

Identification And Functional Analysis Of Novel *PSEN1* Mutations In Japanese Early-onset Alzheimer`s Disease

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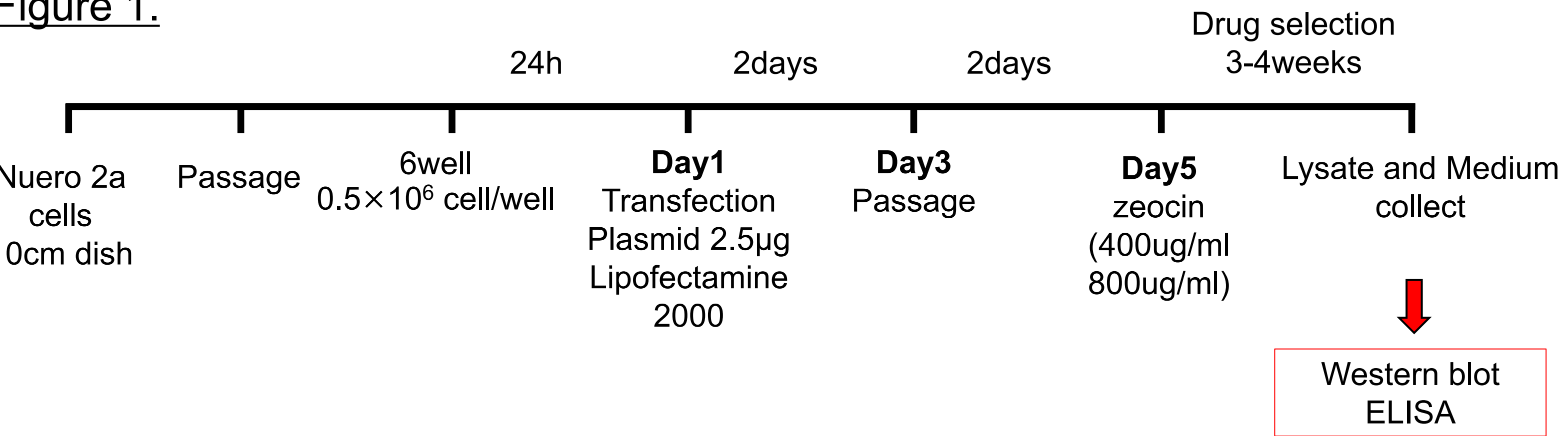
Introduction

Dominantly inherited Alzheimer’s disease(DIAD) is characterized by early-onset dementia and presents with a variety of symptoms. Mutations in *PSEN1*, which encodes presenilin-1, are the most common cause of DIAD. Individuals carrying *PSEN1* mutations typically develop symptoms in their 40s. However, the genetic basis of DIAD in Japanese patients is not fully understood. We identified novel *PSEN1* variants in patients who were diagnosed early onset Alzheimer’s disease. The aim of this study was to identify causative genes in Japanese patients with suspected DIAD and to evaluate the pathogenicity of identified variants using *in silico* analysis and functional assays.

Methods

Whole-exome sequencing (WES) was performed in patients clinically suspected of having DIAD. Candidate variants identified by WES were validated by Sanger sequencing. The pathogenic potential of each variant was assessed using in silico prediction tools, including SIFT, PolyPhen-2, CADD, REVEL and Alpha Missense.

Figure 1.



For functional analyses, N2a cell lines stably expressing wild-type(WT) or mutant *PSEN1* were established, and levels of amyloid-β peptides (Aβ40 and Aβ42) in conditioned media were quantified by enzyme-linked immunosorbent assay (ELISA). I showed how to generate stable cell line expressing PSEN1 WT or mutant *PSEN1* in Figure1.

Results

Table 1. Novel *PSEN1* variants and these clinical features

<i>PSEN1</i> mutation	Age	sex	Onset Age	Family history	APOE
p.Lys380Glu	48	F	44	No	3/4
p.Asp385Glu	45	M	39	Yes	3/3
	57	F	N.A.	Yes	N.A.
p.Leu173del	51	F	39	Yes	2/3
p.Val208del	49	F	46	Yes	3/3

Table 2. *In silico* analysis

<i>PSEN1</i> mutation	CADD score	REVEL	SIFT	PolyPhen-2	Alpha missense	ACMG guideline
p.Lys380Glu	31.0	0.90	Deleterious 0.02	Probably damaging 1.00	Likely pathogenic 1.00	Likely Pathogenic
p.Asp385Glu	23.8	0.90	Deleterious 0.00	Probably damaging 1.00	Likely pathogenic 1.00	Likely Pathogenic
p.Leu173del	18.2	0.65	-	-	-	Likely Pathogenic
p.Val208del	20.2	0.79	-	-	-	Likely pathogenic

Figure 2. Western Blot

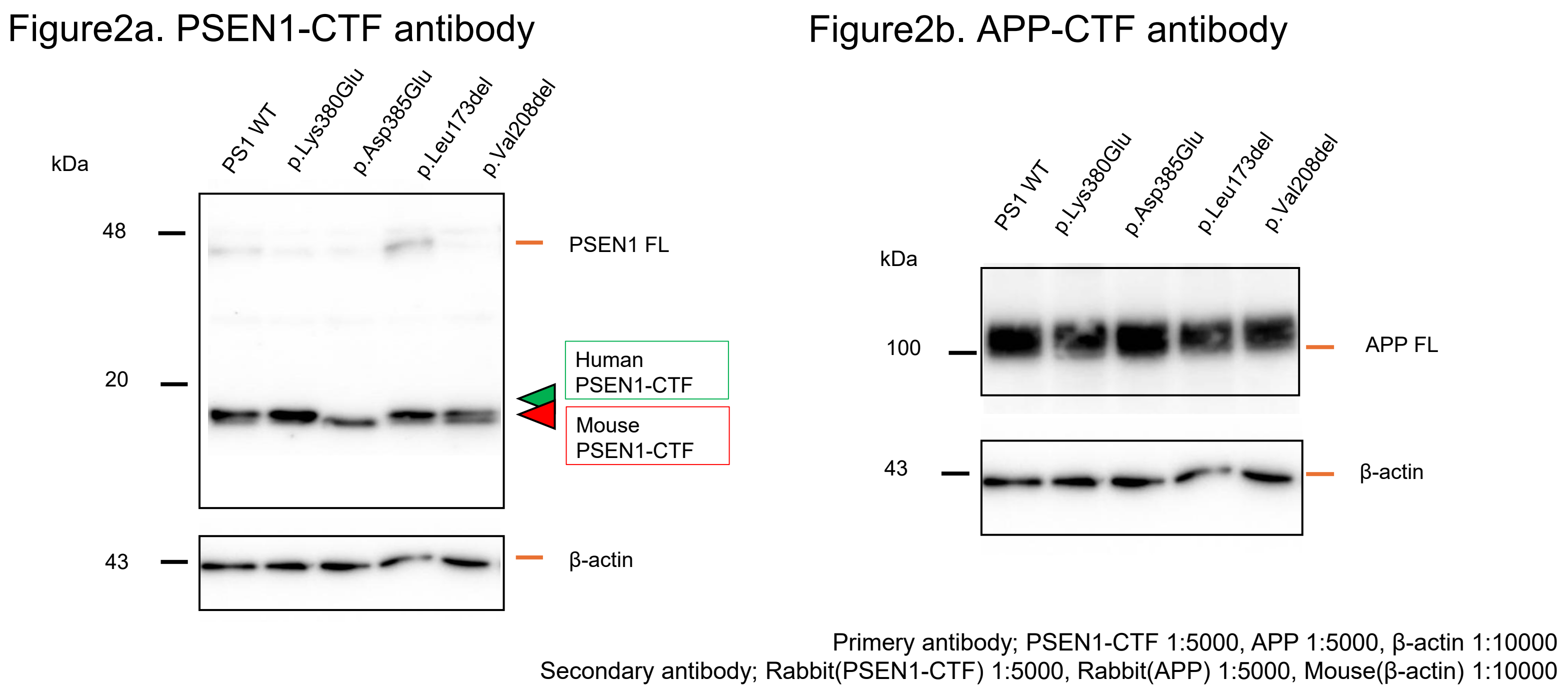
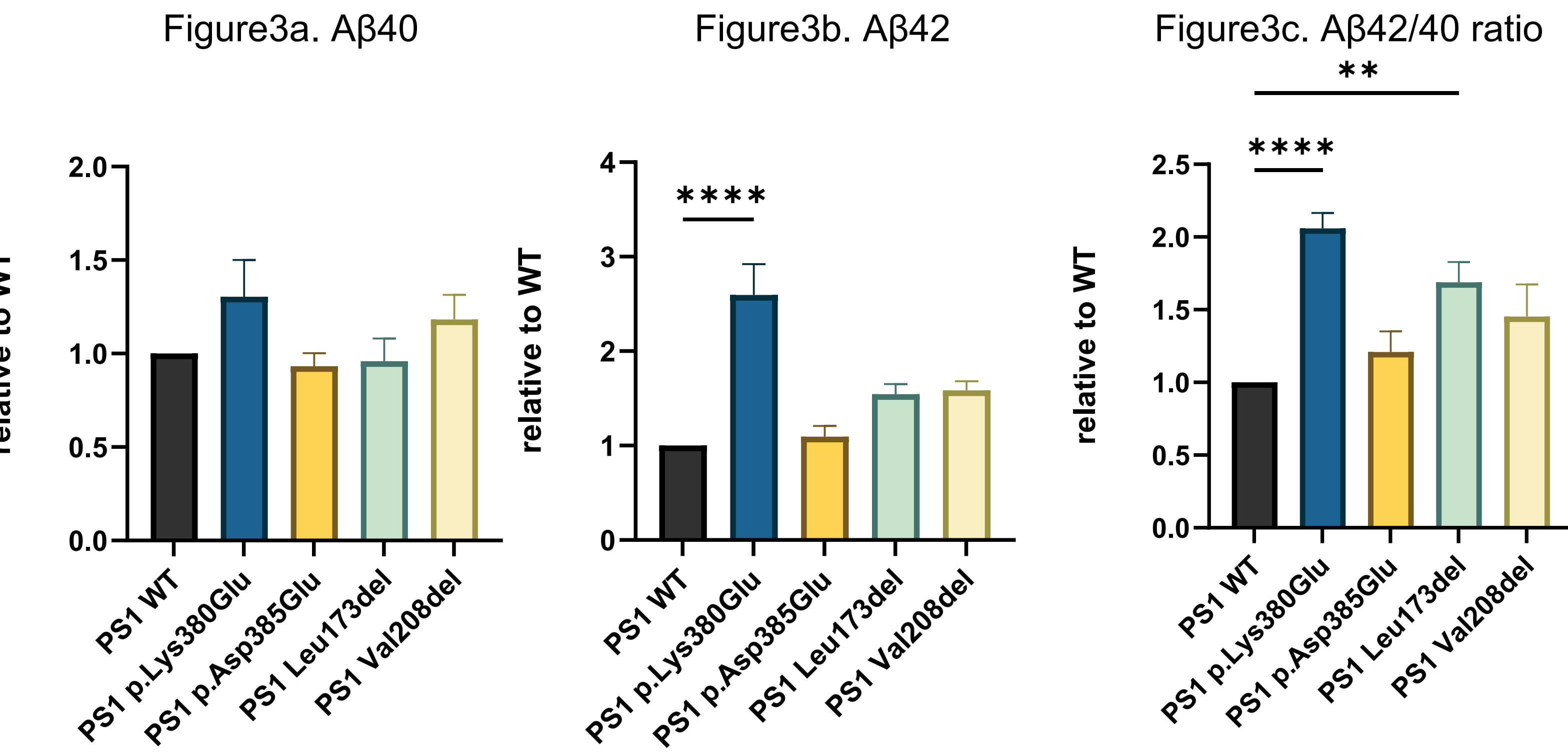


Figure3. ELISA



Analyzed for Aβ40 and Aβ42 by ELISA. Aβ40, Aβ42 and ratio of Aβ42/40 levels expressed relative to PSEN1 WT(n =6).Graphs show mean ± standard error of the mean(SEM). Significance indicated by Dunnett’s t-test(*:p<0.05, **:p<0.01, ***:p<0.001, ****:p<0.0001) compared with *PSEN1* WT.

Conclusions

In this study, we evaluated 4 novel variants in *PSEN1* by *in silico* analysis and cell-based assay. *In silico* analysis shows pathogenicity in 4 variants. According to cell-based assay, *PSEN1* p.Leu173del and p.Lys380Glu produced significant increase in Aβ42/40 ratio compared with WT. On the other hands, *PSEN1* p.Asp385Glu and p.Val208del didn’t show significances. These findings indicate that the novel *PSEN1* variants p.Leu173del and p.Lys380Glu are pathogenic and contribute to DIAD. Other missense mutation leading to an amino acid substitution at position 173rd has reported in 4 papers¹⁾²⁾³⁾⁴⁾. And the pathogenic mechanism leading to AD remains unclear, although there have been previous reports of a mutation at the 380th amino acid⁵⁾. This study provides novel insights into p.Leu173del and p.Lys380Glu. Further studies, including additional functional and clinical investigations, are required to clarify the pathogenic roles of p.Asp385Glu and p.Val208del.

References

1) Sun L, et al. Proc Natl Acad Sci. 2017;24:114(4):e476-e485. 2) Wang G, et al. Journal of Psychiatric Research 2019;113:141–147. 3) Kensaku K, et al.J Neurol 2009;256:1351–1353. 4) Liu L, et al. Alzheimers Dement. 2022;12:19(1):79–96. 5) Jakub J. Int. J. Mol. Sci. 2024; 25: 2564: 1-17.