} \pm S$) Age range (yrs)	65.7 ± 12.6 (41-86)	55.4 ± 11.4 (41-74)	59.4 ± 7.9 (47-70)	0.051
Age of onset ≤65 yr	10/17 (58.8%)	11/14(78.6%)	7/9 (77.8%)	0.415
Time from onset to examination (yrs) ($\bar{x} \pm S$)	2.76 ± 1.39	2.64 ± 2.24	3.78 ± 1.86	0.311
Positive family history	8/17 (47.1%)	12/14 (85.7%)	7/9 (77.8%)	0.055
APOE ε4 carrier	7/17 (41.2%)	2/14 (14.3%)	5/9 (55.6%)	0.100
MMSE ($\bar{x} \pm S$)	16.2 ± 7.6	19.3 ± 8.6	13.0 ± 7.3	0.283
MoCA ($\bar{x} \pm S$)	15.7 ± 6.8	19.3 ± 7.5	14.3 ± 8.0	0.459
ADL ($\bar{x} \pm S$)	37.8 ± 11.7	30.8 ± 7.6	35.7 ± 11.1	0.240
CDR ($\bar{x} \pm S$)	1.7 ± 0.85	1.5 ± 1.1	1.9 ± 1.1	0.701

Table 2. Clinical phenotype and neuroimaging of *APP, PSEN1,* and *PSEN2* mutation carriera.

-	Total	*APP*	*PSEN1*†	*PSEN2*	*p*
Symptoms:	-	-	-	-	-
Amnestic onset	24/40(60%)	11/17(64.7%)	7/14(50.0%)	6/9(66.7%)	0.635
Psychiatric/behavior	21/40(52.5%)	6/17(35.3%)	9/14(64.3%)	6/9(66.7%)	0.172
Irritability	7/40 (17.5%)	1/17 (5.9%)	6/14 (42.9%)	0/9 (0%)	0.008
Delusions	10/40(25.0%)	4/17 (23.5%)	2/14 (14.3%)	4/9 (44.4%)	0.260
Agitation	4/40 (10.0%)	1/17 (5.9%)	0/14 (0%)	3/9 (33.3%)	0.026
Depression	2/40 (5.0%)	0/17 (0%)	2/14 (14.3%)	0/9 (0%)	0.142
Language disorder	7/40(17.5%)	3/17(17.6%)	3/14(21.4%)	1/9(11.1%)	0.817
Movement disorder	9/40(22%)	4/17(23.5%)	3/14(21.4%)	2/9(22.2%)	0.990
Parkinsonism	4/40 (10.0%)	3/17 (17.6%)	0/14 (0%)	1/9 (11.1%)	0.263
Tremor	2/40 (5.0%)	1/17 (5.9%)	0/14 (0%)	1/9 (11.1%)	0.479
Spasticity/pyramidal sign	3/40 (7.5%)	0/17 (0%)	3/14 (21.4%)	0/9 (0%)	0.049
MRI Imaging:	-	-	-	-	-
Bilateral temporal atrophy	27/39(69.2%)	12/17(70.6%)	10/13(76.9%)	5/9(55.6%)	0.558
Bilateral parietal atrophy	28/39(71.8%)	10/17(58.8%)	10/13(76.9%)	8/9(88.9%)	0.237
Severe white matter hyperintensities	12/39(30.8%)	5/17(29.4%)	4/13(30.8%)	3/9(33.3%)	0.979
Vascular changes	6/39(15.4%)	5/17(29.4%)	1/13(7.8%)	0/9(0.0%)	0.091
Fazekas score ($\overline{x} \pm S$)	-	1.1 ± 1.0	0.8 ± 1.2	1.1 ± 1.0	0.822
Abnormal EEG‡	10/20(50.0%)	4/8(50.0%)	2/7(28.6%)	4/5(80.0%)	0.214

Note: †1 patient with *PSEN1* mutation had no MRI imaging due to a metal implant.
‡ Some patients had EEG evaluation.

3.3. Phenotype Differences among *AβPP, PSEN1,* and *PSEN2* Mutation Carriers

The demographic and clinical phenotype differences of the three groups are summarized in Tables **1** and **2** (*p*-value in the last column). Onset age in *PSEN1* carriers was significantly younger than *AβPP* carriers (p-value, 0.026). And that of *PSEN2* carriers was in between (Fig. **1**). *PSEN1* and *PSEN2* carriers had a higher frequency of positive family history than *AβPP* carriers. *PSEN1* mutation group had a lesser frequency of *APOE ε4* carriers than the other two.

The cognition evaluation was indistinguishable among the three groups. Most of the patients were moderately affected. Amnestic onset was the most common in all, and no statistical difference was found among them. As for MRI imaging, parietal atrophy was found more often in the *PSEN1* and *PSEN2* groups, while temporal lobe atrophy was similar in all three mutations. The *AβPP* group had significantly more vascular changes than the other two. The CSF biomarkers profile was indistinguishable among them.

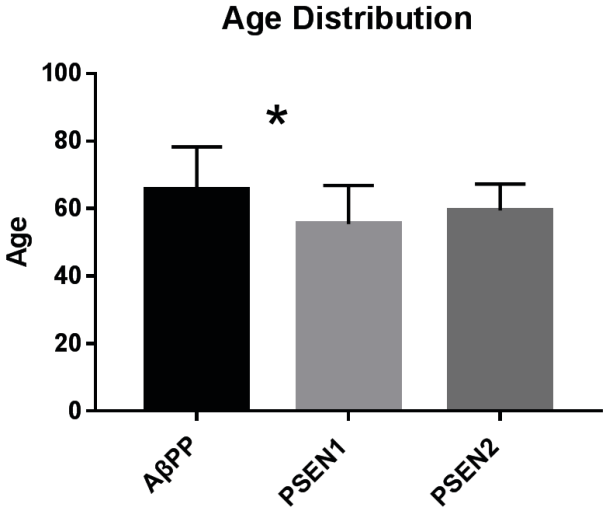

Fig. (1). Onset age was youngest in *PSEN1* mutation carriers. **Note:** *:significant difference between *AβPP* and *PSEN1* group (p=0.026).

3.4. Representative Pedigrees Illustration

The pedigree of Proband 1 is shown in Fig. (**2A**). The patient is amale, heterozygous mutation carrier of *AβPP* (NM_000484.3) Exon16 c.2032G>A p.(Asp678Asn). He presented with memory loss with onset at 64 years of age and was moderately demented on admission. Brain MRI revealed bilateral atrophy of the temporal lobe and parietal

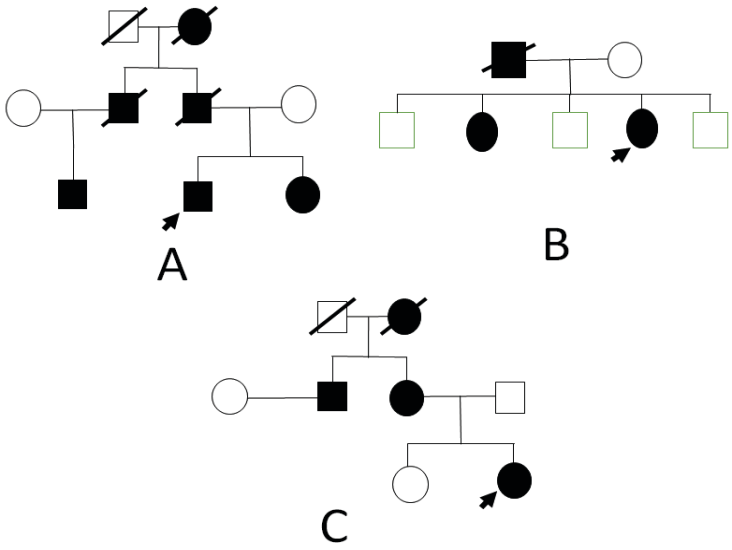

Fig. (2). Pedigrees of the probands. **A**: proband 1; **B**: proband 2; **C**: proband 3. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

lobe (Fig. **3A-C**). His CSF biomarkers were $A\beta_{42}$ 361.3pg/ml, t-tau 883.3pg/ml, p-tau 66.8pg/ml.

Proband 2 is a woman with an age of onset at 41 years old. Her pedigree is shown in Fig. (**2B**). She is a heterozygous mutation carrier of *PSEN1* (NM_000021.3) IVS8 c.869-2A>G. She presented with severe memory loss and non-fluent aphasia. Brain MRI revealed bilateral atrophy of the temporal lobe and parietal lobe (Fig. **3D-G**). Her PiB-PET was positive with amyloid deposition at the frontal lobe and posterior lobes (Fig. **3H**). FDG-PET showed symmetrical hypometabolism at the temporal lobe, parietal lobe, and posterior cingulate gyrus (Fig. **3I**). Her CSF biomarkers were $A\beta_{42}$ 357.0pg/ml, t-tau 1795.1pg/ml, p-tau 148.6pg/ml.

Proband 3 is a woman who presented with memory loss at 47 years of age. She had a positive family history of dementia (Fig. **2C**). She was a heterozygous mutation carrier of *PSEN2* (NM_000447.2) Exon8 c.716T>C p.(Met239Thr). Neuroimaging showed symmetrical atrophy of the parietal lobe without temporal lobe atrophy (Fig. **3J-L**). Her CSF biomarkers were $A\beta_{42}$ 675.1pg/ml, t-tau 892.3pg/ml, p-tau 43.5pg/ml.

4. DISCUSSION

The current definition of Alzheimer's disease relies heavily on biomarkers in CSF and brain imaging. Although genetic information was not included in the biomarkers' profile summarized in the 2018 NIA-AA research framework, symptomatic autosomal dominant mutation carriers were considered to have AD neuropathologic change without the use of biomarkers [14]. In fact, it is generally accepted that a causative gene mutation is the strongest "biomarker." Determination of genetic mutations was important in the clinical diagnosis of AD, especially in early-onset dementia cases. Next-generation sequencing was proved to be a swift, accurate, and cost-effective method for the identification of genetic mutations in clinical diagnosis [15], although the prompt interpretation of results is important. The mutation spectrum reported for AD-CGM showed variations according to different races and areas [16]. The majority of the mutations reported involve *PSEN1,* but other new gene mutations have been reported in different countries and also in China [8, 16]. The AD-CGM mutation rate in our general dementia cohort was around 3.6%, which was in concordance with previous reports. In all the 34 variants detected in the three genes, 23 variants were novel, classified as uncertain significance, or likely pathogenic according to ACMG guidelines. The mutation profile of *AβPP*, *PSEN1,* and *PSEN2* in familial AD was studied in several Chinese reports. The detection rate of novel variants in these studies was around 27.5%-50% [8, 17, 18]. We enrolled more early-onset dementia patients without family history in the cohort, which might result in a relatively higher detection rate of novel variants. The frequencies of the variants in the general population were very low. Most of the novel variants were located in the hotspot region and next to pathogenic variants listed in AlzForum. They were worthy for further validation in our near future research.

The age of symptom onset varied from the third or fourth decade of life to the late 70s or 80s in AD-CGM. We confirmed previous reports that mutations in *PSEN2* had significantly later onset than mutations in *PSEN1* and *AβPP*, and mutations in *PSEN1* had significantly earlier onset than all other groups [19]. We also found *PSEN1* mutation carriers had the youngest age onset in our group.

We found that the clinical phenotype of AD-CGM associated with genetic mutations is extremely heterogeneous. Analysis of the psychological characteristics of the dominantly inherited Alzheimer network (DIAN) cohort concluded that the overall cognitive and personality deficits in mild AD-CGM were similar to sporadic AD, which often began with the hallmark deficit in episodic verbal memory as well as difficulties in some aspects of executive function and visuospatial abilities [20]. The patients in our cohort were mostly in the moderate stage, with a wide spectrum of cognitive domain involvement. Psychiatric/behavior syndrome is more common in the AD-CGM patients in our group, occurring in over half of the cases.

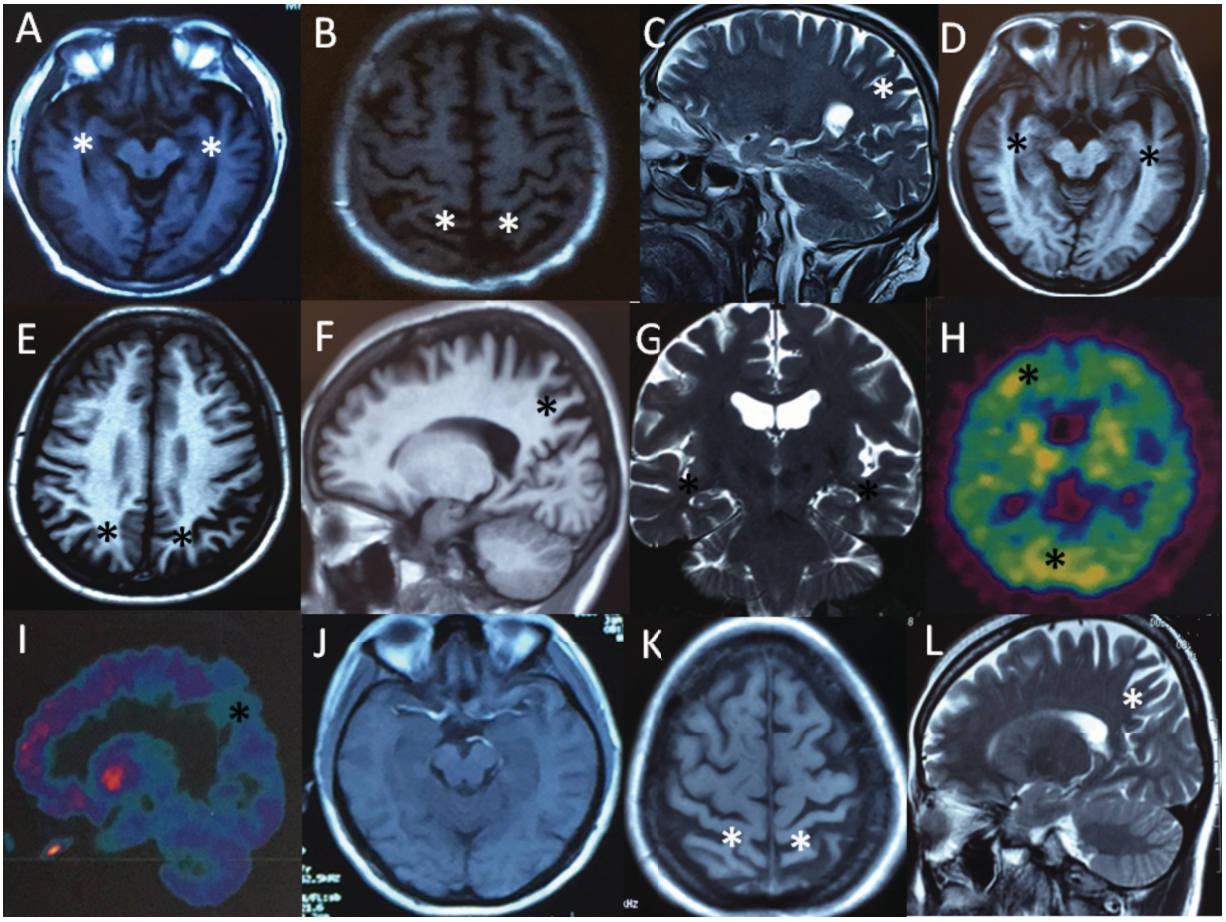

Fig. (3). Representative patients with AD-CGM. (**A-C**): Proband 1 with *AβPP* (NM_000484.3) Exon16 c.2032G>A p. (Asp678Asn), (**A**) symmetrical temporal lobe atrophy; (**B**) symmetrical parietal lobe atrophy; (**C**) paramedian sagittal view of parietal lobe atrophy (white asterisks). (**D-I**): Proband 2 with *PSEN1* (NM_000021.3) IVS8 c.869-2A>G, (**D**) symmetrical temporal lobe atrophy; (**E**) symmetrical parietal lobe atrophy; (**F**) paramedian sagittal view of parietal lobe atrophy; (**G**) coronal view of bilateral temporal lobe atrophy; (**H**) positive PiB-PET; (**I**) hypo-metabolism of temporal and parietal lobe;(dark asterisks).. (**J-L**): Proband 3 with *PSEN2* (NM_000447.2) Exon8 c.716T>C p.(Met239Thr), (**J**) no hippocampus atrophy; (**K**) symmetrical parietal lobe atrophy ; (**L**) paramedian sagittal view of parietal lobe atrophy (white asterisks). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The genotype-phenotype relationship occurring in *PSEN1* mutation carriers has been widely discussed in the literature [21-23]. A large pedigree of E280A *PSEN1* mutation in Columbia showed that the most frequent presentation was memory loss followed by behavior and personality changes and progressive loss of language ability. In the final stages, gait disturbances, seizures, and myoclonus were frequent [24]. Later on, the AD phenotype of *PSEN1* has proved to be very broad, including not only cognitive decline, behavioral and psychiatric symptoms of dementia but also parkinsonism, myoclonus and epileptic seizures, spastic paraparesis, features suggestive of the phenotype of frontotemporal dementia, progressive aphasia, and cerebellar ataxia [23, 25]. It was reported that atypical symptoms occurred more often in mutations after codon 200 of *PSEN1* [4, 6]. Also, *PSEN1* mutation carriers were significantly more likely to exhibit myoclonus, corticobulbar deficits, aphasia, and spasticity [26]. In our cohort, a non-amnestic presentation was also foundmore in *PSEN1* mutation carriers than others, and language symptoms were most prominent. Also, spasticity/pyramidal sign was most common in *PSEN1* mutation carriers in our cohort.

The phenotype of *AβPP* and *PSEN2* mutations was relatively less complicated, with most of them presenting with amnestic symptoms, while behavior symptoms were more common in *PSEN2* mutations [6, 26]. *AβPP* mutation carriers were significantly more likely to present with stroke, both ischemic and hemorrhagic [26]. Literature also proved that several *AβPP* mutations were associated with amyloidosis and caused significant cerebral amyloid angiopathy-like symptoms in AD-CGM [27]. In our cohort, *AβPP* mutation carriers had more frequent vascular lesions, which supported the underlying mechanism. *PSEN2* mutation carriers experienced more behavior and psychiatric symptoms, especially agitation, than those with *AβPP* mutation.

The atrophy pattern on MRI was different in AD-CGM and sporadic AD. More neocortical loss, especially in the posterior area, was found in AD-CGM. Among different genes, there was evidence that *AβPP* subjects had smaller hippocampal volume than *PSEN1* subjects; also, *AβPP* subjects had more medial temporal lobe atrophy, and conversely, *PSEN1* subjects showed more neocortical loss [28]. We did not find atrophy pattern difference between *AβPP* and *PSEN1* carriers in our group, probably because of the

late disease stage; however, significant parietal lobe atrophy was found. White matter hyperintensities (WMH) were common in AD-CGM. Researchers found increased posterior WMH in the *PSEN1* post codon 200 groups, and WMH correlated with the severity of cerebral amyloid angiopathy and cotton wool plaque pathology at post-mortem [29]. WMH was also prominent in our cohort of AD-CGM, but we found no difference among the three gene mutations. Some of our AD-CGM patients with WMH could easily be misdiagnosed as vascular dementia clinically.

The *APOE ε4* allele is a well-known genetic risk factor for AD. In some reports, *APOE ε4* allele carriers were more likely to develop AD at an earlier age than subjects without the *ε4* allele in *PSEN1* mutation AD-CGM, suggesting the *APOE ε4* allele could also modify the age-onset and disease progression of AD-CGM [30]. However, we found no modifications induced by the *APOE ε4* allele in our patients, in agreement with previous research [19]. In contrast, we found more *ε4* carriers in the *AβPP* and *PSEN2* groups than in the *PSEN1* group.

In French AD-CGM cohort research works, CSF biomarkers profile was consistent with an AD diagnosis (cut-off: $A\beta_{42}$<500pg/mL, t-tau>350pg/mL, and p-tau>60 pg/mL) in 90% of families carrying mutations on known genes, and all mutation carriers had abnormal CSF biomarkers, at least one of the three indexes (t-tau, p-tau, $A\beta_{42}$) [31, 32]. The CSF biomarkers profile in our cohort was also consistent with AD, supporting AD pathologic changes and the pathogenicity of genetic variations.

There were some limitations in this study that need future work. Firstly, many of the variations found were of uncertain significance according to ACMG guidelines, and future functional validation would be needed. Secondly, the sample size was still not satisfying, although AD-CGM was rare. Multi-centric research would help in the future. Thirdly, future follow-up studies in our cohort should obtain neuropathology definition of the phenotypes.

CONCLUSION

In conclusion, we described the genotype and phenotype heterogeneity observed in the PUMCH-cohort. Early-onset was a common feature. *PSEN1* mutation carriers had the youngest age of onset, followed by *PSEN2* and *AβPP* mutation carriers. The most common symptoms were amnestic syndrome, followed by psychiatric symptoms and movement disorder, similar to sporadic AD. Parietal and posterior atrophy was more prominent in AD-CGM and especially in *PSEN1* and *PSEN2* mutation carriers, while *AβPP* mutation carriers had more vascular changes. The CSF biomarkers profile was indistinguishable from sporadic AD. Our recommendation is to screen for genetic mutations using NGS in atypical early-onset dementia cases.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethics committee of Peking Union Medical College Hospital approved the study, China (No. JS1836).

HUMAN AND ANIMAL RIGHTS

The study was done in accordance with the ethical standards of the Helsinki Declaration of 1975.

CONSENT FOR PUBLICATION

All patients or their caregivers were informed of the purpose of the study and the future publication of the article. Written consent was obtained from each patient or their caregiver(s).

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

REFERENCES

[1] Sherrington R, Rogaev EI, Liang Y, *et al.* Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 1995; 375(6534): 754-60. http://dx.doi.org/10.1038/375754a0 PMID: 7596406

[2] Levy-Lahad E, Wasco W, Poorkaj P, *et al.* Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 1995; 269(5226): 973-7. http://dx.doi.org/10.1126/science.7638622 PMID: 7638622

[3] Goate A, Chartier-Harlin MC, Mullan M, *et al.* Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 1991; 349(6311): 704-6. http://dx.doi.org/10.1038/349704a0 PMID: 1671712

[4] Ryan NS, Nicholas JM, Weston PSJ, *et al.* Clinical phenotype and genetic associations in autosomal dominant familial Alzheimer's disease: A case series. Lancet Neurol 2016; 15(13): 1326-35. http://dx.doi.org/10.1016/S1474-4422(16)30193-4 PMID: 27777022

[5] Wu L, Rosa-Neto P, Hsiung GY, *et al.* Early-onset familial Alzheimer's disease (EOFAD). Can J Neurol Sci 2012; 39(4): 436-45. http://dx.doi.org/10.1017/S0317167100013949 PMID: 22728850

[6] Ryan NS, Rossor MN. Correlating familial Alzheimer's disease gene mutations with clinical phenotype. Biomarkers Med 2010; 4(1): 99-112. http://dx.doi.org/10.2217/bmm.09.92 PMID: 20387306

[7] Shea YF, Chu LW, Chan AO, Ha J, Li Y, Song YQ. A systematic review of familial Alzheimer's disease: Differences in presentation of

clinical features among three mutated genes and potential ethnic differences. J Formos Med Assoc 2016; 115(2): 67-75.
http://dx.doi.org/10.1016/j.jfma.2015.08.004 PMID: 26337232

[8] Jiang B, Zhou J, Li HL, *et al.* Mutation screening in Chinese patients with familial Alzheimer's disease by whole-exome sequencing. Neurobiol Aging 2019; 76: 215.e15-21.
http://dx.doi.org/10.1016/j.neurobiolaging.2018.11.024 PMID: 30598257

[9] American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington, VA: American Psychiatric Association 2013.

[10] McKhann GM, Knopman DS, Chertkow H, *et al.* The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7(3): 263-9.
http://dx.doi.org/10.1016/j.jalz.2011.03.005 PMID: 21514250

[11] Tan JP, Li N, Gao J, *et al.* Optimal cutoff scores for dementia and mild cognitive impairment of the Montreal Cognitive Assessment among elderly and oldest-old Chinese population. J Alzheimers Dis 2015; 43(4): 1403-12.
http://dx.doi.org/10.3233/JAD-141278 PMID: 25147113

[12] Richards S, Aziz N, Bale S, *et al.* Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17(5): 405-24.
http://dx.doi.org/10.1038/gim.2015.30 PMID: 25741868

[13] Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. AJR Am J Roentgenol 1987; 149(2): 351-6.
http://dx.doi.org/10.2214/ajr.149.2.351 PMID: 3496763

[14] Jack CR Jr, Bennett DA, Blennow K, *et al.* NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018; 14(4): 535-62.
http://dx.doi.org/10.1016/j.jalz.2018.02.018 PMID: 29653606

[15] Beck J, Pittman A, Adamson G, *et al.* Validation of next-generation sequencing technologies in genetic diagnosis of dementia. Neurobiol Aging 2014; 35(1): 261-5.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.07.017 PMID: 23998997

[16] Bagyinszky E, Youn YC, An SS, Kim S. Mutations, associated with early-onset Alzheimer's disease, discovered in Asian countries. Clin Interv Aging 2016; 11: 1467-88.
http://dx.doi.org/10.2147/CIA.S116218 PMID: 27799753

[17] Gao Y, Ren RJ, Zhong ZL, *et al.* Mutation profile of APP, PSEN1, and PSEN2 in Chinese familial Alzheimer's disease. Neurobiol Aging 2019; 77: 154-7.
http://dx.doi.org/10.1016/j.neurobiolaging.2019.01.018 PMID: 30822634

[18] Jia L, Fu Y, Shen L, *et al.* PSEN1, PSEN2, and APP mutations in 404 Chinese pedigrees with familial Alzheimer's disease. Alzheimers Dement 2020; 16(1): 178-91.
http://dx.doi.org/10.1002/alz.12005 PMID: 31914229

[19] Ryman DC, Acosta-Baena N, Aisen PS, *et al.* Symptom onset in autosomal dominant Alzheimer disease: A systematic review and meta-analysis. Neurology 2014; 83(3): 253-60.
http://dx.doi.org/10.1212/WNL.0000000000000596 PMID: 24928124

[20] Storandt M, Balota DA, Aschenbrenner AJ, Morris JC. Clinical and psychological characteristics of the initial cohort of the Dominantly Inherited Alzheimer Network (DIAN). Neuropsychology 2014; 28(1): 19-29.
http://dx.doi.org/10.1037/neu0000030 PMID: 24219606

[21] Larner AJ, Doran M. Clinical phenotypic heterogeneity of Alzheimer's disease associated with mutations of the presenilin-1 gene. J Neurol 2006; 253(2): 139-58.
http://dx.doi.org/10.1007/s00415-005-0019-5 PMID: 16267640

[22] Larner AJ, Doran M. Genotype-phenotype relationships of presenilin-1 mutations in Alzheimer's disease: An update. J Alzheimers Dis 2009; 17(2): 259-65.
http://dx.doi.org/10.3233/JAD-2009-1042 PMID: 19221408

[23] Larner AJ. Presenilin-1 mutations in Alzheimer's disease: An update on genotype-phenotype relationships. J Alzheimers Dis 2013; 37(4): 653-9.
http://dx.doi.org/10.3233/JAD-130746 PMID: 23948899

[24] Lopera F, Ardilla A, Martínez A, *et al.* Clinical features of early-onset Alzheimer disease in a large kindred with an E280A presenilin-1 mutation. JAMA 1997; 277(10): 793-9.
http://dx.doi.org/10.1001/jama.1997.03540340027028 PMID: 9052708

[25] Mengel D, Liu L, Yamamoto R. A novel V272D presenilin mutation associated with logopenia, disorientation, and apraxia in an autosomal-dominant Alzheimer's disease family. Neurobiol Aging 2019; 1: e1-3.
PMID: 31500908

[26] Tang MX, Ryman DC, McDade E, Jasielec MS, Buckles VD, Cairns NJ, *et al.* Neurological manifestations of autosomal dominant Alzheimer's disease from the DIAN cohort and a meta-analysis. Lancet Neurol 2016; 15: 1317-25.
http://dx.doi.org/10.1016/S1474-4422(16)30229-0 PMID: 27777020

[27] Roks G, Van Harskamp F, De Koning I, *et al.* Presentation of amyloidosis in carriers of the codon 692 mutation in the amyloid precursor protein gene (APP692). Brain 2000; 123(Pt 10): 2130-40.
http://dx.doi.org/10.1093/brain/123.10.2130 PMID: 11004129

[28] Scahill RI, Ridgway GR, Bartlett JW, *et al.* Genetic influences on atrophy patterns in familial Alzheimer's disease: A comparison of APP and PSEN1 mutations. J Alzheimers Dis 2013; 35(1): 199-212.
http://dx.doi.org/10.3233/JAD-121255 PMID: 23380992

[29] Ryan NS, Biessels GJ, Kim L, *et al.* Genetic determinants of white matter hyperintensities and amyloid angiopathy in familial Alzheimer's disease. Neurobiol Aging 2015; 36(12): 3140-51.
http://dx.doi.org/10.1016/j.neurobiolaging.2015.08.026 PMID: 26410308

[30] Pastor P, Roe CM, Villegas A, *et al.* Apolipoprotein Eepsilon4 modifies Alzheimer's disease onset in an E280A PS1 kindred. Ann Neurol 2003; 54(2): 163-9.
http://dx.doi.org/10.1002/ana.10636 PMID: 12891668

[31] Wallon D, Rousseau S, Rovelet-Lecrux A, *et al.* The French series of autosomal dominant early onset Alzheimer's disease cases: Mutation spectrum and cerebrospinal fluid biomarkers. J Alzheimers Dis 2012; 30(4): 847-56.
http://dx.doi.org/10.3233/JAD-2012-120172 PMID: 22475797

[32] Lanoiselée HM, Nicolas G, Wallon D, *et al.* APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. PLoS Med 2017; 14(3): e1002270.
http://dx.doi.org/10.1371/journal.pmed.1002270 PMID: 28350801