TITLE: γ-Secretase activity, clinical features, and biomarkers of autosomal dominant Alzheimer's disease: cross-sectional and longitudinal analysis of the Dominantly Inherited Alzheimer Network observational study (DIAN-OBS)

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ABSTRACT

Background:

Genetic variants that cause autosomal dominant Alzheimer's disease are highly penetrant but vary substantially with respect to age at symptom onset (AAO; ranging

from ages <30 to >70), rates of cognitive decline, and biomarker changes. Most pathogenic variants that cause autosomal dominant Alzheimer's disease are in *PSEN1,* which encodes the catalytic core of γ-secretase, an enzyme complex critical to the production of β-amyloid. We investigated whether the heterogeneity seen in AAO and biomarker trajectories in *PSEN1* pathogenic variant carriers can be predicted based on the effects of individual *PSEN1* variants on γ-secretase activity and β-amyloid production.

Methods: γ-secretase activity was measured in 161 *PSEN1* variants using genetically-modified HEK293T cells. A summary measure of γ-secretase activity (GSC; $\frac{\text{short A}\beta 37+38+40}{\text{long A}\beta 42+43}$) was calculated for each variant and compared to clinical history-derived AAO. Further, using cross-sectional and longitudinal data from 190 pathogenic variant carriers participating in the Dominantly Inherited Alzheimer's Network Observational Study (DIAN-Obs; sites across the United States, Europe, South America, and Asia; data collected between Feb 29, 2008 and July 1, 2020) we assessed relationships between variant-level γ-secretase activity and *in vivo* clinical, cognitive, cerebrospinal fluid, and neuroimaging measures.

Findings: Variations in γ-secretase activity across the 161 *PSEN1* mutations examined were highly predictive of AAO (r[159] = 0.58, p < 0.0001). Variations in γ-secretase activity were also strongly correlated with all examined clinical (CDR-SB: B[SE] = -0.05[0.02], p = 0.003), cognitive (including MMSE: B[SE] = 0.08[.03], p = 0.004), and *in vivo* biomarker measures (including cortical Aβ PET burden: (B[SE] = -0.03[0.01], p < 0.0001, MRI-based hippocampal volume: B[SE] = 37.35[6.3], p < 0.0001, and CSF pT217: B[SE] = -0.009[0.002], p =0.0007) in human *PSEN1* carriers.

Interpretation: Our findings suggest that clinical heterogeneity in autosomal dominant Alzheimer's disease can be at least partly explained by different effects of *PSEN1* variants on γ-secretase activity and downstream β-amyloid production. In addition to supporting a critical link between β-amyloid production and Alzheimer's disease progression, these results support targeting the γ-secretase complex as a therapeutic approach and the incorporation of γ-secretase activity measures into autosomal dominant Alzheimer's disease clinical trials. Lastly, these results suggest cell-based models such as those used here may represent a powerful and clinically relevant tool to assess Alzheimer's disease genetic variants of unknown significance or to predict symptom onset in cases with limited family history.

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INTRODUCTION

Although only ~1% of all people who have Alzheimer's disease have autosomal dominant forms,[1] autosomal dominant Alzheimer's disease plays a disproportionately large role in our understanding of the biological basis of both late-onset, sporadic forms of Alzheimer's disease and genetically-driven forms of Alzheimer's disease. All known autosomal dominant Alzheimer's disease-causing mutations appear to impact either the expression level or metabolism of Amyloid Precursor Protein (APP) or by affecting activity of the γ-secretase complex. The study of autosomal dominant Alzheimer's disease mutations has broadly influenced the field, including the development of many transgenic animal models of Alzheimer's disease and in the development Alzheimer's disease therapeutics.

Despite the nearly complete penetrance of autosomal dominant Alzheimer's disease - causing pathogenic variants, the age at which patients develop symptoms of cognitive impairment (AAO) and the rate at which Alzheimer's disease progresses varies substantially across the many known autosomal dominant Alzheimer's disease variants. Indeed, AAO for autosomal dominant Alzheimer's disease mutations can vary from the 20s to the 70s,[1,2] suggesting that, while all variants appear to be penetrant, different mutations may be more or less potent in driving Alzheimer's disease progression.

An increase in production of longer, aggregation-prone Aβ fragments (e.g., Aβ 42 and 43) relative to shorter, non-aggregating fragments (e.g., Aβ 37 and 38) by neurons is a critical initiating pathogenic event in both late onset[3] and autosomal dominant[4,5] Alzheimer's disease. The relative balance between production of aggregating and non-aggregating forms of Aβ influences neurotoxicity[6] and is a direct result of the efficiency and kinetics with which the γ-secretase proteolytic complex cleaves APP[7–11]. Subtle alterations in γ-secretase activity can lead to profound neurodegenerative and cognitive consequences, while modulation of γ-secretase has therapeutic potential.[6,10,12–15] Over 200 pathogenic variants in *Presenilin-1* (*PSEN1*), the key catalytic subunit in the γ-secretase complex, have been identified and are the most common causes of autosomal dominant Alzheimer's disease[16]. Similar to heterogeneity in clinical and Alzheimer's disease biomarker progression seen in late onset Alzheimer's disease, there is striking heterogeneity in AAO and rates of cognitive and biomarker change[17–20] across carriers of *PSEN1* pathogenic variants.

Intriguingly, prior work suggests some pathogenic *PSEN1* variants might not significantly alter the absolute amounts of Aβ42 production[21,22], indicating that more thorough investigation is needed to understand how these pathogenic variants lead to autosomal dominant Alzheimer's disease and the mechanisms that cause earlier versus later disease onset and progression. Findings from our group[23] and others[15,24] suggest that probing the ratios of short-to-long Aβ peptide production across autosomal dominant Alzheimer's disease pathogenic variants may help us understand the observed heterogeneity in these populations. Petit and colleagues[24] investigated the utility of examining ratios of short-to-long Aβ peptide production by γ-secretase for predicting estimated AAO by performing an analysis of 25 autosomal dominant Alzheimer's disease-causing *PSEN1* variants and generating Aβ profiles *in vitro*; they

showed that a ratio of short-to-long Aβ peptide production by the γ-secretase complex may be predictive of AAO.

Here, we examine a large set of *PSEN1* pathogenic variants with AAO ranging from the 20s to the 70s and functionally characterize APP processing by the γ-secretase complex for each variant. We then compare this mutation-level characterization of γ-secretase function to cross-sectional and longitudinal clinical, cognitive, biofluid, and neuroimaging data from people affected by autosomal dominant Alzheimer's disease, and thereby test whether the heterogeneity seen in by autosomal dominant Alzheimer's disease can be partly explained by mutation-level variations in the enzymatic activity of the γ-secretase complex.

METHODS

Study design

We performed cross-sectional and longitudinal analyses of data from participants who were enrolled in the Dominantly Inherited Alzheimer Network observational study (DIAN-Obs). Data from DIAN-Obs data freeze version 15 (last data from June 30, 2020) were utilized. We included individuals with *PSEN1* pathogenic variants and relevant genetic, clinical, imaging, and cerebrospinal fluid (CSF) data. Participants in DIAN-Obs provided informed consent prior to the completion of any study procedures in accordance with the local institutional review boards of each participating site. DIAN-Obs study procedures have received ethics approval at Washington University (MO, USA) and all participating sites.

Participants

Data were included from 190 people carrying *PSEN1* pathogenic variants. Within this sample, 56 unique *PSEN1* pathogenic variants were represented. These 56 variants were characterized via cell-based methods (see Figure 1A, Table 1, and appendix pg 10). Detailed protocols for DIAN-Obs have previously been published and are described in supplemental methods.

Clinical evaluators were blind to the mutation status of participants. As part of their enrollment in DIAN-Obs, participants undergo comprehensive clinical and cognitive evaluations. Clinical Dementia Rating (CDR®) Sum of Boxes (SB) was assessed for each participant using structured interviews, as previously described[25]. Mini-Mental State Examination (MMSE) and Wechsler Memory Scale-Revised Logical Memory Delayed Recall[26] are sensitive global cognition and memory performance and were selected *a priori* as our cognitive measures of interest.

Procedures

Functional assays of γ-secretase activity were performed for 161 unique *PSEN1* variants, including 56 unique variants represented in DIAN-Obs and data from the remaining, non-overlapping, 105 unique variants previously characterized by our group,[23] and wild-type *PSEN1*. Using methods detailed appendix pg 1-2 and a prior report using this model system, each *PSEN1* variant or normal *PSEN1* was sub-cloned and placed into the PcDNA 3.1 expression vector[23]. Subsequently, each variant was co-

transfected with a plasmid encoding wild-type human APP-C99 (99 amino acid fragment of the APP C-terminus) into HEK293T cells genetically depleted of *PSEN1* and *PSEN2*[23]. Creation of this cell line and transient transfection procedures are described in appendix pg 1 and 22. Conditioned media from transfected HEK cells was harvested and diluted with 1% BSA in wash buffer (TBS supplemented with 0.05% Tween). Aβ37, 38, 40, 42, and 43 immunoassays were performed in triplicate and averaged as previously described[23] (see appendix pg 2). As the wildtype or variant PSEN1 were transiently expressed in the cells, to normalize the amount of secreted Aβ peptides from different transfectants, we further used a ratio between a specific secreted Aβ and the total secreted Aβ C-terminal variants (37+38+40+42+43) as has been done previously[23,24]. We leveraged prior literature[3,23,24,27] suggesting a measure of the efficiency of successive cleavage (γ-processivity) of Aβ fragments can be used to summarize γ-secretase activity. Building on the work of Petit and colleagues[24], γ-secretase activity composite was summarized as a ratio of $\frac{\text{short A}\beta 37+38+40}{\text{long A}\beta 42+43}$. To aid in the interpretability of the ratio, we used this ratio as a percent relative to the production seen in the same model system following transfection with wildtype *PSEN1 (*Gamma Secretase Composite; GSC = *variant PSEN1* γ-secretase activity composite / wildtype PSEN1 γ-secretase activity composite * 100)*.*

For DIAN-Obs study participants, AAO was determined through structured interviews to determine the age at onset of progressive cognitive decline for the participant and their first degree relative(s)[25]. For the remaining 105 unique variants previously characterized by our group[23] (but not represented in DIAN-Obs) the previously reported AAO (derived from literature review)[23] was used for the current analyses.

Imaging and Fluid Biomarker Analyses

Detailed protocols for Aβ and brain metabolism positron emission tomography (PET), magnetic resonance imaging (MRI), and CSF studies are described in supplemental methods (appendix pg 3-5 and 23) and in previously published work[20,25,28–30]. Cerebral Aβ load was measured using [^{11}C]Pittsburgh Compound B (PiB) PET, and brain glucose metabolism was measured with [^{18}F] Fluorodeoxyglucose (FDG)-PET. Partial volume corrected values were used in all analyses. FreeSurfer v 5.3 defined regional measures (appendix pg 23) were derived from MRI data. CSF Aβ42, Aβ40, and phosphorylated-tau181 levels were measured using an automated immunoassay system (LUMIPULSE G1200, Fujirebio, Malvern, PA, USA). Phosphorylated-tau 217 (pT217/T217) values were derived from immunoprecipitation-mass spectrometry (IP-MS) as previously described[28] (appendix pg 5).

Statistical Analyses

Individuals from DIAN-Obs were included in cross-sectional analyses if they had completed at least one Aβ PET scan, MRI scan, and at least one cognitive assessment (Figure 1A). A subset of individuals also had [^{18}F] FDG-PET (n=162) and CSF (n=157) available for cross-sectional analyses. Longitudinal data were available from 154 DIAN-Obs participants (appendix pg 10).

Statistical analyses were performed using R version 4.0.3. As described in more detail in the supplemental methods (appendix pg 6-8). Pearson correlations were used to assess associations of GSC with AAO. Next, in order to demonstrate the potential utility of using the GSC to predict an approximate AAO for novel pathogenic variants and/or when a clinical history is not available, we first generated a linear model that defined the linear relationship of γ-secretase activity to the literature-derived AAO in the 105 PSEN1 variants characterized previously and not represented in DIAN-Obs ($\widehat{AAO} \sim 28.14 + 0.27 * \mathrm{GSC}$). We next used this equation to predict AAO for the 56 variants included in the DIAN-Obs (AAO_{gsc}; appendix pg 11-15).

Linear mixed effect models (LMM) including fixed effects for age, self-reported sex, and GSC were employed to assess associations between the cell-based γ-secretase activity and multi-modal biomarker and clinical data from DIAN-Obs. Similar to prior work from DIAN-Obs, a random effect for family membership was included in each linear mixed effects model. Separate models were used to assess associations with each clinical, cognitive, or biomarker outcome measure, focusing on PiB-PET, FDG-PET, CSF Aβ 42/40, CSF $\log_{10}$(phosphorylated-tau 181), CSF $\log_{10}$(pT217/T217), hippocampal volume, CDR-SB, MMSE, and logical memory delayed (see supplemental methods; appendix pg 6-8). Years of education was included as an additional fixed effect term in models examining cognitive and clinical outcomes. The output of each LMM is reported as unstandardized beta coefficients (B), the corresponding standard error [SE], and p-value.

LMM were similarly constructed but made use of an annualized rate of change for each outcome of interest (appendix pg 6). Additional analyses made use of a tertile split of GSC scores to visualize how clinical, cognitive, and biomarker trajectories can be understood in the context of γ-secretase activity. Exploratory, regional analyses comparing PiB-PET standardized uptake value ratios (SUVRs), grey matter volumes, and FDG-PET SUVRs across *PSEN1* carriers with variants in the lowest (most pathogenic; GSC ≥ 33.7% relative to wildtype) vs. highest (least pathogenic; GSC ≤ 51.6% relative to wildtype) tertiles of GSC.

Lastly, to visualize how variant differences in γ-secretase activity may lead to distinct patterns of autosomal dominant Alzheimer's disease progression, we plotted the core clinical, cognitive, and biomarker measures across the lifespan after splitting *PSEN1* pathogenic variants into tertiles based on GSC using data from the DIAN-Obs study. To complement this group-level analysis and to model how cell-based γ-secretase activity scores may predict individual disease trajectories in clinical and clinical research settings, we also used all available data to visualize putative disease trajectories for 2 hypothetical individuals carrying mutations with 25% and 75% GSC, respectively.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

RESULTS

Grouping together *in vitro* HEK293T cell-based Aβ production for all 161 variants (Figure 1B and appendix pg 11-17), median (sd) levels relative to wildtype *PSEN1* for Aβ37, 38, and 40 were 46.49% (44.4), 51.49% (51.4), and 85.13% (64.2). This indicates that, as a group, *PSEN1* pathogenic variants decrease the processive γ-cleavage of Aβ by γ-secretase (γ-processivity), leading to a decrease in these fully processed (shorter) Aβ peptides. Additionally, relative levels of Aβ42 and 43 across variants were higher compared to that observed with wildtype *PSEN1* (median (sd) = 182.41% (221.1) and 158.96% (1174.4), respectively; Figure 1B).

Next, we assessed relationships between the relative concentrations of Aβ species, using the GSC, produced by each variant and AAO. Lower GSC values (Pearson's correlation [degrees of freedom], p-value: r[159] = 0.58, p < 0.0001) were associated with earlier AAO (Figure 1C).

We further examined the potential utility of using the GSC to predict an approximate AAO for novel pathogenic variants and/or when a clinical history is not available. There was high correlation between the history-derived AAO and the GSC-derived AAO (AAO_{gsc}) in the subset of variants represented in DIAN-Obs (r [54] = 0.59, p < 0.0001; appendix pg 11-15).

The location of the *PSEN1* mutation with respect to the affected region within the PSEN1 protein (Figure 1D) did not directly map onto the history derived AAO determined for each DIAN-Obs participant (colored circles, Figure 1D). Furthermore, for sites within the protein for which multiple variants were present (e.g., Met146 for which there were 3 variants characterized) substantial variation in GSC was observed based on the substituted amino acid (Figure 1D and appendix pg 11-15).

In the DIAN-Obs study sample (n = 190 people, representing 56 unique PSEN1 variants), lower GSC (decreased γ-secretase activity relative to wildtype) was associated with higher levels of mean cortical Aβ burden (B[SE] = -0.03[0.01], p < 0.0001; Figure 2A and appendix pg 18) as assessed by Aβ PET, after controlling for demographic factors. Exploratory regional analyses revealed that the significant association between variant-level GSC and Aβ-PET signal was observed in many cortical regions, especially the precuneus and the cingulate regions (Figure 2D).

We next examined associations between the variant-level GSC and commonly used imaging biomarkers of neurodegeneration. Both MRI-based hippocampal volume (B[SE] = 37.35[6.3], p < 0.0001; Figure 2B and appendix pg 18) and precuneus FDG-PET signal (B[SE] = 0.004[0.001], p = 0.001; Figure 2C and appendix pg 18) were highly associated with the GSC, after controlling for demographic factors. Exploratory regional analyses revealed that the cell-based GSC significantly was associated with widespread grey matter volume differences across *PSEN1* variant carriers, most notably in the hippocampus, amygdala, temporal regions, precuneus, and posterior cingulate (Figure 2E), as well as differences in glucose metabolism including the precuneus, parietal, and frontal regions (Figure 2F).

Using established CSF measures of Alzheimer's disease pathology, we observed that variants with lower GSC were associated with lower CSF Aβ 42/40 (B[SE] = 5.32e-04[1.4e-04], p = 0.0004; Figure 2G and appendix pg 19), higher CSF ELISA $\log_{10}$(phosphorylated-tauT181) levels (B[SE] = -0.007[0.002], p = 0.0003; Figure 2H and appendix pg 19), and higher CSF IP-MS $\log_{10}$(pT217/T217) levels (B[SE] = -0.009[0.002], p =0.0007; Figure 2I and appendix pg 19).

The cell-derived GSC levels were associated with MMSE (B[SE] = 0.08[.03], p = 0.004; Figure 2J and appendix pg 20), CDR-SB (B[SE] = -0.05[0.02], p = 0.003; Figure 2K and appendix pg 20), and logical memory-delayed scores (B[SE] = 0.09[.02], p = 0.0006; Figure 2L and appendix pg 20).

We next assessed how longitudinal rates of change in core biomarker, clinical, and cognitive measures of interest (Figure 3A, C, E, and G) may be related to cell-based measures of γ-secretase activity, summarized using the GSC. Variants with lower (more pathogenic) GSCs were associated with faster increase in β-amyloid PET signal (B[SE] = -7.5e-04[3e-04], p = 0.005; Figure 3B and appendix pg 21), as well as more rapid decreases in hippocampal volume (B[SE] = 4.19[0.8], p < 0.0001; Figure 3D and appendix pg 21), MMSE (B[SE] = 0.02[0.01], p = 0.002; Figure 3F and appendix pg 21), and logical memory-delayed (B[SE] = 0.004[0.001], p = 0.0003; Figure 3H and appendix pg 21).

Visualization of the core clinical, cognitive, and biomarker measures across the lifespan depicted within low-, middle-, and high- GSC *PSEN1* pathogenic variant in corresponding pathogenic variant carriers in DIAN-Obs (Figure 4A-C),as well as visualizations of the putative disease trajectories for 2 hypothetical individuals carrying mutations with 25% and 75% GSC, respectively (Figure 4D-I) demonstrate that variants with lower GSC (i.e., greater impairment in γ-secretase activity) exhibit an earlier shift in the age at which cognitive symptoms and biomarker changes become evident. Notably, a similar pattern was seen when using estimated years to symptom onset (EYO; calculated by subtracting history-derived AAO from the participant's age) rather than age (appendix pg 24). This suggests that cell-based assessments of γ-secretase add to the prediction of disease trajectories even after history-derived information about AAO is included in statistical models.

However, while a shift in the timing of symptom and biomarker changes was seen across a range of γ-secretase activity, it is notable that elevations in pathological Aβ burden (higher Aβ PET or lower CSF Aβ42) and tau (higher CSF phosphorylated-tau181) appear to be early events across all tertiles and precede changes in neurodegenerative markers (hippocampal volume, FDG PET) and clinical measures of impairment (CDR-SB). Together, these results suggest that, while mutation-level variations in γ-secretase activity may impact AAO and rate of autosomal dominant Alzheimer's disease progression, the ordering of clinical, cognitive, and biomarker changes is not itself fundamentally altered by differences in γ-secretase activity.

DISCUSSION:

Using a cell-based model system to assess the production of a broad set of Aβ peptides, we observed that mutation level differences in γ-secretase processing of APP strongly predicted the age at which progressive cognitive symptoms are manifest (AAO) for a given variant. In addition to predicting AAO, we observed that a summary measure of γ-secretase activity (GSC) was associated with the rates of functional and cognitive decline. GSC was also associated with all fluid and imaging biomarker measures we examined, including CSF measures of Alzheimer's disease pathology, Aβ PET, FDG-PET, and MRI gray matter volumes. These results suggest that accounting for mutation-level γ-secretase dysfunction may clarify treatment effects in autosomal dominant Alzheimer's disease clinical trials and may also be useful in predicting approximate AAO and disease trajectories for *PSEN1* pathogenic variant carriers that have novel variants or carry a pathogenic variant that lacks sufficient clinical history. Mechanistically, these results highlight the importance of γ-secretase activity on Alzheimer's disease pathobiology and provide support for γ-secretase modulation as a potentially powerful Alzheimer's disease therapeutic and preventative strategy[31].

These results here are concordant with recent findings from Petit and colleagues[24], who used virally induced mouse embryonic fibroblasts to characterize 25 *PSEN1* variants, finding correlations between variant-level Aβ production and AAO. Building on this prior work and using the same composite measure of variant level γ-secretase activity, we used a high-throughput human cell-based model system to characterize a large set of *PSEN1* variants, including some with available cross-sectional and longitudinal data in corresponding *PSEN1* variant carriers. Specifically, we observed that within two datasets – 105 *PSEN1* variants with available AAO, and an additional 56 *PSEN1* variants with full clinical and biomarker data – that a composite measure of Aβ peptide production and γ-secretase activity ($\frac{\mathrm{A\beta 37+38+40}}{\mathrm{A\beta 42+43}}$) expressed as a percent relative to wildtype PSEN1 (GSC) was predictive of AAO. In contrast, initial work by Sun and colleagues[32], using a cell-free assay, showed no clear relationship between variant-level Aβ 42/40 production and AAO. However, the cell-free assay used in this prior study destabilizes PSEN1[33], which may lead to more severe pathogenic variants not producing Aβ. Furthermore, an extended re-analyses of these data[34], after removing an outlier, contradicted these initial results and support the current finding that γ-secretase activity and Aβ production ratios are associated with AAO.

We observed a small number of pathogenic variants (Ala164Val, Thr147Pro, Glu318Gly, Val82Leu, Glu69Asp, and Asp40Del) with apparent GSC greater than 100% (i.e., greater than that observed with wildtype *PSEN1* in our model system). Further work will be needed to understand this observation, especially as there is no cognitive, clinical, or biomarker data for these variants available from the DIAN-Obs study[35–41]. Of note, out of these six variants, three (Ala164Val, Thr147Pro, and Glu69Asp) are not classified as clearly pathogenic, one (Asp40Del) is considered of uncertain significance, one (Glu318Gly) has been re-classified as benign, and one (Val82Leu) is considered

likely pathogenic. Furthermore, our results for these variants are concordant with previous *in vitro* work[24,32] suggesting low or uncertain pathogenicity.

Previous reports investigating variant-dependent clinical and biomarker heterogeneity in *PSEN1* pathogenic variant carriers have utilized location-dependent characteristics including whether a pathogenic variant is located prior-to or post-codon 200 of PSEN1 [19,20], within hydrophilic loop 1 of PSEN1[9], and whether the pathogenic mutation impacts a cytoplasmic vs transmembrane protein domain within PSEN1[42]. We used a more direct approach to characterize individual variants beyond location or the part of the protein affected by the underlying mutation, focusing on the functional consequences of each variant on γ-secretase activity. In the current study, we observed GSC scores could differ even within a particular codon based on which amino acid substitution is present (e.g., Met146). This observation points out an important limitation of categorizing individual pathogenic variants based on location alone and underscores the need for further work to better characterize how different variants affecting the same codon can lead to putatively differential changes in protein structure and differential γ-secretase activity.

We found that the GSC measure explained substantial *in vivo* biomarker heterogeneity across *PSEN1* pathogenic mutation carriers using available data from DIAN-Obs. Focusing on associations between the GSC and Aβ PET, we observed that individuals who carried pathogenic variants in the lowest (most pathogenic) tertile had, on average, 1.4 SUVR units greater Aβ PET signal compared to those with variants in the highest (least pathogenic) tertile, after accounting for age and sex. Putting these differences in the context of the conventionally used threshold for Aβ PET positivity in this cohort and with this Aβ PET tracer (cortical PiB PET SUVR positive at >= 1.42 SUVR), carriers of pathogenic variants in the lowest (most pathogenic) tertile of the GSC would, on average, become amyloid positive ~15 years sooner than those carrying *PSEN1* variants in the highest (least pathogenic) tertile (estimated mean age of 23 and 37 years for low and high tertiles, respectively. Consistent with cross-sectional results, analysis of longitudinal data demonstrated that Aβ PET signal increased more quickly in individuals carrying *PSEN1* variants with lower (more pathogenic) GSC scores compared to those with higher (less pathogenic) composite scores. Though further work is needed to elucidate regional topology differences in Aβ deposition trajectories, the results here also suggest that variants with low GSC may have greater striatal Aβ PET signal compared to carriers of *PSEN1* variants with high GSC values.

CSF measures of Alzheimer's disease pathology in *PSEN1* carriers also had significant associations with the variant-level GSC, including CSF measures of Aβ42/40, phosphorylated-tau181, and phosphorylated-tau217. In addition to associations with core measures of Alzheimer's disease pathobiology, GSC correlated with FDG PET and structural MRI, two commonly used neuroimaging measures of neurodegeneration. Together with the described associations with AAO and functional impairment measures, this broad pattern of association between γ-secretase activity and core Alzheimer's disease and neurodegenerative markers further support the hypothesis that heterogeneity in autosomal dominant Alzheimer's disease can broadly be understood in

the context of mutation-level differences in γ-secretase activity. Importantly, despite variations in AAO and biomarker trajectories across variants, the results here also support prior observations that pathologic changes in Aβ and tau consistently precede neurodegenerative changes and clinical impairment across *PSEN1* variants[23,24,32]. Additionally, it remains quite possible that different formulations of the GSC will be better optimized to predict particular biomarker trajectories in clinical trial settings.

Complementary work is underway to translate these findings to fluid biomarkers of Aβ. Recent studies investigating CSF and blood-based Aβ monomers[25,46], oligomers[47,48] and ratios of short-to-long Aβ monomers [49] (e.g., Aβ 37/42[23]), have highlighted the potential utility of evaluating Aβ peptides outside of Aβ40 and Aβ42 in CSF and blood. To this end, work from our group suggests that the Aβ 37/42 ratio in CSF better distinguishes cognitively normal individuals from those with Alzheimer's disease as compared to the CSF Aβ42/40 ratio[23]. However, interpreting Aβ monomer levels in biofluids can be complex as Aβ production, clearance, and deposition are all processes affecting biofluid peptide concentrations *in vivo*. Notably, the cell-based model system used here allows us to focus on the extent to which each variant impacts the production of pathogenic Aβ species without the potentially confounding effects of Aβ clearance and deposition that may impact *in vivo* biofluid measures of Aβ.

The results here need to be considered in the context of certain limitations. Though AAO information was available on all *PSEN1* variants examined, only a subset had available clinical, cognitive, and biomarker data from the DIAN-Obs study. Accordingly, data from new pathogenic variants in DIAN-Obs and in the broader scientific literature will need to be considered. Similarly, tau PET data will need to be considered in future analyses as they become available. Additionally, it will be important to determine if processing of γ-secretase substrates besides APP, such as Notch[43–45], add to the information derived from Aβ production as it relates to heterogeneity in biomarker, clinical, and cognitive progression of autosomal dominant Alzheimer's disease. Furthermore, the model system used here cannot assess how changes in γ-secretase activity within individuals over time may impact disease progression, and further work will be needed to assess changes in γ-secretase activity over the lifespan of *PSEN1* mutation carriers. Lastly, it will be important to extend the cell-based model system to allow for the characterization of pathogenic variants in *PSEN2* and *APP*, as ~20% of autosomal dominant Alzheimer's disease-causing variants are found within these genes.

Despite these limitations, these findings indicate that variant-level differences in γ-secretase activity are highly associated with core measures of clinical, biomarker, and cognitive, progression of autosomal dominant Alzheimer's disease, and suggest that these variant-level differences explain a large portion of the heterogeneity seen across *PSEN1* pathogenic mutation carriers. As the biochemical measures of γ-secretase activity used here are derived outside the context of family polygenic factors, age, and estimated years to symptom onset, they offer a potentially unique channel of information with respect to disease progression compared to these other factors and may thus represent useful complements to these measures in autosomal dominant Alzheimer's

disease clinical trials. Similarly, as these cell-based measures are divorced from family polygenic factors and age, they may prove to be valuable in gauging the impact of genetic and lifestyle factors that may confer resilience or risk for autosomal dominant Alzheimer's disease onset and progression. Cell-based measures of γ-secretase activity may be particularly useful to assess pathogenicity, potential AAO, and predicted Alzheimer's disease trajectories for newly discovered *PSEN1* pathogenic variants or for variants lacking sufficient clinical history.

Importantly, our results showing γ-secretase activity, derived from a cell-model system, is associated with pathogenicity of disease also supports the continued investigation of γ-secretase modulators as an Alzheimer's disease therapeutic strategy. By demonstrating an association between the processing of APP by γ-secretase in a cell-based model system and autosomal dominant Alzheimer's disease trajectories *in vivo*, the results here provide support for the hypothesis that dysregulation of APP processing is central to Alzheimer's disease pathogenesis.

DIAN Data Accessibility:

Due to the rarity of dominantly inherited Alzheimer's disease, individual-level data from DIAN cannot be shared publicly, as it would compromise participant anonymity. This limitation has been validated by the Institutional Review Board (IRB) and confirmed with the NIH. Nevertheless, this data remains accessible for qualified researchers upon request. Requests can be submitted through the following link: DIAN Biospecimen Request Form.

Author Contributions:

Study design and conceptualization: SAS, LL, DJS, JPC
Data collection and curation: SAS, LL, APS, CDF, RL, JPB, ZS, NJM, CDC, TLSB, GSD, MRF, BAG, JJH, CRJ, MJ, CMK, JHL, JL, RJP, PRS, CX, KAJ, EM, RJB, RAS, DJS, JPC
Data analysis and interpretation: SAS, LL, DJS, JPC
Visualization: SAS, LL, JPC
Writing – original draft: SAS, LL, DJS, JPC
Writing – review & editing: SAS, LL, APS, CDF, RL, JPB, ZS, NJM, CDC, TLSB, GSD, MRF, BAG, JJH, CRJ, MJ, CMK, JHL, JL, RJP, PRS, CX, KAJ, EM, RJB, RAS, DJS, JPC
Verified Underlying Data: SAS, LL, JPC

AUTHORS' CONFLICT OF INTERESTS

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All other authors have nothing to disclose.

RESEARCH IN CONTEXT

Evidence before this study: We searched PubMed from September 1,1990 to September 1, 2023 for relevant articles in English relating to autosomal dominant Alzheimer's disease pathogenic variants that affect γ-secretase processing of amyloid precursor protein (APP). Search terms included: "dominantly inherited Alzheimer's disease", "autosomal dominant Alzheimer's disease", "familial Alzheimer's disease", "gamma-secretase", "amyloid precursor protein", "presenilins", "γ-secretase", "amyloid", "PSEN1", "APP", "Aβ42", and "Aβ40". Previous studies have suggested an increase in production of longer, aggregation-prone Aβ fragments (e.g., Aβ 42 and 43) relative to shorter, non-aggregating fragments (e.g., Aβ 37 and 38) is a critical initiating pathogenic event in both late-onset Alzheimer's disease and autosomal dominant Alzheimer's disease. Intriguingly, prior work suggests some pathogenic *PSEN1* variants may not significantly alter the absolute amounts of Aβ42 production, indicating that more thorough investigation is needed to understand how these pathogenic variants lead to autosomal dominant Alzheimer's disease and the mechanisms that cause earlier vs. later disease onset and progression. To this end, findings from our group and others suggest that probing the ratios of short-to-long Aβ peptide production across autosomal dominant Alzheimer's disease pathogenic variants may help us understand the observed heterogeneity in these populations.

Added value of this study: Findings from the current study indicate that variant-level differences in γ-secretase activity are highly associated with core measures of clinical, biomarker, and cognitive, progression of autosomal dominant Alzheimer's disease, and suggest that these variant-level differences explain a large portion of the heterogeneity seen across *PSEN1* pathogenic mutation carriers. As the biochemical measures of γ-secretase activity used here are derived outside the context of family polygenic factors, age, and estimated years to symptom onset, they offer a potentially unique channel of information with respect to disease progression compared to these other factors and may thus represent useful complements to these measures in autosomal dominant Alzheimer's disease clinical trials. Cell-based measures of γ-secretase activity may be particularly useful to assess pathogenicity, potential AAO, and predicted Alzheimer's disease trajectories for newly discovered *PSEN1* pathogenic variants or for variants lacking sufficient clinical history.

Implications of all of the available evidence: The cell-based methods used here represent a powerful tool to better understand the observed heterogeneity in Alzheimer's disease progression in clinical and research settings, as well as a means of assessing autosomal dominant Alzheimer's disease variants of unknown significance or in cases with limited family history. Our findings suggest that clinical heterogeneity in autosomal-dominant Alzheimer's disease can be at least partly explained by varying effects of PSEN1 variants on γ-secretase activity. If these findings are confirmed in prospective longitudinal studies, assessing γ-secretase activity across PSEN1 variants or within individuals at risk for Alzheimer's disease might eventually be useful for prognosis and identifying new therapeutic approaches.

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TABLE AND FIGURE LEGENDS

Table 1. Cross-sectional study sample characteristics for DIAN-Obs participants.

Variable	N = 190[1]
Age (yrs)	**39.0 (32.0, 48.0)**
Sex	
Male	**109 (57%)**
Female	**81 (43%)**
APOE4	
Non-Carrier	**55 (29%)**
Carrier	**135 (71%)**
Education (yrs)	**14.0 (12.0, 16.0)**
EYO (yrs)	**-5.5 (-13.1, 1.4)**
AAO (yrs)	**44.5 (40.6, 51.4)**
CDR Global	
CDR 0	**110 (58%)**
CDR 0.5	**44 (23%)**
CDR 1+	**36 (19%)**
Race	
white	**163 (86%)**

[1] Median (IQR); n (%)

Median (IQR) values presented unless otherwise noted.

EYO = Estimated years to symptom onset; AAO= Estimated age at symptom onset; CDR = Global Clinical Dementia Rating Scale

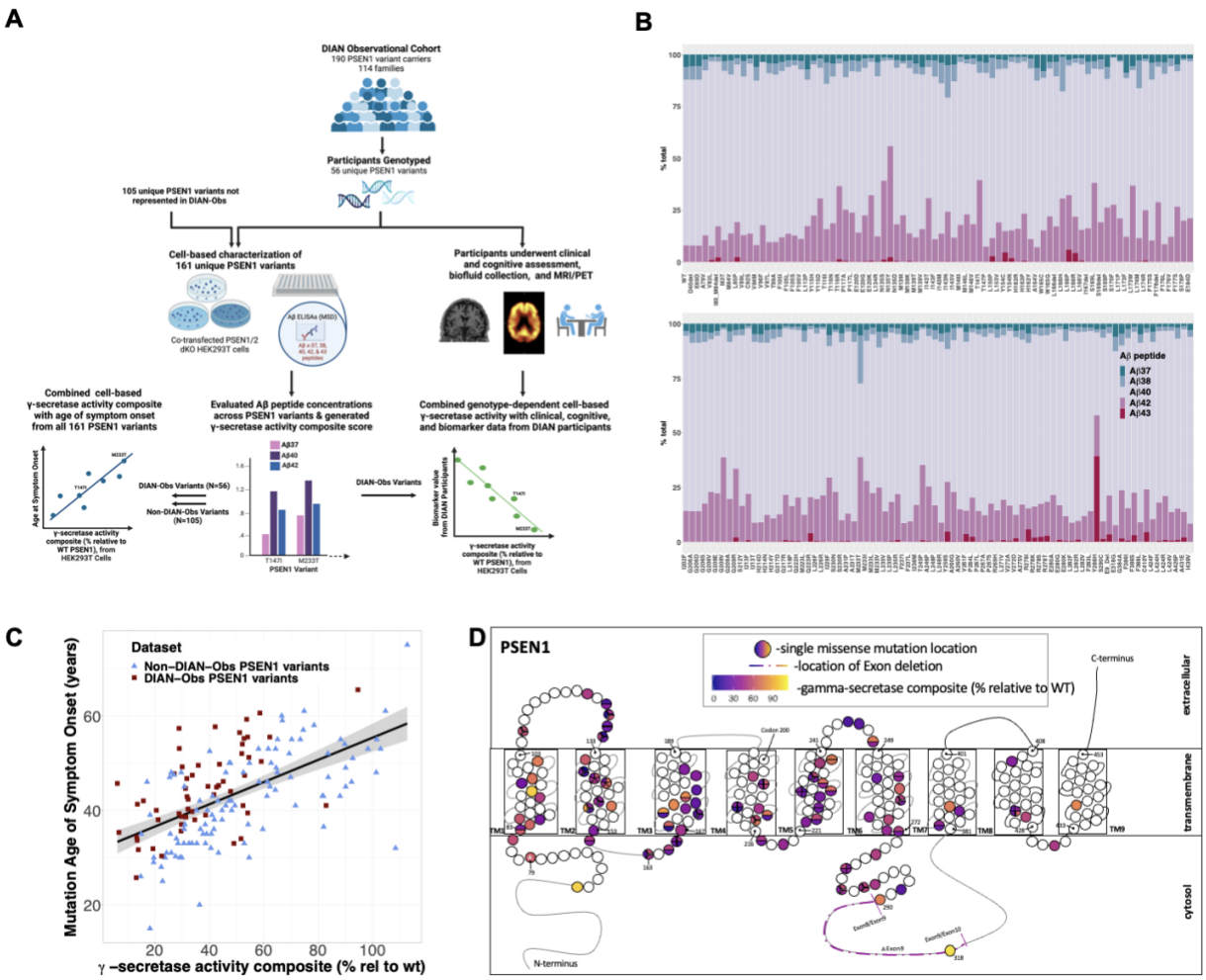

Figure 1. Cell-based measures of γ-secretase activity are associated with age of symptom onset across two cohorts representing 161 unique *PSEN1* variants. (A) Schematic summarizing the characterization of variant-level Aβ production and γ-secretase activity, and correlations with age of symptom onset, cognitive, and biomarker data for each variant (created with BioRender.com). **(B)** Bar chart showing the individual Aβ monomer levels (% relative to total Aβ) for each of the 161 PSEN1 pathogenic variants examined as well as wildtype (WT) PSEN1. (**C**) γ-secretase activity was summarized as a ratio of short (fully γ-secretase processed) to long (incompletely γ-secretase processed) Aβ fragments ($\frac{\text{short Aβ37+38+40}}{\text{long Aβ42+43}}$) . This γ-secretase activity composite (% relative to wildtype PSEN1; GSC) correlated with age of symptom onset in 161 *PSEN1* variants, including 105 *PSEN1* variants not represented in DIAN-Obs (blue triangles) and 56 newly characterized *PSEN1* variants represented in DIAN-Obs (red squares). Individual data points are jittered to maintain blinding. The shaded area around the solid black linear fit line represents one s.e.m. from a linear regression. **(D)** Visualization of GSC scores for each of the 161 *PSEN1* variants characterized.

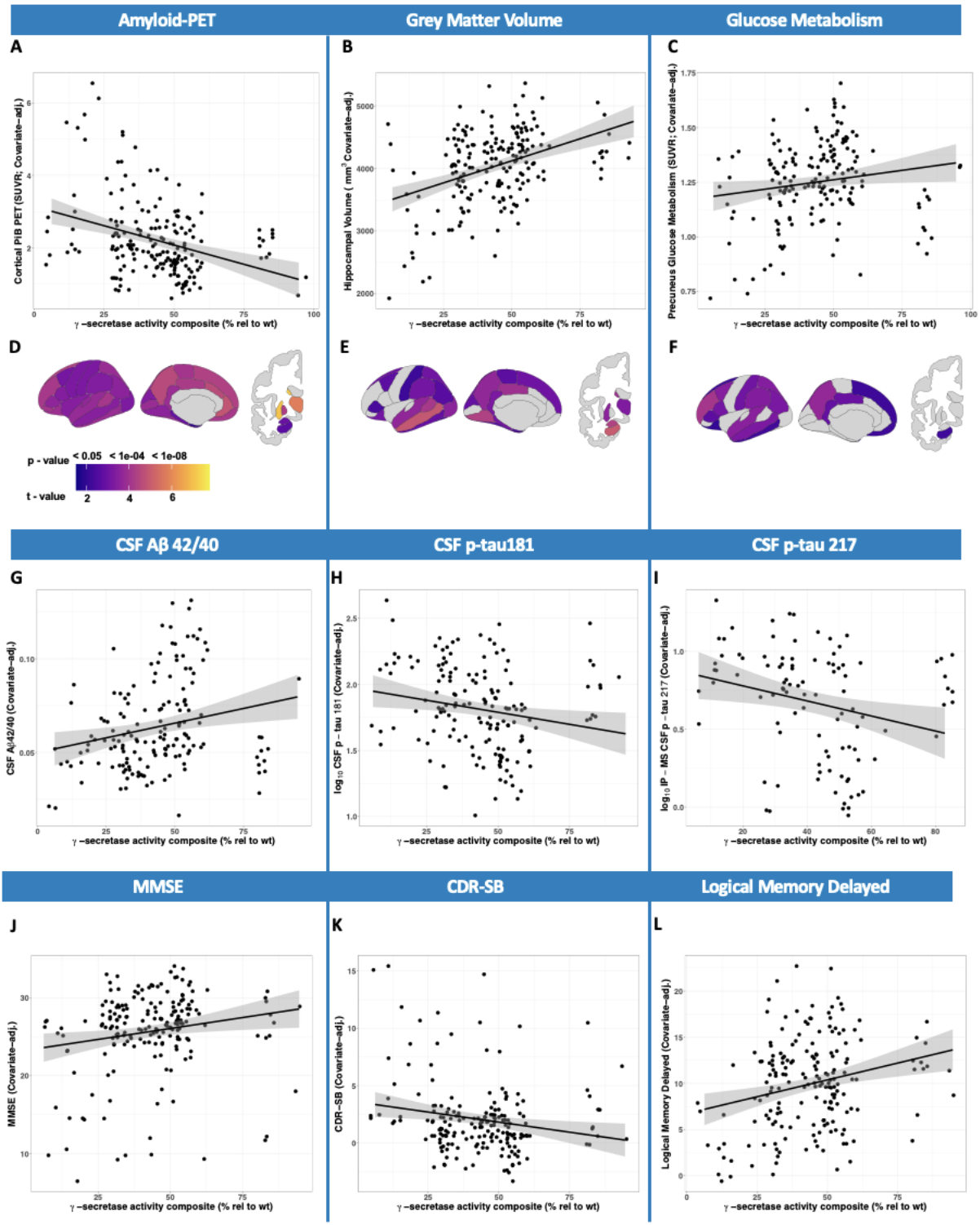

Figure 2. Lower γ-secretase activity across variants is associated with more abnormal biomarker, clinical, and cognitive measures in autosomal dominant Alzheimer's disease. Association between variant-level γ-secretase activity composite scores (% relative to wildtype PSEN1; GSC) and in *vivo* imaging biomarker data from carriers of corresponding *PSEN1* pathogenic variants is shown for cross-sectional Aβ

PET (N = 190, covariate-adjusted PiB-PET SUVR cortical composite, **A**), covariate-adjusted bilateral hippocampal grey matter volume derived from structural MRI (N = 190, **B**), and covariate-adjusted bilateral precuneus FDG-PET SUVR (N = 162, **C**) from *PSEN1* pathogenic variant carriers (56 unique variants represented across 190 mutation carriers). Exploratory, regional analyses comparing PiB-PET SUVRs (**D**), grey matter volumes (**E**), and FDG-PET SUVRs (**F**) across a range of GSC, with colors indicating t- and p-values derived from the comparison of neuroimaging data from *PSEN1* carriers with variants in the lowest (most pathogenic; GSC < 33.7% relative to wildtype) vs. highest (least pathogenic; GSC > 51.6% relative to wildtype) tertiles of GSC. Individual data points are jittered to maintain blinding. Associations between γ-secretase activity composite scores (GSC) for each *PSEN1* variant and cross-sectional CSF Aβ 42/40 (N = 157, **G**), covariate-adjusted CSF $\log_{10}$ pT181 (N = 157, **H**), covariate-adjusted CSF $\log_{10}$ pT217/T217 (N = 99, **I**), covariate-adjusted MMSE (N = 190, **J**), age-adjusted CDR-SB (N = 190, **K**), and covariate-adjusted logical memory-delayed (N = 186, **L**) values from *PSEN1* pathogenic variant carriers are shown. Individual data points are jittered to maintain blinding. The shaded area around the solid black linear fit line represents one s.e.m. from a linear regression.

CSF = Cerebrospinal Fluid; pT = phosphorylated tau; MMSE = Mini Mental State Examination scores; CDR-SB = Clinical Dementia Rating Scale- Sum of Boxes.

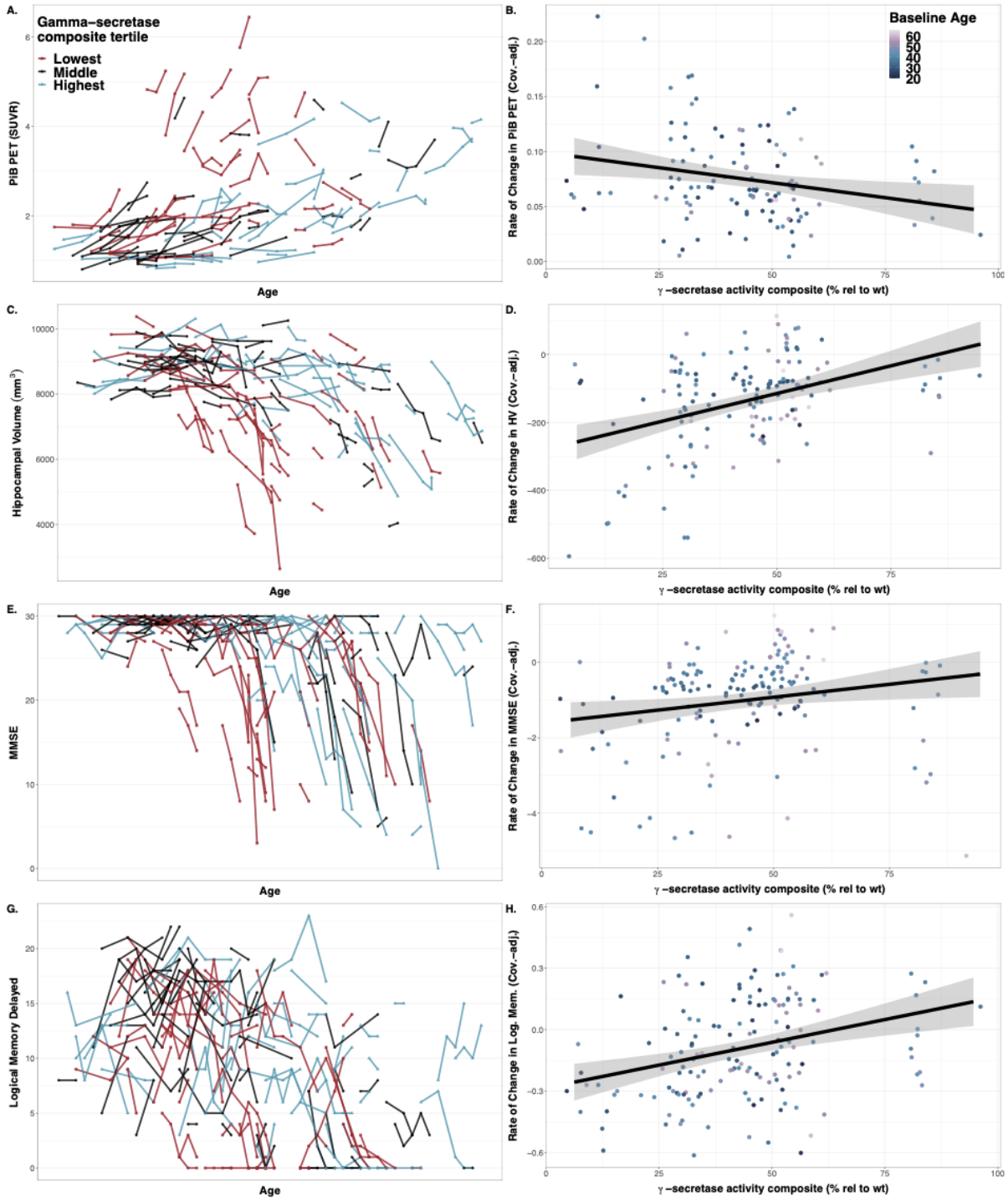

Figure 3. Lower γ-secretase activity across *PSEN1* variants is associated with more rapid worsening in biomarker, clinical and cognitive measures in autosomal dominant Alzheimer's disease. Individual longitudinal measures are plotted for PiB-PET SUVR (N = 119, Aβ burden; **A**), hippocampal volume (N = 141, HV; mm^3; **C**), Mini Mental Status Exam (N = 154, MMSE; **E**), and logical memory delayed (N = 152, Log. Mem.; **G**). Colors indicate whether an individual's *PSEN1* variant is in the low (red; most

pathogenic tertile; GSC < 33.7%), middle (black; intermediate pathogenicity; GSC of 33.7% – 51.6%), or high (blue; least pathogenic, GSC > 51.6%) tertile for γ-secretase activity composite score (% relative to wildtype; GSC). To facilitate statistical comparison, covariate-adjusted, individual-level annualized rate of change slopes for each biomarker and cognitive outcomes of interest were extracted from linear mixed effects models and plotted against GSC (Aβ PET – **B**; Hippocampal Volume – **D**; MMSE – **F**; Logical Memory – **H**). Individual data points are jittered to maintain blinding. The shaded area around the solid black linear fit line represents one s.e.m. from a linear regression.

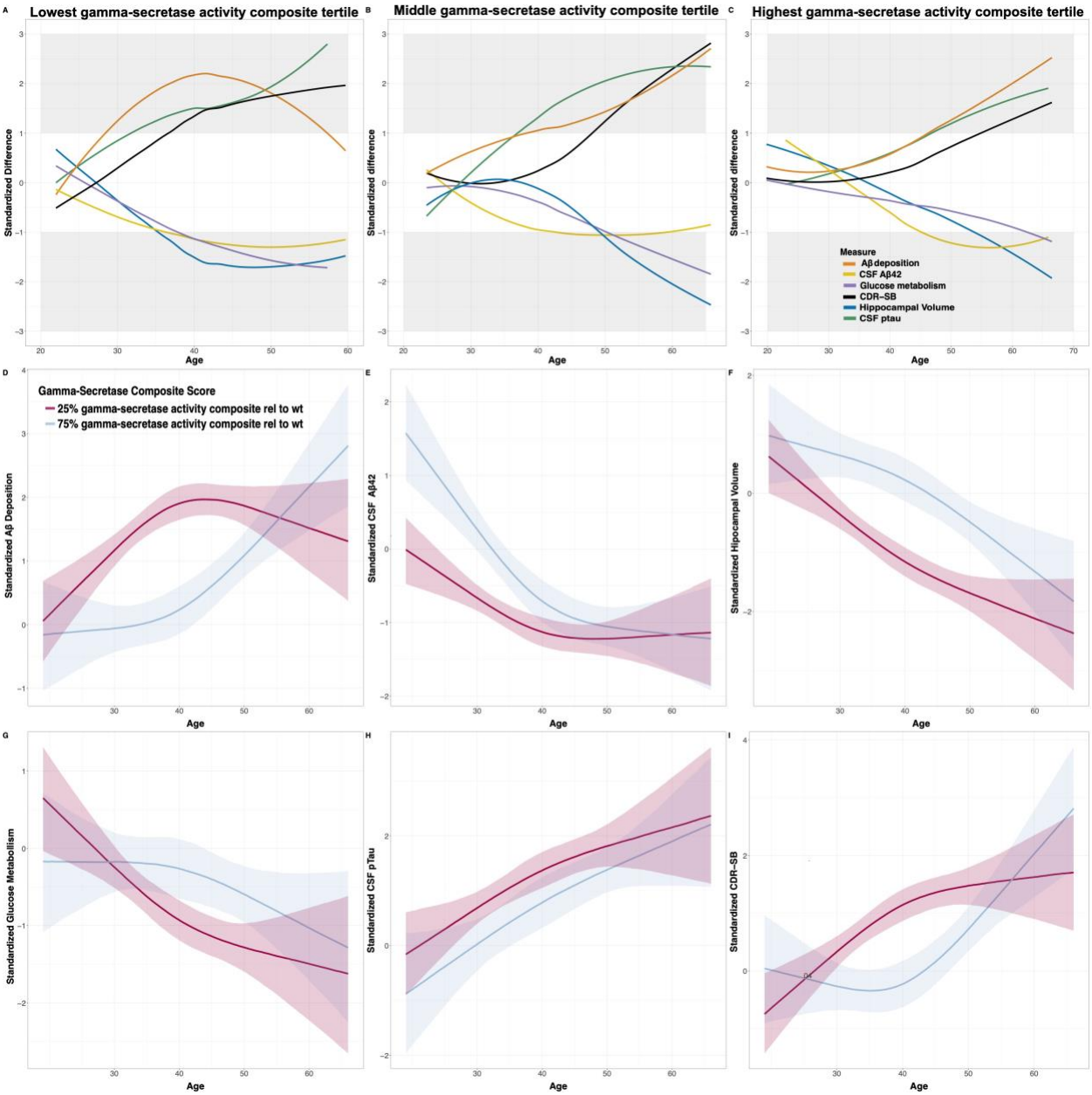

Figure 4. Variant-level variations in γ-secretase activity are broadly associated with the clinical and biomarker course and can be used to predict progression for novel *PSEN1* pathogenic variants. To visualize how variant-level differences in γ-secretase activity may broadly alter the clinical, cognitive, and biomarker course of autosomal dominant Alzheimer's disease, we plotted the standardized differences in core clinical, cognitive, and biomarker measures across the lifespan for DIAN-Obs participants carrying *PSEN1* pathogenic variants in the lowest (**A**, most pathogenic tertile; GSC < 33.7% relative to wild type), middle (**B,** intermediate pathogenicity; GSC = 33.7% – 51.6% relative to wildtype), or highest (**C**; least pathogenic, GSC > 51.6% relative to wildtype) tertile for γ-secretase activity (GSC). All values are shown as z-scores relative to mutation non-carriers from DIAN-Obs. This group-level analysis was followed by an individual-level visualization of disease trajectories for pathogenic variant

801 carriers of 2 possible variants with 25% and 75% of wildtype γ-secretase activity (GSC),
802 respectively (D-I).