Genetic analysis of Vietnamese patients with early-onset Alzheimer’s disease

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ABSTRACT

Purpose of the study: Alzheimer’s disease (AD) is the most common type of dementia and its prevalence is rapidly increasing worldwide. Early-onset Alzheimer’s disease (EOAD) constitutes of patients with age of onset earlier than 65 year-old and is known to be associated with genetic mutations. In this study, we reported the first genetic analysis of Vietnamese patients with EOAD.

Materials and methods: We analyzed targeted sequencing data obtained from a cohort of 51 Vietnamese EOAD patients to identify pathogenic variants in twenty nine well-characterized neurodegenerative genes.

Results: We identified four missense mutations in APP/PSEN1 genes from six individuals, which accounts for 11.8% of all tested cases. Three of these mutations were previously reported as pathogenic and one mutation in the APP gene was newly identified and might be specific for Vietnamese patients. Our study also found eight individuals carrying homozygous APOE ε4 allele, the main risk factor gene for late-onset AD.

Conclusions: Our findings showed that mutation rate in APP/PSEN genes in Vietnamese EOAD patients is consistent with that in other ethnic groups. Although further functional studies are required to validate the pathogenesis of the new mutations, our study demonstrated the necessity of genetic screening for EOAD patients as well as additional genetic data collection in Vietnamese population.

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Introduction

Alzheimer’s disease (AD) is the most common neurodegenerative disorder in dementia and poses a major challenge to public health worldwide. Genetic predisposition is a known risk factor for AD and contributes to two subgroups: early-onset and late-onset Alzheimer’s disease (EOAD and LOAD) [1]. While EOAD is less prevalent (5% of all AD cases), its molecular mechanism is better characterized than that of LOAD [2]. Original studies in the 1990s had showed that few mutations of amyloid-beta protein precursor (APP) gene were associated with familial AD [3, 4]. Following studies in APP protein processing had identified mutations in amyloid-beta protein precursor (APP), presenilin-1 (PSEN1), presenilin-2 (PSEN2) genes as the main cause of EOAD development [5, 6].

Insights into pathogenic mutations within EOAD genes enable early diagnosis and effective therapies for mutant-carrying individuals through genetic screening tests. However, some pathogenic mutations are rare and their prevalence and distribution may vary among populations and ethnic groups. Thus, profiling the mutation spectrum of specific ethnic groups will not only expand the general understanding of the genetic landscape of EOAD but also guide the design of cost-effective and comprehensive screening programs.

As one of the most popular and cost-effective technologies, high-throughput sequencing has become an essential tool for genome-wide associated study (GWAS) of many types of diseases including AD. Multiple studies have recently revealed a comprehensive genetic landscape of EOAD in Caucasian and Asian populations [7–10]. However, the genetic database of minority populations and ethnic groups remain under-represented. While multiple genetic studies in East Asian ethnic groups (e.g. Japanese,
Chinese, Korean, Thai, Filipino and Malaysian) have been recently reported, the mutation prevalence and spectrum of EOAD-related genes in the Vietnamese remain largely unknown.

In this study, we presented the first genetic study in early-onset Alzheimer’s disease of the Vietnamese population. The genetic analysis was performed on targeted sequencing data of twenty-nine neurodegenerative genes obtained from a cohort of 51 Vietnamese individuals diagnosed with EOAD. We identified a novel candidate pathogenic mutation related to EOAD genes and estimated its prevalence in the Vietnamese population.

Materials and methods

Case selection

A total of 51 EOAD unrelated patients who met the following criteria were recruited: (1) ethnic Vietnamese, (2) reported objective cognitive decline and had a clinical diagnosis of AD by DSM-5 criteria, (3) from 18–65 years old, and (4) currently outpatient. The exclusion criteria were: (1) other causes of cognitive impairment supported by magnetic resonance imaging (MRI), (2) individuals with potentially hindering medical or mental disorders. The participants have approved and given written informed consent to the anonymous reuse of their genomic data for this study. All genomic data were de-identified and aggregated for genetic analysis of the population.

Targeted sequencing assay

A panel of genes known to be associated with the inherited forms of AD was used for targeted sequencing in the Vietnamese cohort [9]. The candidate genes were evaluated in the following order: i) EOAD-causing genes (APP, PSEN1, PSEN2); ii) known risk factor genes for Alzheimer’s disease (APOE, TREM2, S100A9, CR1, BIN1, CLU, CTNNA3, DNMBP, SORL1, BACE1, PICALM, GAB2, LPR6, ADAM10, ABCA7, CD33, TOMM40); iii) causal genes for Frontotemporal dementia (C9orf72, MAPT, GRN, DCTN1, FUS, UBQLN2, VCP, TARDBP, TBK1).

DNA was extracted from blood samples using GeneJet Whole Blood genomic DNA Kit (Thermo Fisher, USA). Sequencing libraries were prepared from genomic DNA using the NEBNext Ultra II FS DNA library prep kit (New England Biolabs, USA) following the manufacturer’s instructions. Concentration of sequencing libraries was quantified using QuantiFlour dsDNA system (Promega, USA). Equal amounts of libraries were pooled together and hybridized with xGen Inherited Diseases Panel (IDTDNA, USA). Targeted sequencing was done on the NextSeq 550 platform using paired-end 2 x 75 bp Reagent Kit from Illumina (San Diego, CA, USA).

Variant calling analysis

Quality control of sequencing data was done with FastQC (version 0.11.9) [11]. Pair-end reads were aligned to the human reference genome (build GRCh38) using BWA package BWA and SAMtools packages [12,13]. Duplicate reads were then removed using Picard tools (Broad Institute). Germline variants were called using GATK 3.8 [14]. A custom pipeline with call to BWA, Picard, and GATK packages were built to perform the above-mentioned analysis steps. The final variant call set was annotated against dbSNP (version 151) and ClinVar (version 20191105) databases and analyzed for their potential consequences using Ensemble VEP annotator package [15–17]. Only non-synonymous and splicing acceptor/donor mutations in the exons were reported.

Sanger sequencing for variant validation

Sanger sequencing was used to confirm possible pathogenic mutations identified by next-generation sequencing. Custom primers were designed and synthesized by IDTDNA to amplify the region around the mutations. Sanger sequencing was performed on an ABI 3500 DNA analyzer at Center of Molecular Biomedicine (University of Medicine and Pharmacy at Ho Chi Minh City). Chromatograms were analyzed with CodonCode Aligner software (version 9.0.0) [18].

Results

Study cohort

The cohort of this study included 51 EOAD patients recruited from the University Medical Center at Ho Chi Minh City. The ages and clinical information of the participants are summarized in Table 1, supplementary material. The average age of onset (AOO) was 58 years with the youngest at 39 years old. The female versus male ratio was 62.7% versus 37.3%, which is consistent with previous observation on the disparity of gender in EOAD patients [19].

APP/PSEN1/PSEN2 variants in the study cohort

In APP gene, two missense mutations p.Val717Ile and p.Gly383Glu were identified in three patients (Table 1).
The APP p.Val717Ile, the most frequent variant in multiple ethnic groups, was found as heterozygous variant in two patients whose dementia symptoms started at the age of 51 and 39 (Figure 1a). Both patients’ families have known history of dementia with multiple affected relatives. A novel mutation p.Gly383Glu (submitted to Clinvar) was found in a female patient who exhibited signs of dementia at the age of 55 (Figure 1b). The APP p.Gly383Glu was predicted as deleterious and probably damaging by the PolyPhen and Sorting Intolerant From Tolerant (SIFT) packages, respectively. Although in silico predictions seemed to support pathogenicity of this mutation, biological function study is required to validate its impact. Therefore, this mutation was considered a variant of uncertain significance according to ACMG classification criteria. This Gly-to-Glu substitution in exon 9 was located in the dimerization domain, where mutations were rarely reported. In a genetic analysis of EOAD in four East Asian ethnic groups, Giau et al. had also reported two APP mutations in the dimerization domain (p.Glu145Lys and p.Pro484Ser) in two Korean patients [9]. Even though the variants were not identical, these variants were in the same dimerization domain and identified in Asian EOAD patients, suggesting a possible association of these variants with AD.
In presenilin-1 (PSEN1), we identified two mutations (p.Pro264Leu and p.Lys311Arg) in three patients (Table 1). The p.Pro264Leu mutation was observed in two patients: a 53-years-old male and a 56-years-old female (Figure 1c). This mutation has been reported in many EOAD patients from Caucasian to Asian ethnic groups, all with similar AOO range [7, 8, 10]. A female patient with age of onset at 59 years carried the p.Lys311Arg variant (Figure 1d). Interestingly, this patient has been shown to disrupt amyloid-β production and tau phosphorylation [10, 20]. To the best of our knowledge, this marks the first time this mutation is reported in a sporadic EOAD case.

**Genetic variants in other AD-associated genes**

In 45 cases without APP and PSEN1 mutations, 16 patients carried at least one copy of APOE ε4 allele, of which eight were homozygous APOE ε4 allele. In an unpublished study of late-onset Alzheimer’s disease, we identified only 6 subjects carrying one ε4 allele from a control group of 28 healthy individuals (aged 65 and older). Therefore, the frequency of APOE ε4 allele carrier was higher in EOAD cases compared to the control group. The ε4 allele rates (35.5% for one ε4 allele, 17.8% for two ε4 allele) was consistent with previous report in Chinese population [10], suggesting that APOE ε4 allele could also contribute to the development of EOAD cases without APP/PSEN1 mutations.

In the additional twenty five genes associated with AD, we identified seven missense mutations (1 in BIN1, 1 in CR1, 1 in CLU, 1 in PICALM, 3 in SORL1) and one stop-gained mutation in SORL1 (Table 2). Each of these eight mutations was identified in a single patient and none of these patients had homozygous form of APOE ε4 allele. Genome database reported all eight mutations with very low frequencies in multiple ethnic groups (<1%). Since there was no information of reported pathogenicity, we used Ensemble VEP annotator to predict potential effects of these variants. The stop-gained mutation was predicted to be high impact and might cause loss-of-function in mutant cells. Three missense variants (rs374782165 and rs751110498 in SORL1, rs144391901 in BIN1) were predicted as deleterious/probably damaging by both PolyPhen and SIFT tools. Those high-impact coding variants may be critical for clinical interpretation. Since additional functional and/or segregational data is required to establish its pathogenicity, we classified these variants as Variants of Uncertain Significance for now.

**Segregation of the APP pVal717Ile mutation in a family**

To further analyse the segregation of APP p.Val717Ile in a family, we performed the same targeted sequencing assay in all first-degree relatives of a male patient AZ108 carrying APP p.Val717Ile mutation (Figure 2). In this family, the deceased mother of the proband had developed cognitive impairment at the age of 44 but was not properly diagnosed due to lack of healthcare at the time while his father did not present any symptom of dementia. The most interesting case in this segregation analysis is the sister of proband’s mother, who also expressed symptom of dementia at early age. Unfortunately, we have not been able to perform test for this case yet due to her health problem and transportation difficulty. Out of the five siblings of the proband, four were found to also carry the same APP p.Val717Ile mutation as the proband. However, none of the four siblings showed symptoms of cognitive impairment at the time of writing this paper. Additionally, the spouse of the proband did not carry this mutation and one of the two offsprings of the proband is positive for the APP p.Val717Ile mutation.

**Discussion**

Early-onset Alzheimer’s disease is the form of AD with relatively clear genetic markers for molecular diagnosis. However, the mutation prevalence and spectrum of EOAD-related genes in the Vietnamese remain

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**Table 2. Mutations identified in sixteen AD-associated genes in Vietnamese EOAD patients.**

| Genes | DNA change | Protein change | Effect | PolyPhen | SIFT | rsID | AOO* Gender |
|-------|------------|----------------|--------|----------|------|------|-------------|
| BIN1  | c.787C > T | p.Arg263Trp     | Missense| Deleterious| Probably_damaging | rs144391901 | 51/M |
| CR1   | c.4681G > A | p.Val1561Met   | Missense| Tolerated | Probably_damaging | rs41274768 | 57/F |
| CLU   | c.984C > G | p.Asp328Glu     | Missense| Tolerated | Benign | rs9331939 | 58/F |
| PICALM| c.475A > G | p.Met159Val     | Missense| Deleterious| Benign | rs37304649 | 59/M |
| SORL1 | c.1871C > T | p.Pro624Leu     | Missense| Deleterious | Probably_damaging | rs374782165 | 63/F |
| SORL1 | c.2857C > T | p.Arg953Cys     | Missense| Tolerated | Probably_damaging | rs47925344 | 55/F |
| SORL1 | c.5395C > T | p.Arg1799Ter    | Stop_gain | – | – | rs759988544 | 62/F |
| SORL1 | c.5665A > C | p.Thr1889Pro    | Missense| Deleterious | Possibly_damaging | rs751110498 | 62/M |

*AOO: age of onset. Gender: F (Female), M (Male).
largely unknown, thus hinders the effort to design a cost-effective assay for detection and/or diagnostic of EOAD in this population. In this study, we aimed to provide a first genetic analysis of mutations in EOAD patients from Vietnam. We recruited a cohort of 51 Vietnamese individuals who were from 18–65 years old and had a clinical diagnosis of AD according to DSM-5 criteria. One limitation in assessment of our cohort was the lack of cerebrospinal fluid biomarker measurements. To minimize the number of atypical EOAD forms, we applied more stringent DSM-5 criteria for possible Alzheimer’s disease. Specifically, we used Mini-Mental State Examination (MMSE: cut-off score is 24) as a global dementia screening combined with 5 independent function tests (word list recall, visuo-motor speed, attention, language and visuoconstruction test) to determine patients with possible Alzheimer’s disease. If one of the function tests revealed atypical presentation, we removed these cases from our cohort. In our study, we also added causal genes for frontotemporal dementia for better differential diagnosis.

We next performed targeted sequencing for a panel of twenty-nine genes including 3 known EOAD-causing genes (APP, PSEN1, PSEN2), 17 known risk factor genes for Alzheimer’s disease and 9 known genes for Frontotemporal dementia (a type of dementia with similar symptoms as AD). We identified six patients carrying mutations in APP or PSEN1 genes but not in PSEN2 gene. While two mutations (APP p.Val717Ile and PSEN1 p.Pro264Leu) were known pathogenic mutations for EOAD [21], the other two mutations have not been found to associate with EOAD. Thus, the estimate detection rate of APP/PSENs mutations in Vietnamese EOAD patients was 11.8%, similar to the mutation rate reported by multiple genetic studies (ranging from 6% to 16%) [7–10].

We reported a novel missense mutation of APP gene (p.Gly383Glu) in a female patient (G1771) with AOO of 55 year. This novel mutation was also confirmed using Sanger sequencing. Although this mutation locates outside of the amyloid-beta region, both PolyPhen and SIFT tools predict it as likely pathogenic. This patient started to develop language impairment three years ago despite no history of dementia in her family. When her MRI scans revealed mild cerebral atrophy, cognitive function assessment by DSM-5 scale on this patient confirmed the AD diagnosis. Interestingly, her parents did not show any sign of dementia while her siblings may be too young to express any symptom at the moment (Figure 3a). Follow-up checking showed deleterious dementia status and corticobasal degeneration (CBD) in patient G1771. Future clinical and segregational analyses will provide more information about the mutation’s impact in the family. In addition, further biological assessment of this novel mutation is required to validate its classification as a pathogenic variant.

Figure 2. Segregation analysis of four generations in a family case (AZ108) carrying APP p.Val717Ile mutation showed five other members with same mutation. Family members in the second generation (II-1, II-2, II-5) expressed signs of dementia at very young age despite lack of AD diagnosis. Proband: III-2.
We also found a previously reported pathogenic mutation of *PSEN1* gene (p.Lys311Arg) in one EOAD patient (AZ037). This mutation was reported to increase amyloid Aβ42/Aβ40 ratio and tau phosphorylation *in vitro* [20]. Interestingly, this mutation was only discovered in four Chinese LOAD patients [10]. However, the patient also carried homozygous *APOE* ε4 allele in addition to *PSEN1* p.Lys311Arg mutation. While *APOE* ε4 genotypes may contribute to early progressing of Alzheimer’s disease, it requires more studies to investigate potential epistasis between *APOE* and *PSEN1* genes. At present, only the mother of this patient was reported with dementia symptoms while other immediate family members have not showed any dementia signs (Figure 3b). Further genetic segregation of this family is required to understand penetrance of this mutation in EOAD.

Segregation analysis in the family of a case with *APP* p.Val717Ile revealed four siblings of the proband carrying the same mutation (Figure 2). Genetic counseling had been provided to the family and closely monitoring for signs of dementia would help early diagnosis for these carrier members. This result demonstrated the advantage of genetic studies and their important role to guide cost-effective and comprehensive screening programs for EOAD specific to the Vietnamese population.

Our study also reported eight mutations in five AD-associated genes (*BIN1, CR1, CLU, PICALM, SORL1*) from eight different EOAD patients. Functional prediction tools suggested that these mutations might have damaging impact to the proteins. Most recent studies have placed *SORL1* as a major genetic determinant in both EOAD and LOAD [22–24]. *In vitro* cell culture analyses found that loss-of-function mutations in
**Ethics approval and consent to participate**

The institutional ethics committee of the University Medical Center, Ho Chi Minh City, Vietnam approved this study. All human genetic data of the participants was processed following the standard guidelines set by the University Medical Center, Ho Chi Minh City, Vietnam. All the participants have approved and provided written consent to the anonymous re-use of their genomic data for this study.

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**References**

[1] Dourlen P, Kilinc D, Malmanche N, et al. The new genetic landscape of Alzheimer's disease: from amyloid cascade to genetically driven synaptic failure hypothesis? Acta Neuropathol. 2019;138(2):221–236.

[2] Giri M, Zhang M, Lu Y. Genes associated with Alzheimer's disease: an overview and current status. Clin Interv Aging. 2016;11:665–681.

[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):704–706.

[4] Murrell J, Farlow M, Ghetti B, et al. A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science. 1991;254(5028):97–99.

[5] Campion D, Flaman JM, Brice A, et al. Mutations of the presenilin 1 gene in families with early-onset Alzheimer's disease. Hum Mol Genet. 1995;4(12):2373–2377.

[6] Chávez-Gutiérrez L, Bammens L, Benilova I, et al. The mechanism of γ-Secretase dysfunction in familial Alzheimer disease. Embo J. 2012;31(10):2261–2274.

[7] Lanoiselee HM, 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:e1002270.

[8] Giau VV, Bagyinszky E, Youn YC, et al. PSEN1, and PSEN2 mutations in Asian patients with early-onset Alzheimer Disease. JMS. 2019;20(19):4757.

[9] Giau VV, Bagyinszky E, Yang YS, et al. Genetic analyses of early-onset Alzheimer's disease using next generation sequencing. Sci Rep. 2019;9(1):8368.

[10] 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):178–191.

[11] FastQC. [https://www.bioinformatics.babraham.ac.uk/projects/fastqc/](https://www.bioinformatics.babraham.ac.uk/projects/fastqc/).
[12] Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997v2 [q-bio.GN]. 2013.

[13] Li H, Handsaker B, Wysoker A, 1000 Genome Project Data Processing Subgroup, et al. The sequence alignment/map format and SAMtools. Bioinformatics. 2009;25(16):2078–2079.

[14] Van der Auwera GA, et al. From FastQ data to high confidence variant calls: the genome analysis toolkit best practices pipeline. Curr. Protoc. Bioinformatics. 2013;43:11.10.1–11.10.33.

[15] Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res. 2001;29(1):308–311.

[16] Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014;42(Database issue):D980–D985.

[17] McLaren W, Gil L, Hunt SE, et al. The Ensembl variant effect predictor. Genome Biol. 2016;17(1):122.

[18] Aligner C. https://www.codoncode.com/aligner/.

[19] Chene G, BA. Gender and incidence of dementia in the Framingham heart study from mid-adult life. Alzheimers Dement. 2015;11(2015):310–320.

[20] Dong J, Qin W, Wei C, et al. A novel PSEN1 K311R mutation discovered in Chinese families with late-onset Alzheimer’s disease affects amyloid-β production and Tau phosphorylation. JAD. 2017;57(2):613–623.

[21] Alzforum mutation database. https://www.alzforum.org/mutations.

[22] Cuccaro ML, Carney RM, Zhang Y, et al. SORL1 mutations in early- and late-onset Alzheimer disease. Neurol Genet. 2016;2(6):e116.

[23] Holstege H, van der Lee SJ, Hulsman M, et al. Characterization of pathogenic SORL1 genetic variants for association with Alzheimer’s disease: a clinical interpretation strategy. Eur J Hum Genet. 2017;25(8):973–981.

[24] Campion D, Charbonnier C, Nicolas G. SORL1 genetic variants and Alzheimer disease risk: a literature review and meta-analysis of sequencing data. Acta Neuropathol. 2019;138(2):173–186.