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Systematic validation of variants of unknown significance in *APP*, *PSEN1* and *PSEN2*

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease that is clinically characterized by progressive cognitive decline. More than 200 pathogenic mutations have been identified in *amyloid- β precursor protein (APP)*, *presenilin 1 (PSEN1)* and *presenilin 2 (PSEN2)*. Additionally, common and rare variants occur within *APP*, *PSEN1*, and *PSEN2* that may be risk factors, protective factors, or benign, non-pathogenic polymorphisms. Yet, to date, no single study has carefully examined the effect of all of the variants of unknown significance reported in *APP*, *PSEN1* and *PSEN2* on A β isoform levels *in vitro*. In this study, we analyzed A β isoform levels by ELISA in a cell-based system in which each reported pathogenic and risk variant in *APP*, *PSEN1*, and *PSEN2* was expressed individually. In order to classify variants for which limited family history data is available, we have implemented an algorithm for determining pathogenicity using available information from multiple domains, including genetic, bioinformatic, and *in vitro* analyses. We identified 90 variants of unknown significance and classified 19 as likely pathogenic mutations. We also propose that five variants are possibly protective. In defining a subset of these variants as pathogenic, individuals from these families may eligible to enroll in observational studies and clinical trials.

Keywords

APP; PSEN1; PSEN2; Alzheimer's disease; Cell-based assays; Pathogenicity algorithm

1. Introduction

Alzheimer's disease (AD) is characterized clinically by progressive cognitive decline and neuropathologically by progressive neuronal loss and the accumulation of amyloid plaques

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Declaration of Competing Interest

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and neurofibrillary tangles. Mutations in amyloid- β precursor protein (*APP*), presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) are the pathogenic cause of autosomal dominant AD (ADAD). Rare recessive mutations in *APP* (A673V and E693) also cause early onset AD (Di Fede et al., 2009; Giaccone et al., 2010; Tomiyama et al., 2008).

While more than 200 pathogenic mutations have been identified in *APP*, *PSEN1*, or *PSEN2*, more than 90 additional variants have been identified where the pathogenicity remains in question (reviewed in (Karch et al., 2014; Cruts et al., 2012)). The uncertainty in pathogenicity may be due to several reasons. In some cases, variants in *APP*, *PSEN1* or *PSEN2* have been identified in families with several generations of AD. In these cases, pathogenicity can be evaluated by segregation analysis: the presence of the variant in multiple individuals with clinically or pathologically confirmed AD and the absence of the variant in healthy, older family members. However, in many cases only the single proband has DNA available. Alternatively, there may be limited or no family history. Challenges also arise when young, healthy individuals are found to be variant carriers. To assess the pathogenicity of novel variants in *APP*, *PSEN1*, and *PSEN2* when pedigree and clinical data is limited or incomplete, Guerreiro and colleagues (Guerreiro et al., 2010a) proposed a pathogenicity algorithm. We have since modified and expanded this algorithm to evaluate pathogenicity of six novel variants identified through the Dominantly Inherited Alzheimer Network Extended Registry (DIAN-EXR) using genetic, biochemical, biomarker, and clinical data (Hsu et al., 2018). In our modified algorithm, we found that biochemical evidence of a change in A β was informative in assessing pathogenicity where genetic data was limited (Hsu et al., 2018).

To date, more than 90 variants of unknown significance are included in genetic databases for Alzheimer's disease (AD/FTD database and AlzForum Mutations database), in part due to the reliance on genetic information alone for classification of pathogenicity (Cruts et al., 2012). Here, we further modified our pathogenicity algorithm to classify 90 variants of unknown significance for which no family segregation data is available that have been previously reported in the AD/FTD and AlzForum Mutations Databases.

2. Material and methods

2.1. Identification of variants of unknown significance

To identify variants of unknown significance, we queried the AD/FTD ((5)<http://www.molgen.ua.ac.be/ADMutations/>) and AlzForum Mutations Databases (<https://www.alzforum.org/mutations>). Variants classified by the Guerreiro et al. algorithm (Guerreiro et al., 2010a) as being “not pathogenic” or “pathogenic nature unclear” were selected for evaluation by bioinformatic and *in vitro* analyses ($n = 90$; Supplemental Table 1).

2.2. Bioinformatics

To determine whether *APP*, *PSEN1*, and *PSEN2* variants represented rare or common polymorphisms, we investigated two population-based exome sequencing databases: Exome Variant Server (EVS) and Exome Aggregation Consortium (ExAC) browser. The Genome

Aggregation Database (gnomAD) was excluded from this study given that sequencing data from the Alzheimer's Disease Sequencing Project are included in the database and thus would not be representative of a control population. Polymorphism phenotype v2 (PolyPhen-2; (Adzhubei et al., 2010)) and Sorting Intolerant From Tolerant (SIFT) were used to predict whether the amino acid change would be disruptive to the encoded protein.

2.3. In vitro analyses

2.3.1. Plasmids and mutagenesis—The full-length *APP* cDNA (isoform 695) was cloned into pcDNA3.1 (Wang et al., 2004). *APP* variants (Table 1) were introduced into the *APP* cDNA using a QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA). Clones were sequenced to confirm the presence of the variant and the absence of additional modifications. *APP* wild-type (WT) and pathogenic *APP* KM670/671NL, *APPL*723P and *APPK*724N mutations were included as controls. Three variants were not generated due to incompatibility with the cDNA plasmid: *APP* E296K, *APP* P299L, *APP* IVS17 83–88delAAGTAT, *APP* c *18C > T, and *APP* c *372 A > G (N/A; Table 1).

The full-length *PSEN1* cDNA was cloned into pcDNA3.1 myc/his vector (Brunkan et al., 2005). *PSEN1* variants (Table 1) were introduced into the *PSEN1* cDNA and screened as described above. *PSEN1* WT and pathogenic *PSEN1* A79V, *PSEN1* L286V, and *PSEN1* exon 9 deletion (E9) mutations were included as controls. One variant was not generated due to incompatibility with the cDNA plasmid: *PSEN1* N32N. Four additional variants failed at the mutagenesis step and were not modeled in the cellular assay: *PSEN1* L219R, *PSEN1* D333G, *PSEN1* T354I, and *PSEN1* S365Y (N/A; Table 1).

The full-length *PSEN2* cDNA was cloned into pcDNA3.1 vector (Kovacs et al., 1996). *PSEN2* variants (Table 1) were introduced into the *PSEN2* cDNA and screened as described above. *PSEN2* WT and the pathogenic *PSEN2* N141I mutation were included as controls (Walker et al., 2005). Nine variants failed at the mutagenesis step and were not modeled in the cellular assay: *PSEN2* T18M, *PSEN2* R29H, *PSEN2* V101M, *PSEN2* S130L, *PSEN2* H162N, *PSEN1* V214L, *PSEN2* T301M, *PSEN2* K306fs, and *PSEN2* P334R (N/A; Table 1).

In total, we generated 65 plasmids containing variants of unknown significance in *APP*, *PSEN1* or *PSEN2*.

2.3.2. Transient transfection—To assess *APP* variants, we transiently expressed *APP* WT, variant, or mutant *APP* in mouse neuroblastoma cells (N2A). To assess *PSEN1* and *PSEN2* variants, we used mouse neuroblastoma cells in which endogenous *Psen1* and *Psen2* were knocked out by CRISPR/Cas9 (N2A-PS1/PS2 KO; Pimenova and Goate, 2020). We then transiently expressed human *APP* WT (695 isoform) along with the *PSEN1* or *PSEN2* constructs. N2A cells were maintained in equal amounts of Dulbecco's modified Eagle's medium and Opti-MEM, supplemented with 5% fetal bovine serum, 2 mM L-glutamine, and 100 µg/mL penicillin/streptomycin. Upon reaching confluency, cells were transiently transfected with Lipofectamine 2000 (Life Technologies). Culture media was replaced after 24 h, and cells were incubated for another 24 h prior to analysis of extracellular Aβ in

the media. Three independent transfections were performed for each construct and used for subsequent analyses. Six variants exhibited low expression levels when transiently expressed and thus were not included in the A β ELISA analyses: *APPH677R*, *APPG709S*, *PSEN1* H163P, *PSEN2* L143H, *PSEN2* A237V and *PSEN2* A377V (N/A; Table 1).

2.3.3. A β Enzyme-linked immunosorbent assay (ELISA)—Conditioned media was collected and centrifuged at 3000 $\times g$ at 4 °C for 10 min to remove cell debris. Levels of A β 40 and A β 42 in cell culture media were measured by sandwich ELISA as directed by the manufacturer (Life Technologies, Carlsbad, CA, USA). Statistical difference was measured using a one-way ANOVA and post-hoc Dunnett test.

2.3.4. Immunoblotting—Cell pellets were extracted on ice in lysis buffer (50 mM Tris pH 7.6, 2 mM EDTA, 150 mM NaCl, 1% NP40, 0.5% Triton 100 \times , protease inhibitor cocktail) and centrifuged at 14,000 $\times g$. Protein concentration was measured by BCA method as described by the manufacturer (Pierce-Thermo). Standard SDS-PAGE was performed in 4–20% Criterion TGX gels (Bio-Rad). Samples were boiled for 5 min in Laemmli sample buffer prior to electrophoresis (Laemmli, 1970). Immunoblots were probed with 22C11 (1:1000; Millipore) and goat-anti-rabbit-HRP (1:5000; Thermo Fisher).

3. Results and discussion

3.1. The impact of variants of unknown significance in APP, PSEN1 and PSEN2 on A β levels in vitro

Prior cellular studies have largely focused on defining the impact of known pathogenic mutations in *APP*, *PSEN1*, and *PSEN2* on extracellular A β 40 and A β 42 levels (Haass et al., 1994; Haass et al., 1995; Sun et al., 2017). Many common and rare variants occur within *APP*, *PSEN1*, and *PSEN2* that may be risk factors, protective factors, or benign, non-pathogenic polymorphisms (Cruchaga et al., 2012; Sassi et al., 2014). Yet, to date, no single study has systematically examined the impact of these variants of unknown significance on A β isoform levels *in vitro*. The goal of this study was to determine the extent to which variants in *APP*, *PSEN1* and *PSEN2* impact A β isoform levels and to determine the utility of our assay to discriminate between pathogenic and non-pathogenic variants.

We compared extracellular A β 40, A β 42, and A β 42/40 in the media of mouse N2A cells expressing *APP*WT, pathogenic *APP* mutations (KM670/671NL, L723P or K724N) or *APP* containing one of 22 variants of unknown significance (Fig. 1). We found that four of the 22 *APP* variants resulted in a significant increase in the A β 42/40 ratio compared with *APP* WT: *APPS614G*, *APPP620A*, *APPA713T*, and *APPT719N* (Fig. 1; Supplemental Table 2). *APPT719N* was a variant of unknown significance that has recently been classified as pathogenic (Hsu et al., 2018). Consistent with the reported effects of *APP*KM670/671NL, we found that one *APP* variant (*APPP620L*) produced a significant increase in A β 40 and A β 42 without altering the A β 42/40 ratio (Fig. 1C; Supplemental Table 2). Two *APP* variants resulted in a significant increase in A β 42 without significantly altering the A β 42/40 ratio: *APPG708G* and *APPH733P* (Fig. 1; Supplemental Table 2). Those variants that significantly increased the A β 42/40 ratio or A β 40 and A β 42 occur in the juxtamembrane region or amyloid beta domain consistent with known pathogenic mutations; however, some

variants, including *APPS614G*, *APPP620L*, and *APPP620A*, occur outside of the regions that are routinely sequenced (Fig. 1A) (Cruts et al., 2012).

To evaluate variants of unknown significance in *PSEN1* and *PSEN2*, we selected a cell line in which APP is metabolized similarly to neuronal cells and in which endogenous presenilin genes are absent in order to avoid background A β production: N2A-PS1/PS2 KO. The absence of endogenous *Psen1* and *Psen2* allows us to capture effects of known pathogenic mutations in these genes, where a robust reduction in A β 40 results in a shift in the A β 42/40 ratio. We evaluated 19 variants in *PSEN1* and 22 variants in *PSEN2* (Figs. 2 and 3). A β 40, A β 42, and A β 42/40 levels were compared to *PSEN1* WT or *PSEN2* WT, respectively. Six of the 19 *PSEN1* variants resulted in a significant increase in the A β 42/40 ratio: *PSEN1* M84V, *PSEN1* T99A, *PSEN1* QR127G, *PSEN1* H131R, *PSEN1* M146V, and *PSEN1* G378fs (Fig. 2; Supplemental Table 2). Six variants resulted in a significant increase in A β 40 and A β 42 or A β 42 only compared with *PSEN1* WT: *PSEN1* D40, *PSEN1* E69D, *PSEN1* R108Q, *PSEN1* R358Q, *PSEN1* A396T, and *PSEN1* I439V (Fig. 2; Supplemental Table 2). We found that six of 22 *PSEN2* variants resulted in a significant increase in the A β 42/40 ratio: *PSEN2* P123L, *PSEN2* I235F, *PSEN2* L238F, *PSEN2* R284G, *PSEN2* P348L, and *PSEN2* D439A (Fig. 3; Supplemental Table 2). We evaluated several known polymorphisms in *PSEN2* that do not cause AD: *PSEN2* R62H and *PSEN2* R71W (Walker et al., 2005). Cells expressing these benign variants failed to produce a significant change in A β 40 or A β 42 (Fig. 3). Overall, variants of unknown significance in *PSEN1* and *PSEN2* that alter A β were located across the gene. Thus, leveraging multiple types of data (genetic, bioinformatic, and cellular) are most informative in evaluating variants of unknown significance.

While the pathogenicity algorithm is designed to discriminate between pathogenic and non-pathogenic variants, by utilizing the *in vitro* assay, we have the opportunity to discriminate between benign, non-pathogenic variants and those that may confer resilience to AD. A rare variant in *APP*, *APP* A673T, has been reported to confer protection against AD risk (Jonsson et al., 2012; Maloney et al., 2014). *In vitro*, *APP* A673T results in a significant reduction of A β 42 and A β 40 without altering total APP levels by decreasing BACE activity (Maloney et al., 2014). Interestingly, we observed that eight variants in *APP*, *PSEN1*, and *PSEN2* produced significantly lower levels of A β 42 and A β 40 (Figs. 1–3; Supplemental Table 2) without altering total APP levels: *APP* A235V; *APP* Y538H; *APP* V713V; *PSEN1* V191A; *PSEN1* G378fs; *PSEN2* E126fs; *PSEN2* A252T; *PSEN2* V393M (Fig. 4). Thus, we propose that these variants may reduce A β production and confer resilience to AD.

3.2. Pathogenicity

Evaluating pathogenicity of variants of unknown significance requires carefully weighing the clinical phenotype of the variant carrier along with bioinformatic predictions and functional analyses. By evaluating more than 90 variants of unknown significance *in vitro*, we found that only a subset of variants were able to significantly alter A β 40, A β 42, or A β 42/40 in a manner consistent with known pathogenic mutations. Thus, we propose that the impact of variants on extracellular A β levels *in vitro* should be weighed separately in the pathogenicity algorithm. As such, we have revised the pathogenicity algorithm for use when segregation data is unavailable (Fig. 5).

Applying this modified pathogenicity algorithm to the 90 variants of unknown significance, we classified 19 variants as probably pathogenic (*APPP620A*; *APPP620L*; *APPH733P*; *PSENI E69D*; *PSENI M84V*; *PSENI T99A*; *PSENI R108Q*; *PSENI QR127G*; *PSENI H131R*; *PSENI M146V*; *PSENI R358Q*; *PSENI G378fs*; *PSENI A396T*; *PSENI I439V*; *PSEN2 P123L*; *PSEN2 I235F*; *PSEN2 L238F*; *PSEN2 R284G*; *PSEN2 P348L*; Table 1). Many of the variants of unknown significance were identified in single individuals presenting clinically with AD (Guerreiro et al., 2010a; Hsu et al., 2018; Sassi et al., 2014; Nicolas et al., 2016; Guerreiro et al., 2010b; Ikeda et al., 2013; Dobricic et al., 2012; Rogaeva et al., 2001; Lohmann et al., 2012; Blauwendraat et al., 2016). Our *in vitro* assay revealed that nine variants significantly reduced A β 40 and A β 42 levels (Figs. 1C, 2C, 3C). Among these variants, four variants were identified in individuals with AD (Supplemental Table 1), while five variants were identified in individuals with no evidence of neurodegeneration (*APPY538H*, *APPA673T*, *APPA713V*, *PSENI V191A*, *PSEN2 A252T*; Table 1). Thus, we predict that these five variants confer resilience to AD.

4. Conclusions

Here, we applied genetic, bioinformatic, and functional data to an algorithm to assess pathogenicity of variants of unknown significance in *APP*, *PSENI* and *PSEN2*. We propose that 19 variants are probable pathogenic AD mutations. This algorithm was adapted and modified from a pathogenicity algorithm originally proposed by Guerreiro and colleagues (Guerreiro et al., 2010a) to impute pathogenicity when extensive genetic data is missing. We have expanded upon this algorithm in several important ways: (1) expanding the number of controls in the association analyses from 100 to 65,000 by leveraging the EVS and ExAC databases; (2) incorporating cell-based assays to evaluate the impact of novel variants on A β levels; and (3) evaluating the bioinformatic functional findings (*e.g.* conservation between *PSENI* and *PSEN2* and the presence of other mutations at the same residue) independent of the cell-based functional findings. The cell-based assay focused on the impact of variants on A β 42 and A β 40 levels. Some pathogenic mutations have been reported to lead to reduced A β 40 and elevated A β 43 and A β 42 (Chavez-Gutierrez et al., 2012). In many of these mutations, the increase in A β 43 is much greater than A β 42 (Chavez-Gutierrez et al., 2012). Because our assays focus on A β 42 and A β 40, we may not capture the magnitude of the aberrant effect on A β levels. Ultimately, definitive pathogenicity comes from segregation: presence of the variant in multiple family members with autopsy confirmed AD and absence in family members free of disease. Designation of a variant as pathogenic will allow for individuals to enroll in observational studies and clinical trials for AD, with clear applications in clinical and research settings.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.nbd.2020.104817>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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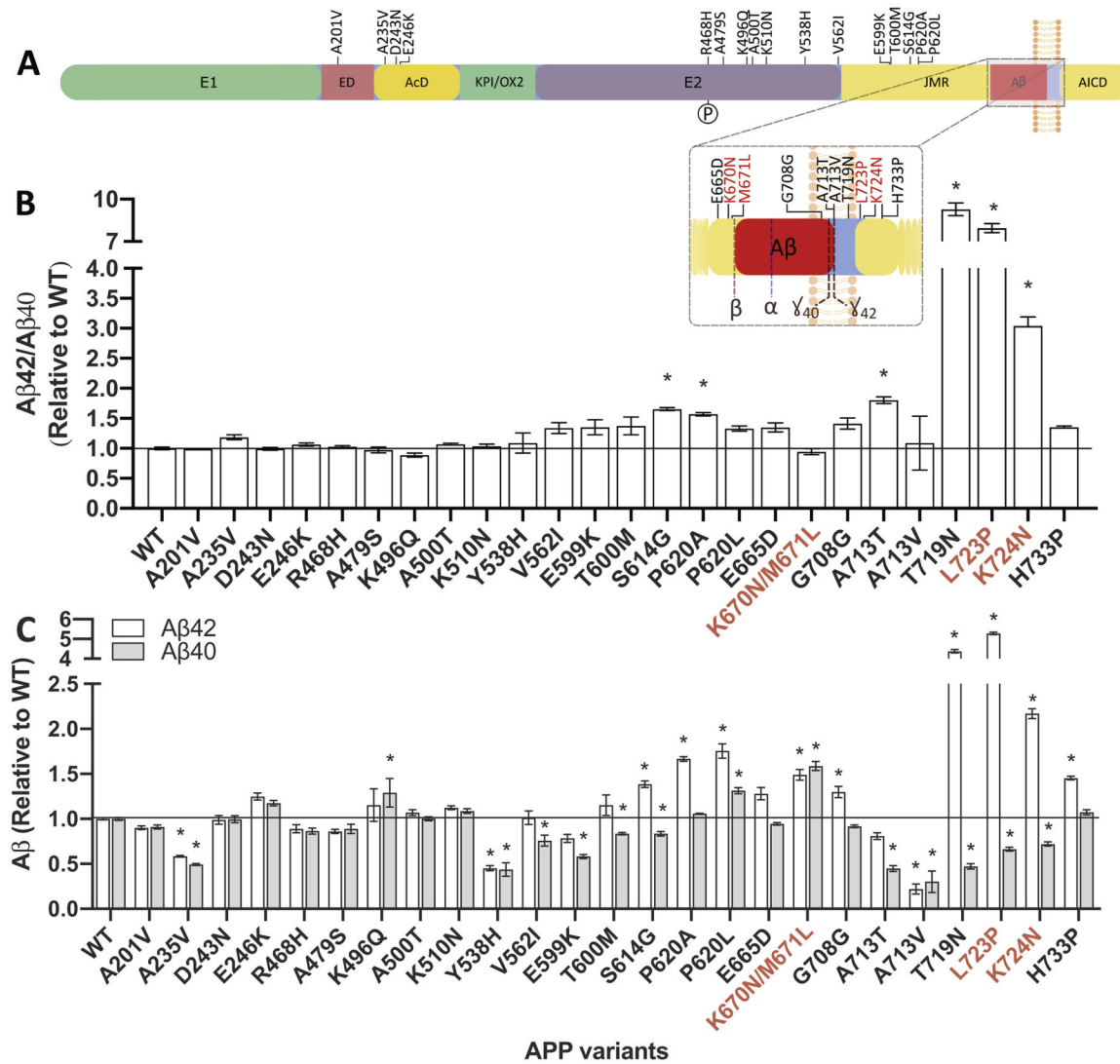


Fig. 1. Impact of APP variants of unknown significance on Aβ levels in vitro.

A. Diagram of the location of variants of unknown significance in APP. B-C. Mouse N2A cells were transiently transfected with plasmids containing APP695 WT, known pathogenic mutations (K670N/M671L, L723P, K724N), or a variant of unknown significance. After 48 h, media was collected and analyzed for Aβ42 and Aβ40 by ELISA. B. Ratio of Aβ42/40 expressed relative to APP WT. C. Aβ42 (white box) and Aβ40 (gray box) levels expressed relative to APP WT. Graphs represent mean ± standard error of the mean (SEM). Significance indicated by Dunnett's *t*-test (*, *p* < .05). Red, known pathogenic mutations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

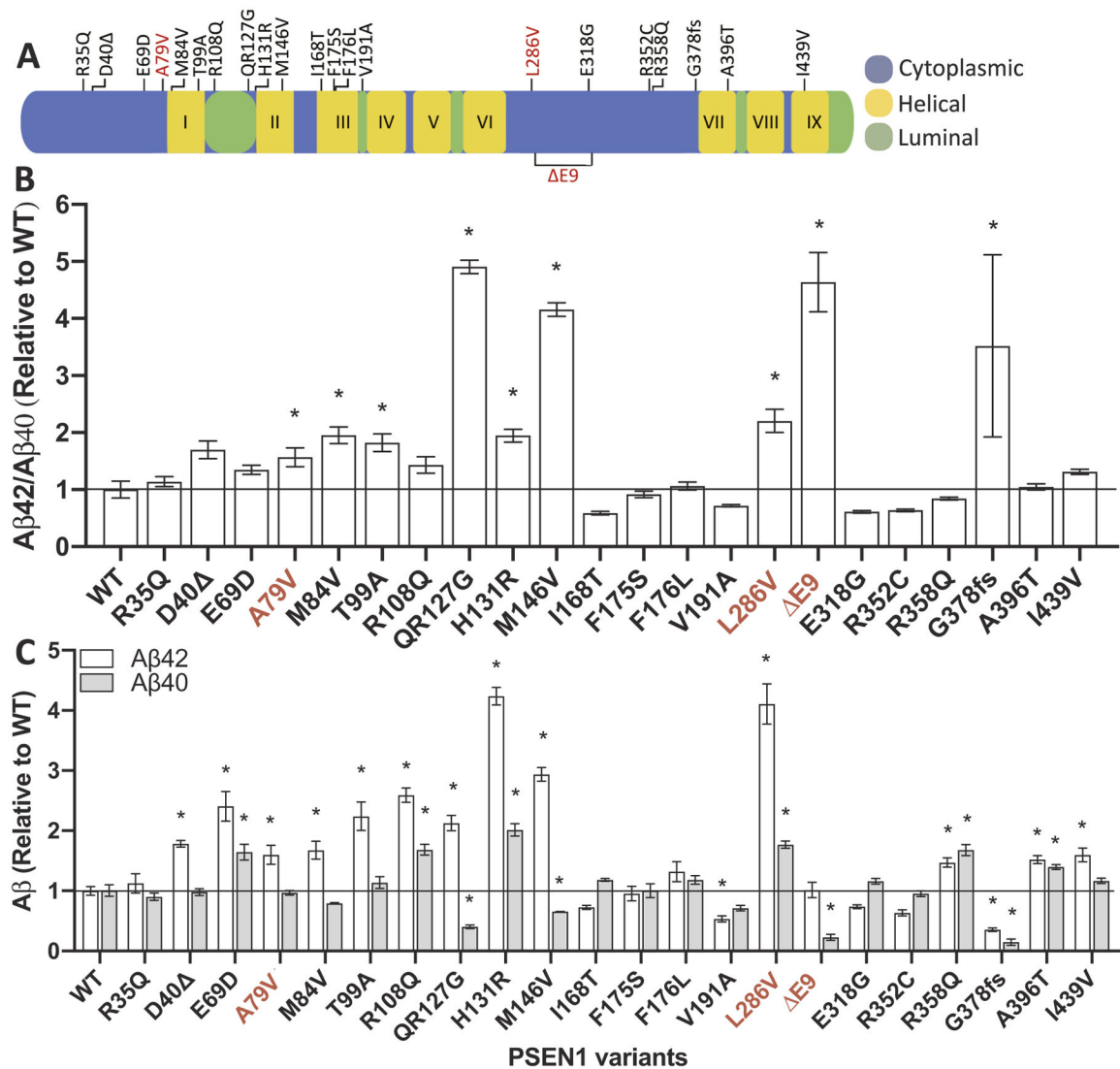


Fig. 2. Impact of *PSEN1* variants of unknown significance on Aβ levels *in vitro*.

A. Diagram of the location of variants of unknown significance in *PSEN1*. B-C. Mouse N2A-PS1/PS2 KO cells were transiently transfected with plasmids containing APP WT and *PSEN1* WT, known pathogenic mutations (A79V, L286V, and E9), or a variant of unknown significance. After 48 h, media was collected and analyzed for Aβ42 and Aβ40 by ELISA. B. Ratio of Aβ42/40 expressed relative to *PSEN1* WT. C. Aβ42 (white box) and Aβ40 (gray box) levels expressed relative to *PSEN1* WT. Graphs represent mean ± SEM. Significance indicated by Dunnett's *t*-test (*, *p* < .05). Red, known pathogenic mutations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

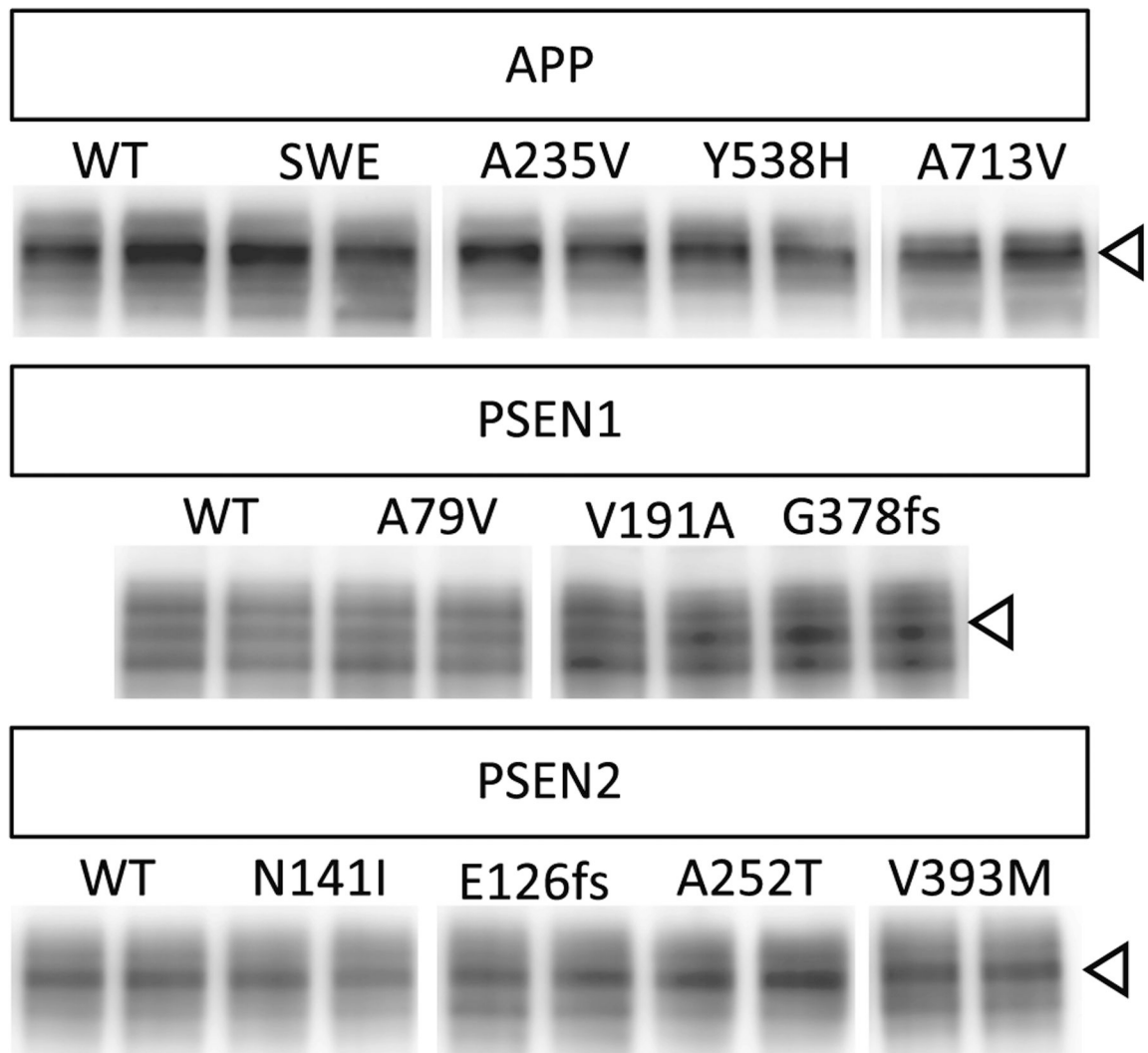


Fig. 4. APP, PSEN1, and PSEN2 variants that reduce A β do not alter total APP levels. Cells transiently overexpressing WT and risk variants for 48 h were analyzed by SDS-PAGE and immunoblotting as described in Methods. Immunoblots were probed with 22C11 (full-length APP; open arrow). The immunoblot is representative of 3 replicate experiments.

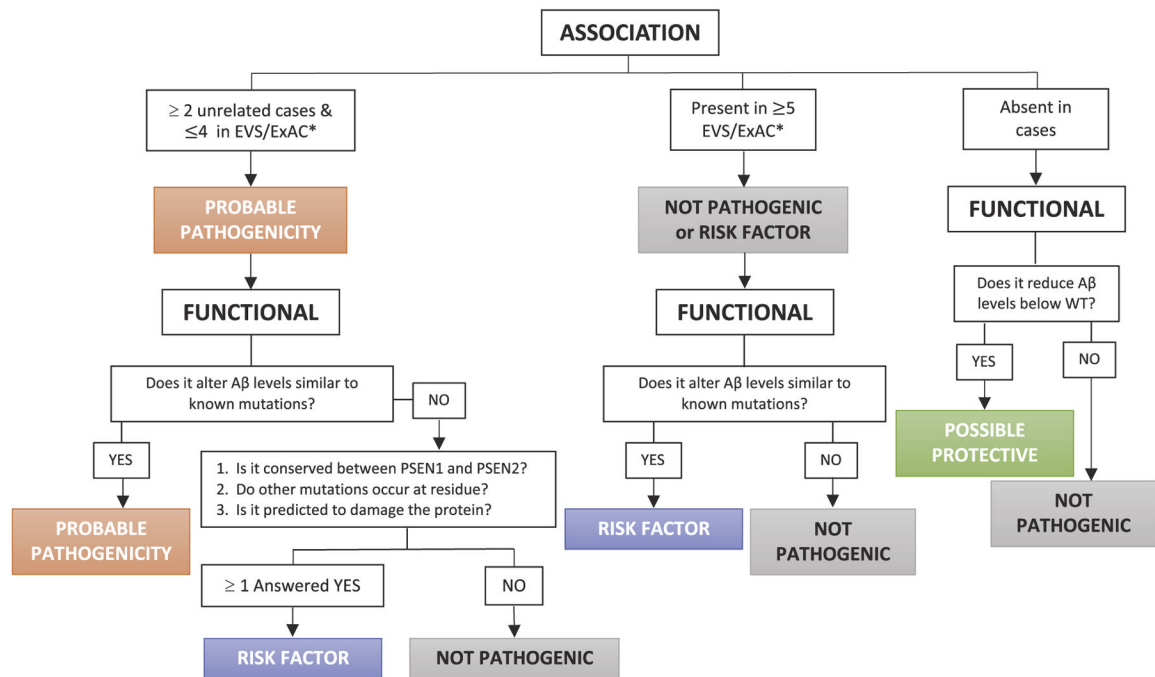


Fig. 5. Algorithm to classify variants of unknown significance in *APP*, *PSEN1* and *PSEN2* when family segregation data is not available.

This model is modified from the algorithm previously proposed by Guerreiro *et al* 2010 and Hsu et al., 2018 (Guerreiro et al., 2010a; Hsu et al., 2018) to focus on variants of unknown significance for which no family segregation data is available. *EVS/ExAC databases should be used to evaluate the presence of a novel variant in the population. GnomAD contains data from the Alzheimer’s Disease Sequencing Project and thus may be enriched for variants that contribute to AD risk.

Table 1

APP, PSEN1 and PSEN2 variants of unknown significance evaluated by the pathogenicity algorithm.

| Variant | 5 alleles in EVS/ExAC | Conserved between PSEN1 and PSEN2 | Known pathogenic mutations | Predicted to damage protein ^a | Increase A β ₄₂ /40 or A β ₄₂ ^b | Predicted pathogenicity ^c |
|---------------------------|-----------------------|-----------------------------------|----------------------------|--|--|--------------------------------------|
| APP A201V | Yes | N/A | No | Benign | No | Not pathogenic |
| APP A235V | Yes | N/A | No | Benign | No | Not pathogenic |
| APP D243N | Yes | N/A | No | Benign | No | Not pathogenic |
| APP E246K | No | N/A | No | Possibly damaging | No | Risk factor |
| APP E296K | No | N/A | No | Probably damaging | N/A | Probable pathogenic; Risk factor |
| APP P299L | No | N/A | No | Probably damaging | N/A | Probable pathogenic; Risk factor |
| APP V340M | No | N/A | No | Probably damaging | No | Not pathogenic |
| APP R468H | No | N/A | No | Probably damaging | No | Not pathogenic |
| APP A479S | Yes | N/A | No | Benign | No | Not pathogenic |
| APP K496Q | No | N/A | No | Probably damaging | No | Not pathogenic |
| APP A500T | No | N/A | No | Probably damaging | No | Not pathogenic |
| APP K510N | No | N/A | No | Probably damaging | No | Not pathogenic |
| APP Y538H | No | N/A | No | Possibly damaging | No | Not pathogenic; Possibly protective |
| APP V562I | No | N/A | No | Benign | No | Not pathogenic |
| APP E599K | Yes | N/A | No | Probably damaging | No | Not pathogenic |
| APP T600M | Yes | N/A | No | Probably damaging | No | Not pathogenic |
| APP S614G | Yes | N/A | No | Benign | Yes | Risk factor |
| APP P620A | No | N/A | No | Possibly damaging | Yes | Probable pathogenic |
| APP P620L | No | N/A | No | Probably damaging | Yes | Probable pathogenic |
| APP E665D | No | N/A | No | Benign | No | Not pathogenic |
| APP A673T | Yes | N/A | Yes | Benign | No | Not pathogenic; Possibly protective |
| APP H677R | No | N/A | No | Possibly damaging | N/A | Probable pathogenic; Risk factor |
| APP G708G | Yes | N/A | No | N/A | Yes | Risk factor |
| APP G709S | Yes | N/A | No | Probably damaging | N/A | Not pathogenic; Risk factor |
| APP A713T | Yes | N/A | No | Probably damaging | Yes | Risk factor |
| APP A713V | Yes | N/A | No | Probably damaging | No | Not pathogenic; Possibly protective |
| APP H733P | No | N/A | No | Probably damaging | Yes | Probable pathogenic |
| APP IVS17 83-88del/AACTAT | No | N/A | No | N/A | N/A | Unknown |

| Variant | 5 alleles in EVS/ExAC | Conserved between PSEN1 and PSEN2 | Known pathogenic mutations | Predicted to damage protein ^e | Increase A β 42 ^b or A β 42/40 ^b | Predicted pathogenicity ^c |
|------------------|-----------------------|-----------------------------------|----------------------------|--|--|--------------------------------------|
| APP c *18C > T | No | N/A | No | N/A | N/A | Unknown |
| APP c *372 A > G | No | N/A | No | N/A | N/A | Unknown |
| PSEN1 N32N | No | No | No | N/A | N/A | Not pathogenic |
| PSEN1 R35Q | Yes | No | No | Benign | No | Not pathogenic |
| PSEN1 D40del | Yes | Yes | No | N/A | Yes | Risk factor |
| PSEN1 E69D | No | No | No | Benign | Yes | Probable pathogenic |
| PSEN1 M84V | No | Yes | Yes | Probably damaging | Yes | Probable pathogenic |
| PSEN1 T99A | No | Yes | No | Probably damaging | Yes | Probable pathogenic |
| PSEN1 R108Q | No | No | No | Benign | Yes | Probable pathogenic |
| PSEN1 QR127G | No | Yes | No | N/A | Yes | Probable pathogenic |
| PSEN1 H131R | No | No | No | Benign | Yes | Probable pathogenic |
| PSEN1 M146V | No | Yes | Yes | Probably damaging | Yes | Probable pathogenic |
| PSEN1 H163P | No | Yes | Yes | Probably damaging | N/A | Probable pathogenic; Risk factor |
| PSEN1 I168T | No | No | No | Benign | No | Not pathogenic |
| PSEN1 F175S | No | Yes | No | Probably damaging | No | Not pathogenic |
| PSEN1 F176L | No | No | No | Benign | No | Not pathogenic |
| PSEN1 V191A | No | Yes | No | Possibly damaging | No | Not pathogenic; Possibly protective |
| PSEN1 L219R | No | Yes | Yes | Probably damaging | N/A | Probable pathogenic; Risk factor |
| PSEN1 E318G | Yes | Yes | No | Benign | No | Not pathogenic |
| PSEN1 D333G | Yes | No | No | Possibly damaging | N/A | Not pathogenic |
| PSEN1 R352C | Yes | No | No | Possibly damaging | No | Not pathogenic |
| PSEN1 InsR352 | No | No | No | N/A | N/A | Unknown |
| PSEN1 T354I | No | No | No | Probably damaging | N/A | Probable pathogenic; Risk factor |
| PSEN1 R358Q | No | Yes | No | Probably damaging | Yes | Probable pathogenic |
| PSEN1 S365Y | No | No | No | Possibly damaging | N/A | Probable pathogenic; Risk factor |
| PSEN1 G378fs | No | Yes | Yes | N/A | Yes | Probable pathogenic |
| PSEN1 A396T | No | No | Yes | Probably damaging | Yes | Probable pathogenic |
| PSEN1 I439V | No | Yes | Yes | Benign | Yes | Probable pathogenic |
| PSEN2 T18M | Yes | No | No | Probably damaging | N/A | Probable pathogenic; Risk factor |
| PSEN2 R29H | No | No | No | Probably damaging | N/A | Probable pathogenic; Risk factor |

| Variant | 5 alleles in EVS/ExAC | Conserved between PSEN1 and PSEN2 | Known pathogenic mutations | Predicted to damage protein ^e | Increase Aβ42/40 or Aβ42 ^b | Predicted pathogenicity ^c |
|--------------|-----------------------|-----------------------------------|----------------------------|--|---------------------------------------|--------------------------------------|
| PSEN2 G34S | Yes | No | No | Benign | No | Not pathogenic |
| PSEN2 R62C | Yes | Yes | No | Possibly damaging | No | Not pathogenic |
| PSEN2 R62H | Yes | Yes | No | Benign | No | Not pathogenic |
| PSEN2 P69A | Yes | Yes | No | Benign | No | Not pathogenic |
| PSEN2 R71W | Yes | Yes | No | Benign | No | Not pathogenic |
| PSEN2 K82R | No | Yes | No | Probably damaging | No | Risk factor |
| PSEN2 A85V | No | Yes | No | Probably damaging | No | Risk factor |
| PSEN2 V101M | No | Yes | No | Probably damaging | N/A | Not pathogenic; Risk factor |
| PSEN2 P123L | No | Yes | No | Probably damaging | Yes | Probable pathogenic |
| PSEN2 E126fs | No | Yes | Yes | N/A | No | Risk factor |
| PSEN2 S130L | Yes | No | No | Possibly damaging | N/A | Not pathogenic; Risk factor |
| PSEN2 V139M | Yes | No | No | Possibly damaging | No | Not pathogenic |
| PSEN2 L143H | No | No | No | Probably damaging | N/A | Not pathogenic |
| PSEN2 R163H | No | No | No | Probably damaging | No | Not Pathogenic |
| PSEN2 H162N | Yes | No | No | Probably damaging | N/A | Not pathogenic; Risk factor |
| PSEN2 M174V | No | No | No | Benign | No | Not pathogenic |
| PSEN2 V214L | Yes | Yes | No | Probably damaging | N/A | Not pathogenic; Risk factor |
| PSEN2 I235F | No | Yes | No | Probably damaging | Yes | Probable pathogenic |
| PSEN2 A237V | Yes | Yes | No | Probably damaging | N/A | Not pathogenic; Risk factor |
| PSEN2 L238F | No | Yes | No | Probably damaging | Yes | Probable pathogenic |
| PSEN2 A252T | Yes | Yes | No | Possibly damaging | No | Not pathogenic; Possibly protective |
| PSEN2 A258V | No | No | No | Benign | No | Not pathogenic |
| PSEN2 R284G | No | Yes | No | Probably damaging | Yes | Probable pathogenic |
| PSEN2 T301M | Yes | No | No | Possibly damaging | N/A | Not pathogenic; Risk factor |
| PSEN2 K306fs | No | No | No | N/A | N/A | Unknown |
| PSEN2 P334A | No | No | No | Benign | No | Not pathogenic |
| PSEN2 P334R | Yes | No | No | Benign | N/A | Not pathogenic |
| PSEN2 P348L | No | No | No | Benign | Yes | Probable pathogenic |
| PSEN2 A377V | Yes | Yes | No | Probably damaging | N/A | Not pathogenic |
| PSEN2 V393M | Yes | Yes | No | Probably damaging | No | Not pathogenic |

| Variant | 5 alleles in EVS/ExAC | Conserved between PSEN1 and PSEN2 | Known pathogenic mutations | Predicted to damage protein ^a | Increase A β 42/40 or A β 42 ^b | Predicted pathogenicity ^c |
|-------------|-----------------------|-----------------------------------|----------------------------|--|---|--------------------------------------|
| PSEN2 T421M | No | Yes | No | Probably damaging | No | Not pathogenic |
| PSEN2 D439A | Yes | Yes | No | Probably damaging | Yes | Risk factor |

^aBenign, assigned by PolyPhen to mean not damaging.

^bN/A, cell-based data not available (see Materials and Methods).

^cUnknown indicates there is not sufficient functional/bioinformatic evidence to assign pathogenicity. Two assignments are made when functional/bioinformatics data is not complete.