Title: Misfolded α-synuclein in autosomal dominant Alzheimer's disease

Running head: Misfolded α-syn in ADAD

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Abstract

We analysed Lewy body (LB) pathology in 18 autosomal dominant Alzheimer's disease (ADAD) brains via immunohistochemistry (ICH). Real-time quaking induced conversion (RT-QuIC) was used to detect misfolded α-synuclein (α-syn) in 18 living ADAD CSF samples. Concomitant LB pathology was present in 44% ADAD brains. Only 6% CSF samples were positive for misfolded α-syn. In an additional AD sample, including sporadic AD, we observed that all patients with confirmed LB presented misfolded α-syn in post-mortem CSF regardless the LB staging. In conclusion, misfolded α-syn in CSF was scarce in symptomatic living individuals with ADAD, in contrast to brain tissue. These results might suggest a late appearance of LB pathology in ADAD.

Introduction

Autosomal dominant Alzheimer's disease (ADAD) is a rare form of Alzheimer's disease (AD) caused by mutations in *PSEN1*, *PSEN2* or *APP* genes. Both sporadic AD (sAD) and ADAD are characterised by extracellular amyloid-β (aβ) plaque accumulation and intracellular aggregation of hyperphosphorylated tau proteins ending up in synaptic and neuronal loss [1]. AD brains can also contain other pathological aggregates, such as Lewy Bodies (LBs), whose main component is misfolded α-synuclein (α-syn) [2]. LB are observed in 25% to 50% of both sAD and ADAD brains [3, 4]. However, the pathological mechanisms linking α-syn aggregation and AD and the timing of α-syn accumulation in these subjects are not well understood.

Aggregated abnormal α-syn might be used as a biomarker for α-synucleinopathies [5]. The Real-time quaking-induced conversion (RT-QuIC) technique has proven to be highly specific and sensitive to detect pathological α-syn seeds in the cerebrospinal fluid (CSF) of patients with LB diseases, even during the premotor stages of the disease [6, 7]. It has yet to be evaluated whether this technique can detect misfolded α-syn when presented as concomitant pathology with other primary proteinopathies, including AD.

The objective of our study was to gain a deeper understanding of the presence of abnormal α-syn in ADAD. We first describe the presence of LB pathology in our ADAD post-mortem cohort and determine the seeding activity of misfolded α-syn in CSF from living symptomatic ADAD patients. Finally, we evaluate the correlation between the detection of α-syn seeding activity with the RT-QuIC method in post-mortem CSF and the presence and staging of LB in AD brains.

Methods

ADAD cohort

The study included a cohort of 18 neuropathologically-confirmed ADAD patients (14 males and 4 females aged 36 – 78 years, mean 54.06 ± 7.52 years; 14 ADAD patients carried a *PSEN1* mutation and 4 carried an *APP* mutation) (Table 1) and 18 living symptomatic ADAD individuals: 17 carrying a *PSEN1* mutation and 1 an *APP* mutation (8 males and 10 females aged 26 – 59 years, mean 44.89 ± 8.46 years) (Table 2).

The Hospital Clínic de Barcelona Ethics committee approved the study (HCB/2020/1410) and all the participants signed written informed consent for brain or CSF donation for research.

Neuropathology examination

Brain samples were obtained from the Neurological Tissue Bank (NTB), Biobank-Hospital Clínic-FRCB-IDIBAPS, Barcelona, Spain. Neuropathological examination was performed according to standardized protocols [8]. Main primary antibodies in the immunohistochemistry (IHC) protocol

for this study were: anti-βA4 (6F/3D, Dako, Glostrup, Denmark), anti-tau (AT8; Thermo Scientific, USA), anti-α-synuclein (5G4; Analytik Jena, Germany), anti-TDP-43 phospho Ser409/410 (11-9; Cosmo Bio, Japan). Disease evaluation was performed according to international consensus criteria [9, 10].

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All brain donors with AD died in a stage of severe dementia.

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Real-time quaking induced conversion (RT-QuIC) analysis

RT-QuIC buffer composition was as follows: 10 mM phosphate buffer (pH 8), 10 μM Thioflavin-T (ThT), and 0.1 mg/mL human recombinant full-length (1–140 aa) α-syn (Sigma-Aldrich, Cambridge, UK). Reactions were prepared in a black 96-well, optical bottomed plate (Nalgene Nunc International, #265301) with 85 μL of RT-QuIC buffer and 15 μL undiluted CSF for a final reaction volume of 100 μL. Each sample was run in triplicate. The plates were sealed and incubated in a BMG OPTIMA FluoSTAR plate reader at 35°C for 120 h with intermittent shaking cycles: double orbital with 1 min shake (400 rpm), 14 min rest. ThT fluorescence measurements (450 nm excitation and 480 nm emission) were taken every 15 minutes. A positive response was defined as a relative fluorescence unit (rfu) value of 60.000. A positive response in two or more of the replicates was considered positive.

Results

Neuropathological diagnosis in post-mortem ADAD brains

AD staging revealed an A3B3C3 score in 17 subjects (94.4%) and an A3B2C3 score due to Braak IV in 1 subject (5.6%; PM4, Table 1). α-syn aggregates in the form of LB were present in 8 subjects (44.4%). In 4 positive subjects the deposits were present predominantly in amygdala (50%), in 2 deposits were limbic (25%), in 1 neocortical (12.5%) and in 1 were present in olfactory bulb only (12.5%) (Table 1). In addition, TDP-43 concomitant pathology was observed in 1 patient (5.6%, PM9) (LATE-NC stage 2).

RT-QuIC in CSF from living ADAD individuals

Analysis of CSF samples via RT-QuIC from living ADAD showed that only one *PSEN1* mutation carrier was positive for misfolded α-syn (5.6%) (Table 2).

RT-QuIC in post-mortem CSF and IHC results comparison

Seven (63.6%) post-mortem CSF samples from AD patients (1 ADAD and 6 sAD) were positive for misfolded α-syn using the RT-QuIC technique. All of them showed the presence of LB at the IHC at different stages (1 amygdalar, 3 limbic, 3 neocortical). In the same way, all the patients with positive IHC presented positive CSF results. None of the AD cases without LB pathology or the negative control used were RT-QuIC positive even after 120 h (Fig. 1).

Discussion

In the present study, we observed that 44% of ADAD brains showed LB pathology, the majority with predominant amygdala deposits. In contrast, only one ADAD patient carrying one *PSEN1* mutation, 6% of samples tested from symptomatic living ADAD individuals, presented in CSF detectable abnormal α-syn using RT-QuIC technology.

The presence of LB pathology in ADAD in our cohort was similar to previous cohorts [3, 4]. The low level of misfolded α-syn RT-QuIC positivity in CSF from living symptomatic ADAD individuals was unexpected based on previous and own data regarding the post-mortem IHC. This discrepancy could suggest a low sensitivity of the technique to detect abnormal α-syn in these individuals. Another possibility might be that the α-syn pathology appears late in the course of the disease in ADAD, as all the individuals tested were in mild-moderate phases of the disease.

To investigate the cause of this discrepancy, we evaluated post-mortem CSF and correlated the RT-QuIC results with the presence of LB pathology at post-mortem brain, in samples from ADAD and sAD. CSF post-mortem samples from patients with LB pathology were positive for abnormal α-syn using the RT-QuIC technique, unrelated to the stage of LB pathology (amygdalar, limbic, or neocortical) and viceversa. The CSF negative cases were also negative at IHC. Thus, post-

mortem CSF abnormal α-syn RT-QuIC results and the presence of LB pathology showed a complete concordance, supporting that the seeding evaluation of α-syn using RT-QuIC is a reliable technique for the study of the presence of LBs in this population. Thus, the low level of α-syn positivity in CSF from living individuals should not be attributed to low sensitivity of the technique to detect concomitant LB pathology. In this sense, these findings would then support the late appearance of the LB pathology in most ADAD individuals.

The presence of TDP-43 concomitant pathology was rare in this population (5.6%, LATE-NC stage 2), compared to late onset AD [3], which would suggest that even late, the concomitant presence of LB pathology is specific to AD [12], even in young genetic individuals.

We should, however, acknowledge we cannot exclude that the origin of the CSF (intraventricular in the post-mortem in contrast to lumbar CSF in living individuals) might influence the results. Due to the limited number of post-mortem CSF samples from ADAD (2), we included post-mortem sAD samples in the analysis, although the performance of both subtypes of AD seemed quite similar. Because there were only 2 genetic cases in our cohort, we can only speculate that the CSF α-syn RT-QuIC technique might detect a late LB pathology in ADAD.

Altogether, we provide new data about the presence and detection of α-syn aggregates in ADAD that suggest a late appearance of LB pathology in these individuals. The frequency and specificity of this concomitant pathology in post-mortem species in ADAD suggest a direct result of the biological process. Additional studies with a broader sample are needed to confirm these results.

Acknowledgements

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Author Contributions

RSV, LMP and AA designed the study, provided data and obtained funding. LFA and ORC prepared the samples, collected the data, performed the analysis and drafted the manuscript. RR and LN performed the RT-QuIC analysis. AL, MB and DE provided clinical data. All the authors reviewed and approved the final version of the manuscript.

Conflicts of Interest

RSV has served in Advisory boards Meetings for Wave Life Sciences, Ionis and Novo Nordisk and received personal fees for participating in educational activities from Janssen, Roche Diagnostics and Neuroxpharma and funding to her institution for research projects from Biogen and Sage Pharmaceuticals.The other authors do not declare conflicts of interest.

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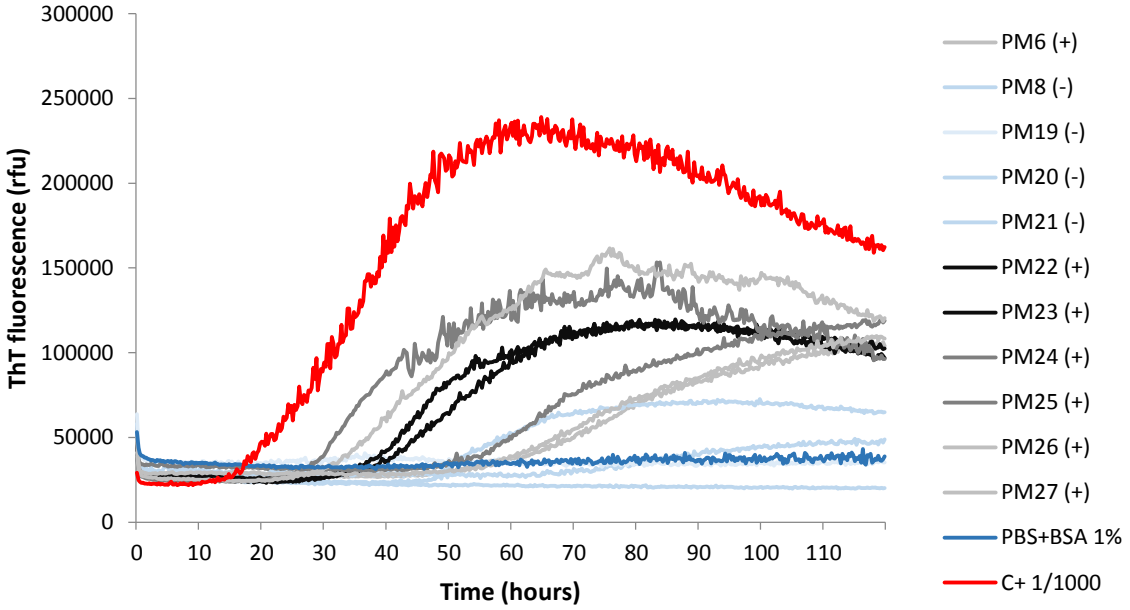
Figure 1. RT-QuIC detection of pathological α-synuclein (α-syn) seeding activity in CSF from 11 AD post-mortem samples



RT-QuIC kinetics measured by relative ThT fluorescence (rfu) from CSF of each individual cases with ADAD (PM6 and PM8) and with sAD (PM19 – 27) at 120 h were positive (+) for one ADAD individual (PM6) and 6 sAD subjects (PM22 – 27). Each curve consists of 3 duplicates. PM: post-mortem. PBS+BSA1%: negative control. C+ 1/1000: positive control.

Tables

Table 1. Neuropathological data from the 18 ADAD post-mortem (PM) subjects

Case #	Age at Death	Sex	Mutation	Lewy body IHC	Brain area
PM1	36	M	*APP I716L*	+	amygdala
PM2	56	M	*APP A713T*	+	amygdala
PM3	68	M	*APP duplication*	+	olfactory bulb
PM4	54	M	*APP duplication*	-	
PM5	44	M	*PSEN1 E120G*	-	
PM6	44	M	*PSEN1 G206D*	+	neocortical
PM7	53	F	*PSEN1 L286P*	-	
PM8	53	M	*PSEN1 L286P*	-	
PM9	56	F	*PSEN1 L286P*	-	*
PM10	48	F	*PSEN1 M139T*	+	amygdala
PM11	53	M	*PSEN1 M139T*	+	limbic
PM12	57	M	*PSEN1 M139T*	-	
PM13	60	M	*PSEN1 M139T*	+	limbic
PM14	64	M	*PSEN1 M139T*	+	amygdala
PM15	56	F	*PSEN1 P264L*	-	
PM16	60	M	*PSEN1 P264L*	-	
PM17	54	M	*PSEN1 V89L*	-	*
PM18	57	M	*PSEN1 V89L*	-	

PM: post-mortem

PSEN: Presenilin

APP: Amyloid-beta precursor protein

*: olfactory bulb not available

Table 2. Misfolded α-syn CSF RT-QuIC results from the 18 AD living (L) subjects and patient characteristics per diagnosis

Case #	Sample Age	Sex	Mutation	Misfolded α-syn	MMSE	CDR
L1	36	F	*APP I716T*	-	N/A	N/A
L2	39	M	*PSEN1 E280A*	-	13	1
L3	51	M	*PSEN1 G209E*	-	24	0.5
L4	59	M	*PSEN1 G209E*	-	28	0
L5	46	F	*PSEN1 G378R*	-	19	2
L6	48	M	*PSEN1 H163R*	-	18	2
L7	55	M	*PSEN1 I439S*	-	20	0.5
L8	59	F	*PSEN1 K239N*	-	20	1
L9	52	M	*PSEN1 K239N*	-	22	0.5
L10	45	M	*PSEN1 L173F*	-	15	2
L11	43	F	*PSEN1 L173F*	-	19	1
L12	37	F	*PSEN1 L286P*	-	28	0.5
L13	39	F	*PSEN1 L286P*	-	24	N/A
L14	42	M	*PSEN1 L286P*	-	24	N/A
L15	47	F	*PSEN1 L235R*	-	10	3
L16	45	F	*PSEN1 L282R*	-	22	0.5
L17	26	F	*PSEN1 S169P*	+	20	1
L18	39	F	*PSEN1 T116I*	-	19	2

L: Living

PSEN: Presenilin

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AD staging revealed an A3B3C3 score in 17 subjects (94.4%) and an A3B2C3 score due to Braak IV in 1 subject (5.6%; PM4, Table 1). α-syn aggregates in the form of LB were present in 8 subjects (44.4%). In 4 positive subjects the deposits were present predominantly in amygdala (50%), in 2 deposits were limbic (25%), in 1 neocortical (12.5%) and in 1 were present in olfactory bulb only (12.5%) (Table 1). In addition, TDP-43 concomitant pathology was observed in 1 patient (5.6%, PM9) (LATE-NC stage 2).

RT-QuIC in CSF from living ADAD individuals

Analysis of CSF samples via RT-QuIC from living ADAD showed that only one *PSEN1* mutation carrier was positive for misfolded α-syn (5.6%) (Table 2).

RT-QuIC in post-mortem CSF and IHC results comparison

Seven (63.6%) post-mortem CSF samples from AD patients (1 ADAD and 6 sAD) were positive for misfolded α-syn using the RT-QuIC technique. All of them showed the presence of LB at the IHC at different stages (1 amygdalar, 3 limbic, 3 neocortical). In the same way, all the patients with positive IHC presented positive CSF results. None of the AD cases without LB pathology or the negative control used were RT-QuIC positive even after 120 h (Fig. 1).

Discussion

In the present study, we observed that 44% of ADAD brains showed LB pathology, the majority with predominant amygdala deposits. In contrast, only one ADAD patient carrying one *PSEN1* mutation, 6% of samples tested from symptomatic living ADAD individuals, presented in CSF detectable abnormal α-syn using RT-QuIC technology.

The presence of LB pathology in ADAD in our cohort was similar to previous cohorts, where they found LB presence in 50% of ADAD post-mortem cases [3, 4]. The low level of misfolded α-syn RT-QuIC positivity in CSF from living symptomatic ADAD individuals was unexpected based on previous and own data regarding the post-mortem IHC. This discrepancy could suggest a low sensitivity of the technique to detect abnormal α-syn in these individuals. Another possibility might be that the α-syn pathology appears late in the course of the disease in ADAD, as all the individuals tested were in mild-moderate phases of the disease.

To investigate the cause of this discrepancy, we evaluated post-mortem CSF and correlated the RT-QuIC results with the presence of LB pathology at post-mortem brain, in samples from ADAD and sAD. CSF post-mortem samples from patients with LB pathology were positive for abnormal α-syn using the RT-QuIC technique, unrelated to the stage of LB pathology (amygdalar, limbic,

or neocortical) and viceversa. The CSF negative cases were also negative at IHC. Thus, post-mortem CSF abnormal α-syn RT-QuIC results and the presence of LB pathology showed a complete concordance, supporting that the seeding evaluation of α-syn using RT-QuIC is a reliable technique for the study of the presence of LBs in ADthis population. In a recent study, the sensibility of post-mortem CSF using the α-syn RT-QuIC technique to detect LBs in 14 patients with LBs as concomitant pathology in neuropathological confirmed AD, was lower, showing that only 64% of the samples were positive [12]. In the same study, however, the sensitivity of the α-syn RT-QuIC ranged from 100% in those cases with cortical LBs to 37,5% in cases with olfactory bulb only or brainstem predominant LB (not included in our sample), suggesting that the positivity of the post-mortem assay depends on the distribution of the LBs. In another study performed in cognitive impaired sporadic patients, 21% of participants with AD presented positive CSF α-syn RT-QuIC, a higher percentage of that observed in our study in ADAD, and much closer to the prevalence (33 – 42%) of LBs in neuropathological studies in sAD [13]. Thus, the low level of α-syn positivity in CSF from living individuals should not be attributed to low sensitivity of the technique to detect concomitant LB pathology. In this sense, these findings would then support the late appearance of the relevant LB pathology in most ADAD individuals.

The presence of TDP-43 concomitant pathology was rare in this population (5.6%, LATE-NC stage 2), compared to late onset AD [3], which would suggest that even late, the concomitant presence of LB pathology is specific to AD [142], even in young genetic individuals.

We should, however, acknowledge we cannot exclude that the origin of the CSF (intraventricular in the post-mortem in contrast to lumbar CSF in living individuals) might influence the results. Due to the limited number of post-mortem CSF samples from ADAD (2), we included post-mortem sAD samples in the analysis, although the performance of both subtypes of AD seemed quite similar. Because there were only 2 genetic casesADAD post-mortem CSF samples in our cohort, we can only speculate that the post-mortem CSF α-syn RT-QuIC technique might detect a late LB pathology in ADADperform in a similar way than in sAD.

In sAD, the positivity of syn RT-QuIC is associated to a faster cognitive decline [13]. At this point, there is not enough information to evaluate the clinical impact of the presence of LB pathology in ADAD. However, we can speculate that introducing α-syn RT-QuIC testing in the evaluation of ADAD individuals could help to stratify patients in terms of prognosis or response to therapeutic interventions.

Altogether, we provide new data about the presence and detection of α-syn aggregates in ADAD that suggest a late appearance of LB pathology in these individuals. The frequency and specificity of this concomitant pathology in post-mortem species in ADAD suggest a direct result of the biological process. Additional studies with a broader sample are needed to confirm these results.

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Author Contributions

RSV, LMP and AA designed the study, provided data and obtained funding. LFA and ORC prepared the samples, collected the data, performed the analysis and drafted the manuscript. RR and LN performed the RT-QuIC analysis. AL, MB and DE provided clinical data. All the authors reviewed and approved the final version of the manuscript.

Conflicts of Interest

RSV has served in Advisory boards Meetings for Wave Life Sciences, Ionis and Novo Nordisk and received personal fees for participating in educational activities from Janssen, Roche Diagnostics and Neuroxpharma and funding to her institution for research projects from Biogen and Sage Pharmaceuticals.The other authors do not declare conflicts of interest.

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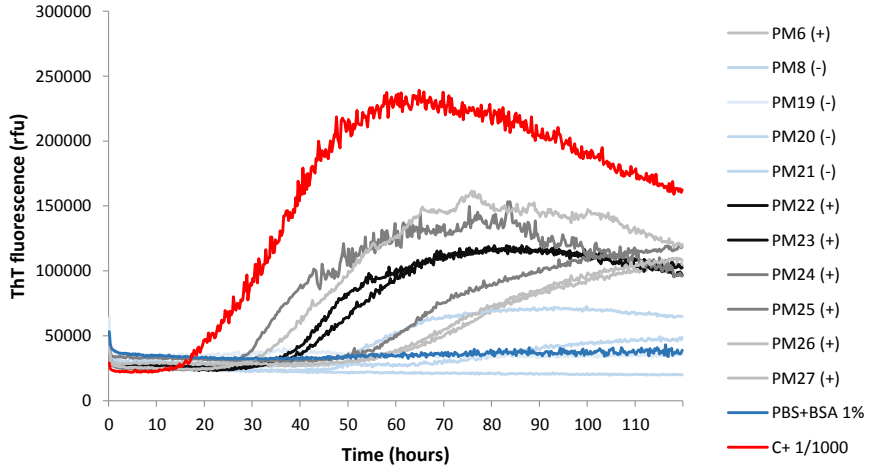
Figure 1. RT-QuIC detection of pathological α-synuclein (α-syn) seeding activity in CSF from 11 AD post-mortem samples



RT-QuIC kinetics measured by relative ThT fluorescence (rfu) from CSF of each individual cases with ADAD (PM6 and PM8) and with sAD (PM19 – 27) at 120 h were positive (+) for one ADAD individual (PM6) and 6 sAD subjects (PM22 – 27). Each curve consists of 3 duplicates. PM: post-mortem. PBS+BSA1%: negative control. C+ 1/1000: positive control.

Tables

Table 1. Neuropathological data from the 18 ADAD post-mortem (PM) subjects

Case #	Age at Death	Sex	Mutation	Lewy body IHC	Brain area
PM1	36	M	*APP I716L*	+	amygdala
PM2	56	M	*APP A713T*	+	amygdala
PM3	68	M	*APP duplication*	+	olfactory bulb
PM4	54	M	*APP duplication*	-	
PM5	44	M	*PSEN1 E120G*	-	
PM6	44	M	*PSEN1 G206D*	+	neocortical
PM7	53	F	*PSEN1 L286P*	-	
PM8	53	M	*PSEN1 L286P*	-	
PM9	56	F	*PSEN1 L286P*	-	*
PM10	48	F	*PSEN1 M139T*	+	amygdala
PM11	53	M	*PSEN1 M139T*	+	limbic
PM12	57	M	*PSEN1 M139T*	-	
PM13	60	M	*PSEN1 M139T*	+	limbic
PM14	64	M	*PSEN1 M139T*	+	amygdala
PM15	56	F	*PSEN1 P264L*	-	
PM16	60	M	*PSEN1 P264L*	-	
PM17	54	M	*PSEN1 V89L*	-	*
PM18	57	M	*PSEN1 V89L*	-	

PM: post-mortem

PSEN: Presenilin

APP: Amyloid-beta precursor protein

*: olfactory bulb not available

Table 2. Misfolded α-syn CSF RT-QuIC results from the 18 AD living (L) subjects and patient characteristics per diagnosis

Case #	Sample Age	Sex	Mutation	Misfolded α-syn	MMSE	CDR
L1	36	F	*APP I716T*	-	N/A	N/A
L2	39	M	*PSEN1 E280A*	-	13	1
L3	51	M	*PSEN1 G209E*	-	24	0.5
L4	59	M	*PSEN1 G209E*	-	28	0
L5	46	F	*PSEN1 G378R*	-	19	2
L6	48	M	*PSEN1 H163R*	-	18	2
L7	55	M	*PSEN1 I439S*	-	20	0.5
L8	59	F	*PSEN1 K239N*	-	20	1
L9	52	M	*PSEN1 K239N*	-	22	0.5
L10	45	M	*PSEN1 L173F*	-	15	2
L11	43	F	*PSEN1 L173F*	-	19	1
L12	37	F	*PSEN1 L286P*	-	28	0.5
L13	39	F	*PSEN1 L286P*	-	24	N/A
L14	42	M	*PSEN1 L286P*	-	24	N/A
L15	47	F	*PSEN1 L235R*	-	10	3
L16	45	F	*PSEN1 L282R*	-	22	0.5
L17	26	F	*PSEN1 S169P*	+	20	1
L18	39	F	*PSEN1 T116I*	-	19	2

L: Living

PSEN: Presenilin

APP: Amyloid-beta precursor protein