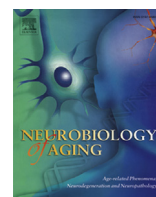


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# Neurobiology of Aging

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## Genetic screening in early-onset Alzheimer's disease identified three novel presenilin mutations



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### ABSTRACT

Mutations in presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), and amyloid precursor protein (*APP*) are major genetic causes of early-onset Alzheimer's disease (EOAD). Clinical heterogeneity is frequently observed in patients with *PSEN1* and *PSEN2* mutations. Using whole exome sequencing, we screened a Dutch cohort of 68 patients with EOAD for rare variants in Mendelian Alzheimer's disease, frontotemporal dementia, and prion disease genes. We identified 3 *PSEN1* and 2 *PSEN2* variants. Three variants, 1 in *PSEN1* (p.H21Profs\*2) and both *PSEN2* (p.A415S and p.M174I), were novel and absent in control exomes. These novel variants can be classified as probable pathogenic, except for *PSEN1* (p.H21Profs\*2) in which the pathogenicity is uncertain. The initial clinical symptoms between mutation carriers varied from behavioral problems to memory impairment. Our findings extend the mutation spectrum of EOAD and underline the clinical heterogeneity among *PSEN1* and *PSEN2* mutation carriers. Screening for Alzheimer's disease-causing genes is indicated in presenile dementia with an overlapping clinical diagnosis.

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### 1. Introduction

Early-onset Alzheimer's disease (EOAD) accounts for 1%–2% of all Alzheimer's disease (AD) cases. It can be caused by mutations in presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), and amyloid precursor protein (*APP*) in an autosomal dominant inheritance pattern (Goate et al., 1991; Levy-Lahad et al., 1996; Sherrington et al., 1995). To date, more than 280 mutations have been found in *PSEN1*, *PSEN2*, and *APP* ([www.molgen.ua.ac.be/ADMutations](http://www.molgen.ua.ac.be/ADMutations)) (Cruts et al., 2012).

Presenilin 1 and presenilin 2 proteins are both key components of gamma secretases, which process APP by cleaving into amyloid beta ( $A\beta$ ) fragments (Karch et al., 2014). Mutation in these genes impairs the proteolytic activity of gamma secretases, resulting in a disbalance of  $A\beta_{40}$  and  $A\beta_{42}$  (Weggen and Beher, 2012). Considerable heterogeneity is found in the clinical presentation of *PSEN1* and *PSEN2* mutation carriers, including initial behavioral, language, and dysexecutive problems, myoclonus, seizures, spasticity, and hallucinations (Jayadev et al., 2010; Ryan et al., 2016). The age at onset among mutation carriers also varies greatly, ranging from 23 to 71 years, even in families with the same mutation (Ryman et al.,

2014). Neuropathologically, *PSEN1* mutation carriers often have more neuronal loss in the frontotemporal cortex than sporadic AD cases (Shepherd et al., 2009). Furthermore, more neocortical senile plaques and higher  $A\beta_{42}/A\beta_{40}$  plaque ratios are observed in *PSEN1* and *PSEN2* mutation carriers than sporadic AD cases.

In this study, we assessed the contribution of rare variants in Mendelian AD (*PSEN1*, *PSEN2*, and *APP*), frontotemporal dementia (FTD; *MAPT*, *GRN*, *TARDBP*, *VCP*, *CHMP2B*, *FUS*, and *TBK1*), and prion disease genes (*PRNP*) in a Dutch cohort of 68 patients with EOAD using whole exome sequencing (WES). We found 3 novel and 2 reported variants in *PSEN1* and *PSEN2*. We describe the clinical and available pathological features of the *PSEN1* and *PSEN2* variant carriers.

### 2. Methods

#### 2.1. Subjects

Patients were included either by referral to the Department of Neurology of the Erasmus Medical Center (Rotterdam, the Netherlands) or by visits to (nursing) homes. Patients underwent a clinical examination, neuropsychological assessment, neuroimaging, and if indicated, a lumbar puncture. The diagnosis of probable AD was established according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's

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Disease and Related Disorders Association (NINCDS-ADRDA) criteria for AD (McKhann et al., 2011). EOAD was defined as an age at onset of  $\leq 65$  years. Family history was defined as positive if the patient had at least one first degree relative with dementia. Cerebrospinal fluid (CSF) profile consistent with AD was defined as  $A\beta_{42} < 550$  pg/mL,  $\tau > 375$  pg/mL, and phospho- $\tau > 53$  pg/mL or  $\tau/A\beta_{42} > 0.52$  (Duits et al., 2015). We selected 68 patients with EOAD for WES based on their initial clinical diagnosis of probable AD and/or CSF profile consistent with AD, as mentioned previously. CSF was present in 41 patients, and pathological diagnosis of AD was found in 4 patients.

The study was approved by the Medical Ethical Committee of the Erasmus Medical Center, and written informed consent was obtained from all participants or their legal representatives.

## 2.2. Genetic analysis

DNA from all samples was prepared with the Illumina TruSeq Paired-End Library Preparation Kit, and 100-base-pair paired-end reads were acquired by sequencing the libraries on a HiSeq 2000. Exomes were captured using NimbleGen SeqCap EZ Exome Capture Kit v2. All data were generated at the Human Genomics Facility (HuGeF; [www.glimdna.org](http://www.glimdna.org)) at the Erasmus Medical Center Rotterdam. Sequencing reads were aligned to the hg19 human genome assembly using BWA-MEM (version 0.7.3a) (Li and Durbin, 2009), followed by duplicate marking and sorting alignments by Picard Tools (version 1.9) (Li and Durbin, 2009). Subsequently, Genome Analysis Tool Kit (version 3.3) was used to perform indel realignment, base quality score recalibration, and variant calling (McKenna et al., 2010). Subsequently, we used variant quality score recalibration from Genome Analysis Tool Kit to filter out low-quality variants using thresholds of 90 for single-nucleotide variants and 50 for indels. All individuals in the WES data were checked for sex concordance using Plink (Purcell et al., 2007). Variants were annotated using ANNOVAR (Wang et al., 2010).

We focused on missense, nonsense, splicing, and frameshift variants in Mendelian AD (*PSEN1*, *PSEN2* and *APP*), FTD (*MAPT*, *GRN*, *TARDBP*, *VCP*, *CHMP2B*, *FUS*, and *TBK1*), and prion disease genes (*PRNP*) as described in previous studies (Blauwendraat et al., 2016; Perrone et al., 2018). Any identified variants with a minor allele frequency of  $\geq 0.1\%$  in the genome aggregation database (gnomAD), Healthy EXomes (HEX), Genome of the Netherlands, and in-house WES data from the Rotterdam Study were filtered out (Guerreiro et al., 2018a,b; Lek et al., 2016; The Genome of the Netherlands, 2014; van Rooij et al., 2017). Subsequently, we interpreted the identified variants using the Alzheimer Disease & Frontotemporal Dementia Mutation Database ([www.molgen.ua.ac.be/admutations/](http://www.molgen.ua.ac.be/admutations/)) (Cruts et al., 2012), AlzForum ([www.alzforum.org/mutations](http://www.alzforum.org/mutations)) database, and a literature search. Combined Annotation Dependent Depletion (CADD) score was used to predict the pathogenicity of

the variants (Kircher et al., 2014). Variants in *PSEN1* and *PSEN2* were further classified according to the algorithm described by Guerreiro et al. (Guerreiro et al., 2010). All identified variants were confirmed by Sanger sequencing. Screening of chromosome 9 open reading frame (*C9orf72*) repeat expansions was performed on selected cases with upper and/or lower motor neuron signs or a family history positive for motor neuron sign.

## 2.3. Histology and immunohistochemistry

The Netherlands Brain Bank performed brain autopsy according to their Legal and Ethical Code of Conduct. Tissue blocks taken from all cortical areas, hippocampus, amygdala, basal ganglia, substantia nigra, pons, medulla oblongata, cerebellum, and cervical spinal cord were embedded in paraffin blocks and subjected to routine staining with hematoxylin and eosin, periodic acid-Schiff reaction, and silver staining. The slides were also immunochemically stained with Anti- $\beta$ -Amyloid, 1–42 (12F4, dilution 1:400; BioLegend), Anti- $\beta$ -Amyloid, 1–40 (11A50-B10, dilution 1:400; BioLegend),  $\alpha$ -synuclein (NCL-ASYN, dilution 1:10,000; Novocastra), and AT8 (MN1020, dilution 1:200; Thermo Fisher Scientific). Braak stage was ascertained according to the revised National Institute on Aging–Alzheimer's Association guidelines (Montine et al., 2012).

## 3. Results

The mean age at onset in our cohort of 68 EOAD cases was 57.7 years (range 51–65). A positive family history was found in 39 cases (57%).

### 3.1. Mutation screening

After filtering, we found 5 rare variants, 3 in *PSEN1* and 2 in *PSEN2* (Table 1). Two variants (p.A79V and p.P264L) in *PSEN1* were previously described in EOAD cases (Campion et al., 1995; Cruts et al., 1998). One single *PSEN1* (p.H21Profs\*2) (Fig. 1) and both *PSEN2* (p.A415S and p.M174I) variants were novel. No rare variants were found in *APP* gene. All variants, except one in *PSEN1* (p.A79V), were unknown in gnomAD, HEX, GoNL, and exome data from the Rotterdam Study. No rare variants in *SORL1*, *TREM2*, or *ABCA7* have been found in the 5 *PSEN* carriers. The main clinical features of the *PSEN1* and *PSEN2* mutations carriers are summarized in Table 2.

### 3.2. Novel *PSEN1* mutation p.H21Profs\*2

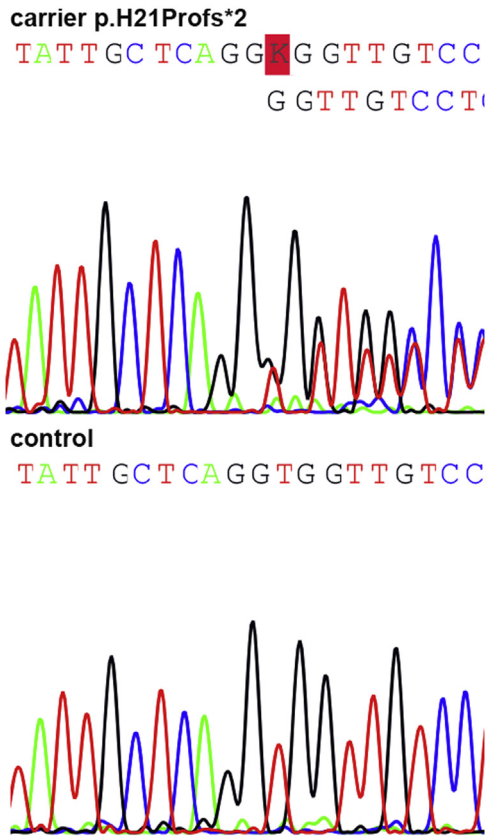
*PSEN1* p.H21Profs\*2 is located in exon 3, which codes for the N-terminal domain. The female carrier, aged 60 years, presented with progressive memory impairment, followed by problems in orientation, housekeeping, verbal expression, and loss of initiative. Neurological examination 1 year after onset showed a Mini-Mental

**Table 1**  
Rare variants in *PSEN1* and *PSEN2* identified in the EOAD cohort

Variant interpretation	Gene	Exon	Nucleotide change	Protein change	Status	Protein domain	GnomAD	CADD phred score
Known pathogenic	<i>PSEN1</i>	4	c.236C>T	p.A79V	rs63749824	N-terminal	$1.44 \times 10^{-02}$	33
	<i>PSEN1</i>	8	c.791C>T	p.P264L	rs63750301	HL-VI a	$4.08 \times 10^{-03}$	35
Probable pathogenic <sup>a</sup>	<i>PSEN2</i>	7	c.522G>A	p.M174I	Novel	HL-I	0	20.8
	<i>PSEN2</i>	13	c.1243G>T	p.A415S	Novel	TM-IX	0	33
Variant of unknown significance	<i>PSEN1</i>	3	c.62delA	p.H21Profs*2	Novel	N-terminal	0	NA

Key: CADD, Combined Annotation Dependent Depletion; EOAD, early-onset Alzheimer's disease; GnomAD, genome aggregation database; HL, hydrophilic loop; NA, not available; *PSEN1*, presenilin 1 (NM\_000021); *PSEN2*, presenilin 2 (NM\_000447); TM, transmembrane.

<sup>a</sup> Pathogenicity classification based on the algorithm proposed by Guerreiro et al. (2010).



**Fig. 1.** Electropherogram of PSEN1 p.H21Profs\*2 variant.

State Examination (MMSE) score of 23/30, mild bradyphrenia, and left-sided cogwheel sign during risperidone medication prescribed for nightmares. Neuropsychological assessment showed deficits in multiple domains including memory, executive functions, and visual perception; however, several tests were prematurely ended due to poor performance. MRI showed global brain atrophy and extensive white matter lesions in the parieto-occipital region. During follow-up, the patient was treated with acetylcholinesterase inhibitors until death, aged 65 years. Family history revealed a mother with dementia with an age at onset of 60 years.

**3.3. Novel PSEN2 mutation p.M174I**

The p.M174I variant in the PSEN2 gene was identified in a female patient who developed memory problems at the age of 53 years. This variant is located in the hydrophilic loop domain of the presenilin 2 protein. The patient developed progressive anomia and

memory impairment within the first 3 years. She had a depressed mood and was apathetic. Neurological examination (2 years after onset) showed an MMSE score of 18/30, short-term memory impairment, dyscalculia, bradyphrenia, and apraxia. Over time, the patient developed behavioral changes, including aggression, restlessness, obsessive thinking, blunted emotions, and binge eating. MRI showed generalized cerebral atrophy most prominent of the frontal lobes; CSF was not available. The clinical diagnosis was either presenile AD with frontal presentation or a behavioral variant of FTD. After admission to a nursing home, she developed hallucinations, seizures, spasticity, and mutism. She died of pneumonia at the age of 64 years. The patient's mother had been diagnosed with AD at the age of 75 years.

Neuropathological examination of the p.M174I PSEN2 carrier revealed severe neuronal loss and gliosis of the frontal, temporal, and parietal cortices, cornu ammonis (CA) region 1 of the hippocampus and to a lesser extent in the other CA regions, subiculum, and occipital cortex. Abundant AT8-positive threads and tangles accompanied by many Aβ-positive senile plaques of variable size and morphology were found in the neocortex, predominantly in the frontal cortex, and to a lesser extent in the parietal and temporal cortices and CA1 of the hippocampus (Fig. 2A–E). Classical, predominantly Aβ<sub>42</sub>-positive plaques with a dense core were seen in 5%–10% of the plaques. Only a small number of plaques were stained Aβ<sub>40</sub>-positive (Fig. 2B and C). The severe involvement of the frontal, temporal, and parietal cortices is consistent with advanced stage AD, Braak stage 6, A3B3C3 (Montine et al., 2012). Furthermore, many α-synuclein-positive Lewy bodies (LBs) were seen (Fig. 2F), especially in the amygdala and parahippocampal cortex and a few in the substantia nigra and brainstem.

**3.4. Novel PSEN2 mutation p.A415S**

The PSEN2 (p.A415S) variant, located in the transmembrane IX domain, was identified in a female patient aged 59 years who presented with memory impairment and gait disturbance. She developed difficulties in verbal expression with problems in word finding, hallucinations, and myoclonus. Her grandmother, aunt, and uncle also had dementia, all with onset age > 65 years. Neurological examination revealed an MMSE score of 17/30, hyperreflexia in the arms and legs, and a spastic gait. Neuropsychological assessment showed impairment in memory, language, perceptuospatial skills, and praxis. MRI showed global cerebral and cerebellar atrophy. Her CSF was compatible with an AD profile with decreased Aβ (203 pg/mL), increased phospho-tau (95 pg/mL), and total tau (551 pg/mL). Cognitive functions including verbal expression and motor functioning deteriorated over the following 3 years, and she was admitted to a nursing home. At a later stage, aged 65 years, she became mutistic and wheelchair bound.

**Table 2**  
Clinical characteristics of the mutation carriers in PSEN1 and PSEN2

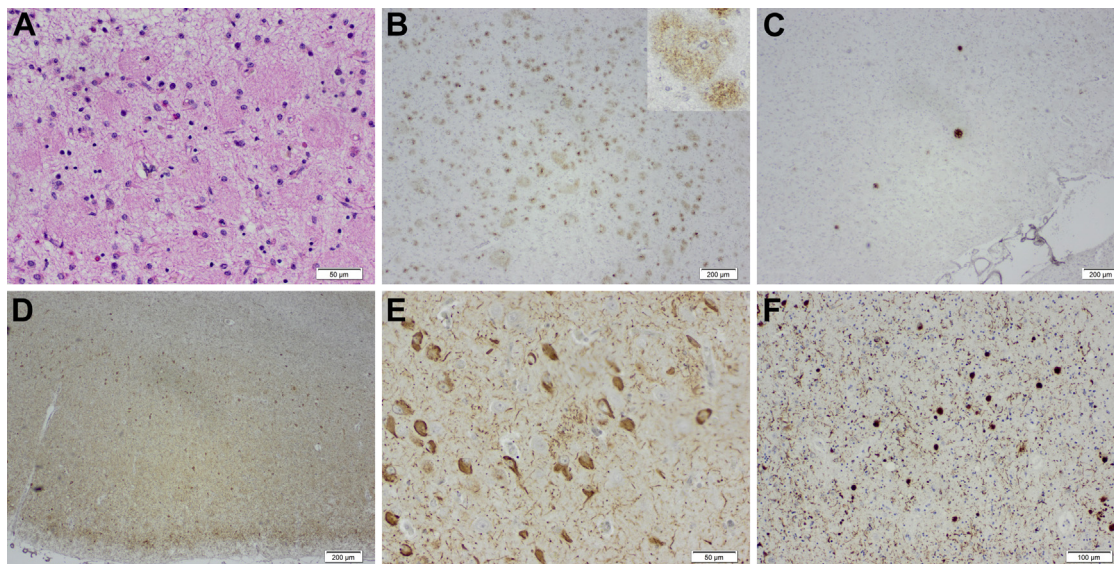
Gene	Protein change	Diagnosis	AAO	Family history	Initial presentation	Behavioral symptoms <sup>a</sup>	Myoclonus	Seizure	Delusions/hallucinations	Spasticity	Extrapyramidal sign
PSEN1	p.H21Profs*2	AD	60	Positive	Memory impairment	No	No	No	No <sup>b</sup>	No	No
PSEN1	p.A79V	AD/DLB	64	Positive	Behavioral changes	Suspicious	No	No	Yes	No	Yes
PSEN1	p.P264L	AD/FTD + PLS	56	Positive	Gait disturbance	Labile affect	No	No	No	Yes	No
PSEN2	p.M174I	AD/FTD	51	Positive	Memory impairment	Depressed	Yes	Yes	Yes	Yes	Yes
PSEN2	p.A415S	AD	59	Negative	Gait disturbance	No	Yes	No	Yes	Yes	Yes

Symptom presentation referred to the clinical presentation at disease onset.

Key: AAO, age at onset; AD, Alzheimer's disease; DLB, Dementia with Lewy bodies; FTD, frontotemporal dementia; PLS, primary lateral sclerosis; PSEN1, presenilin 1; PSEN2, presenilin 2.

<sup>a</sup> Behavioral symptoms at first visit.

<sup>b</sup> Nightmares treated with risperidone.



**Fig. 2.** Pathological features of the *PSEN2* p.M174I mutation carrier. Histological section and immunostaining in the frontal cortex and hippocampus of the *PSEN2* p.M174I carrier. Hematoxylin and eosin staining of the frontal cortex showed many plaques and eosinophilic bodies (A). Extensive numbers of A $\beta$ <sub>42</sub>-immunoreactive (IR) plaques were observed in all layers of the frontal cortex (B), whereas only small numbers of A $\beta$ <sub>40</sub>-IR plaques were found (C). Immunostaining with tau protein showed many AT8-IR tangles and small threads in the frontal (D) and hippocampus (E). Numerous Lewy bodies positive for  $\alpha$ -synuclein were seen in the amygdala (F).

### 3.5. Previous reported *PSEN1* mutations p.A79V and p.P264L

The patient with *PSEN1* p.A79V mutation presented with cognitive impairment, delusions, hallucinations, and parkinsonism suggestive for probable dementia with Lewy bodies (DLBs) when aged 64 years (McKeith et al., 2005). Family history revealed 5 siblings and a father who had AD before the age of 65 years. Three of the affected siblings also suffered from hallucinations. DNA was only available in 1 sister with AD and onset age of 74 years who also carried the *PSEN1* p.A79V mutation.

The second mutation, *PSEN1* p.P264L, was found in a patient aged 56 years who presented with gait disturbance. Memory impairment, behavioral changes, and impairment in word comprehension and word finding were also observed early in the disease process. The clinical diagnosis was suspected behavioral variant of FTD or a frontal variant of AD with primary lateral sclerosis. The patient's grandmother, mother, and 1 sister also had dementia. Screening on *C9orf72* was negative.

## 4. Discussion

We identified 1 novel frameshift deletion in *PSEN1* (p.H21Profs\*2) and 2 novel missense in *PSEN2* (p.A415S and p.M174I) using the WES in a Dutch cohort with EOAD. These novel variants were not present in the public sequencing database and population-match exomes. In addition, we found 2 known *PSEN1* variants (p.A79V and p.P264L), reported as pathogenic in previous studies (Cruchaga et al., 2012). The *PSEN1* and *PSEN2* variant carriers had variable clinical presentation including memory impairment, behavioral changes, and pyramidal and extrapyramidal symptoms.

The first novel variant, *PSEN1* p.H21Profs\*2, is a frameshift mutation resulting in a premature stop codon in the sequences of exon 3, and as a consequence, a truncated protein. Frameshift deletions in *PSEN1* and *PSEN2* were reported as a possible genetic cause of EOAD; however, the pathogenicity is debatable (El Kadmiri et al., 2014; Jayadev et al., 2010; Perrone et al., 2018). Variable phenotypes of the frameshift deletion mutation carriers including AD, mild cognitive impairment, FTD, and amyotrophic lateral sclerosis have been reported. Segregation or functional studies supporting

the pathogenicity of these mutations are scarce. One study reported a reduced presenilin 2 protein expression in the lymphoblast cells of a *PSEN2* (p.G359Lfs\*74) mutation carrier compared with control, but a reduced presenilin 2 protein expression was also found in a mutation carrier with autopsy confirmed frontotemporal lobar degeneration (Perrone et al., 2018). We classified the pathogenicity of our novel variant as a variant of uncertain significance, as we were unable to investigate the functional effect due to the lack of additional blood or brain tissue from the patient.

The second novel variant, *PSEN2* p.M174I, was classified as probable pathogenic according to the algorithm proposed by Guerreiro et al. (Guerreiro et al., 2010) based on reported mutation in the same codon (p.M174V) and the altered A $\beta$  level at neuropathology. However, conflicting results about the pathogenicity of p.M174V have been reported (Cruchaga et al., 2012; Fernandez et al., 2017; Guerreiro et al., 2010; Lohmann et al., 2012). Although the pathogenicity of *PSEN2* p.M174V is unclear, our case carried a different *PSEN2* variant with a high CADD score of 20.8, which is unknown in gnomAD. Furthermore, the presence of a higher number of neocortical senile plaques with a higher A $\beta$ <sub>42</sub>/A $\beta$ <sub>40</sub> ratio in our case is consistent with the reported pathological features of *PSEN1* and *PSEN2* mutation carriers (Shepherd et al., 2009); therefore, this variant may be causative for AD.

The third novel variant, *PSEN2* p.A415S, was classified as probable pathogenic based on (1) conserved residues in *PSEN1* A431; (2) previously reported mutations in the same codon (A431E and A431V) (Matsushita et al., 2002; Rogaeva et al., 2001); and (3) CSF profile with low A $\beta$  and high phospho-tau and total tau indicative for AD. Furthermore, the damaging effect of this variant was also supported by a high CADD score of 33.

The atypical AD symptoms of the mutation carriers in our study have been frequently reported in *PSEN1* carriers (Ryan et al., 2016). Up to 16% of *PSEN1* carriers had an atypical presentation, and about 8% of *PSEN1* mutations carriers presented with behavioral changes at onset. Similar to previous reports, the *PSEN1* p.P264L carrier in our study also had symptoms consistent with spastic paraparesis (Jacquemont et al., 2002). Spastic paraparesis is present in 25% of *PSEN1* cases and is frequently associated with mutations beyond codon 200 (Ryan et al., 2016; Shea et al., 2016).

In addition, 3 of the 5 carriers in our study had delusions or hallucinations during the disease course. In one (*PSEN1* p.A79V) carrier, the clinical diagnosis was suspect for DLBs (McKeith et al., 2005) or AD because of the presence of hallucinations and parkinsonism together with memory impairments. Interestingly, 3 siblings of this carrier also had hallucinations, but prominent cognitive impairment made the clinical diagnosis of AD more likely. The association of *PSEN1* p.A79V with DLBs as phenotype has been reported previously (Meeus et al., 2012). Notably, the DLB phenotype has also been reported in a patient with *PSEN2* p.A85V mutation, which is homolog to *PSEN1* p.A79V (Piscopo et al., 2008). Neuropathological examination of the *PSEN2* p.A85V carrier showed abundant neocortical LBs and AD pathology, suggesting a link between this mutation and LB pathology. Up to 96% of LB pathology has been reported in familial AD with *PSEN1* mutations, whereas lower percentages have been reported in AD with *PSEN2* mutation (64%) and sporadic AD (60.7%) (Hamilton, 2000; Leverenz et al., 2006). However, this difference in LB pathology between these groups has not been confirmed in other studies (Lippa et al., 1998; Ringman et al., 2016). Unfortunately, no brain tissue of the *PSEN1* p.A79V carrier was present to examine the presence of LB pathology. Another possibility for the DLB phenotype in our case may be the presence of another gene mutation for DLBs. Recent studies identified the genetic association of variants in the glucocerebrosidase (*GBA*) gene with DLBs (Geiger et al., 2016; Guerreiro et al., 2018a,b; Nalls et al., 2013). In our cases, screening of *GBA* did not identify any rare variants in this gene (data not shown). Nevertheless, the presence of unidentified genetic factor(s) contributing to the DLB phenotype cannot be ruled out.

We used exome sequencing to screen for mutations in known dementia genes. Although we were able to identify small deletion and missense mutations using this method, large deletion such as DeltaE9 in *PSEN1* (Crook et al., 1998), *C9orf72* repeat expansions, and copy number variations variants can be missed, and thus, we may underestimate the frequency of the mutations. However, these mutations in *PSEN1* and *PSEN2* are rare, and the observed frequency of *PSEN* variants in 7% of our cohort of 68 EOAD cases is in line with those reported earlier by Brouwers et al. (Brouwers et al., 2008). Another limitation is that the follow-up time in most patients was limited to a few years, and we cannot rule out that additional symptoms may have developed later during their disease course. Finally, DNA was only available from a sister with AD of the p.A79V mutation carrier; we were unable to include other family members of the other mutation carriers. Therefore, segregation analysis could not be performed for these mutation carriers.

Our study provides further insights into the genetics of AD by identifying 3 novel mutations in *PSEN1* and *PSEN2* and highlights the clinical heterogeneity of the presenilin mutation carriers. Although overlapping clinical diagnosis with FTD or DLBs was found in some patients, we were unable to identify other probable pathogenic dementia-causing genes besides *PSEN1* and *PSEN2*. Genetic screening of AD causative genes is valuable in patients with clinically suspected EOAD with atypical clinical features, particularly in familial cases.

## Disclosure

The authors declare no actual or potential conflicts of interest.

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