

## Research Report

# What Threshold of Amyloid Reduction Is Necessary to Meaningfully Improve Cognitive Function in Transgenic Alzheimer's Disease Mice?

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### Abstract.

**Background:** Amyloid- $\beta$  plaques (A $\beta$ ) are associated with Alzheimer's disease (AD). Pooled assessment of amyloid reduction in transgenic AD mice is critical for expediting anti-amyloid AD therapeutic research.

**Objective:** The mean threshold of A $\beta$  reduction necessary to achieve cognitive improvement was measured via pooled assessment ( $n=594$  mice) of Morris water maze (MWM) escape latency of transgenic AD mice treated with substances intended to reduce A $\beta$  via reduction of beta-secretase cleaving enzyme (BACE).

**Methods:** Machine learning and statistical methods identified necessary amyloid reduction levels using mouse data (e.g., APP/PS1, LPS, Tg2576, 3xTg-AD, control, wild type, treated, untreated) curated from 22 published studies.

**Results:** K-means clustering identified 4 clusters that primarily corresponded with level of A $\beta$ : untreated transgenic AD control mice, wild type mice, and two clusters of transgenic AD mice treated with BACE inhibitors that had either an average 25% "medium reduction" of A $\beta$  or 50% "high reduction" of A $\beta$  compared to untreated control. A 25% A $\beta$  reduction achieved a 28% cognitive improvement, and a 50% A $\beta$  reduction resulted in a significant 32% improvement compared to untreated transgenic mice ( $p<0.05$ ). Comparatively, wild type mice had a mean 41% MWM latency improvement over untreated transgenic mice ( $p<0.05$ ). BACE reduction had a lesser impact on the ratio of A $\beta_{42}$  to A $\beta_{40}$ . Supervised learning with an 80%–20% train-test split confirmed A $\beta$  reduction was a key feature for predicting MWM escape latency ( $R^2=0.8$  to 0.95).

**Conclusions:** Results suggest a 25% reduction in A $\beta$  as a meaningful treatment threshold for improving transgenic AD mouse cognition.

**Keywords:** Alzheimer's disease, anti-amyloid therapy, artificial intelligence, BACE inhibitor, effect size, machine learning, transgenic mice

## INTRODUCTION

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Dementia is a term used to describe the deterioration of the mind through behavior, memory, and language [1, 2]. One of the most common forms of dementia is Alzheimer's disease (AD). AD is

characterized as the decline in cognition that affects behavior and body function simultaneously [3]. The Amyloid Cascade Hypothesis has long been among the leading theories of causation of AD [4]. It postulates that the accumulation of amyloid- $\beta$  (A $\beta$ ) protein in the brain is a key event in the pathogenesis of AD [3, 4]. A $\beta$  is produced by the cleavage of amyloid- $\beta$  protein precursor (A $\beta$ PP) by  $\beta$ -secretase (BACE) and  $\gamma$ -secretase enzymes. Two major forms of A $\beta$  are produced: A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub>. A $\beta$ <sub>40</sub> is more abundant, but A $\beta$ <sub>42</sub> is more prone to aggregation and is believed to be the main component of amyloid plaques [5]. Additionally, A $\beta$  can exist in both soluble and insoluble forms, which may have different effects on the brain. Insoluble A $\beta$  refers to A $\beta$  that has aggregated into fibrils, which can form amyloid plaques in the brain. These plaques are a hallmark of AD and are believed to disrupt communication between neurons, leading to neuronal dysfunction and death. Soluble A $\beta$ , on the other hand, refers to A $\beta$  that has not aggregated into fibrils and remains in a soluble form in the brain [6, 7]. Soluble A $\beta$  can also have toxic effects on neurons and has been implicated in synaptic dysfunction and neuronal damage [8, 9].

A recent in-depth review on the role of A $\beta$  in AD presented evidence from multiple studies that patients with more severe AD pathology typically have significantly higher levels of A $\beta$  [10]. The levels of soluble A $\beta$  are often considered a better predictor of the degree of neurodegeneration than the levels of insoluble A $\beta$  [11]. As such, targeting soluble A $\beta$  may be a potential therapeutic strategy. A recent meta-analysis of 14 randomized trials illustrated that the pooled estimate for the effect of reducing amyloid levels by 0.1 standardized uptake value ratio units was an improvement in the Mini-Mental State Examination score of 0.03 (95% confidence interval -0.06 to 0.1) points [12].

A recent report discussed the great potential for anti-amyloid therapies like lecanemab and aducanumab to become an eventual standard of care treatment for clinical AD [13]. Patients who received lecanemab had a significantly slower rate of cognitive and functional decline compared to those who received a placebo. However, the increased risk of amyloid-related imaging abnormalities with lecanemab warrant close monitoring and additional longitudinal follow-up [14]. Future research is necessary to determine if and when at-risk, asymptomatic patients should initiate an anti-amyloid therapy to potentially stave off AD before clinical symptoms manifest.

Transgenic mice present a unique experimental paradigm to expedite clinical research and successful therapeutic translation and have been pivotal to AD research. For a recent comprehensive review on transgenic AD mouse models, please see Sanchez-Varo et al. [15]. Briefly, mouse models enable the assessment of multi-scalar, multi-factorial pathological mechanisms and initial drug safety profiles that cannot be easily or ethically tested in humans. Transgenic AD mice are genetically engineered to express human genes that are associated with the development of AD. By first testing in transgenic mice, researchers can more quickly and cost effectively screen drug candidates and potential doses necessary for a positive outcome. As such, understanding the pooled effect size of amyloid reduction with anti-amyloid therapy in transgenic AD mice is pivotal for AD mechanism examination and improving anti-amyloid drug protocols.

Transgenic AD mouse cognitive function can be measured with a variety of tests. One of the most popular cognitive assessments is the Morris water maze (MWM), which tests spatial memory and learning. In the MWM, transgenic AD mice undergo repeated training to find a hidden platform within a cold-water bath [16]. Mice use visual and spatial clues to "remember" where the platform is located so they can more quickly escape the cold water. After a pre-determined number of trials, mice are timed to see how quickly they find the platform. The amount of time it takes to find the platform is called the MWM escape latency. Faster MWM escape latency is analogous to improved cognitive performance.

The absolute levels of A $\beta$  may not always reflect the severity of the disease. For example, in some cases, mice with relatively low levels of A $\beta$  may still show signs of cognitive impairment, while mice with high levels of A $\beta$  may not [17]. Similarly, meta-analysis of multiple experimental mouse studies, which examined only A $\beta$  or the ratio of A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> found a weak trend with cognition [18]. Further, pooled analysis comparing the impact of A $\beta$  and phosphorylated tau found that the impact of phosphorylated tau far exceeded A $\beta$  in transgenic AD mice [19]. However, there has not been a pooled estimate of amyloid reduction in transgenic mice treated with substances meant to decrease A $\beta$ . Comparing the multi-study pooled estimate of A $\beta$  reduction to the analogous pooled clinical estimate of A $\beta$  reduction [12] would provide critical insight for future translational research.

The goal of this study was to determine the mean threshold of A $\beta$  reduction necessary to achieve a specific quantitative threshold of cognitive improvement in transgenic AD mice treated with anti-amyloid therapies. Prior transgenic AD mice studies that used therapeutic substances to directly or indirectly reduce BACE have widely varied in their ability to reduce A $\beta$  and improve cognition. The present study determines the mean A $\beta$  reduction necessary to achieve meaningful cognitive improvement in transgenic AD mice treated with substances that either directly or indirectly reduce or inhibit BACE. Knowing such aggregate effect sizes is crucial for planning future anti-amyloid preclinical and clinical therapeutic studies. Here we perform statistical analysis and machine learning on a carefully curated pool of published transgenic AD mouse data to determine the average threshold of A $\beta$  reduction necessary to achieve meaningful improvement in MWM escape latency.

## METHODS

The following two questions guided the methodological framework: 1) By how much does A $\beta$  need to be reduced (via BACE inhibitors) before there is a meaningful decrease in transgenic AD mouse MWM escape latency (e.g., improved cognition)? 2) What

measured features are most important to predicting transgenic AD mouse MWM escape latency? Statistical methods were used in tandem with machine learning algorithms to infer answers from the aggregated experimental data.

### Inclusion and exclusion criteria

The experimental data collected for this study was extracted and curated from the peer-reviewed, published literature. Studies were identified through key term searches in PubMed and PubMed Central. The terms searched were “Alzheimer’s disease”, “amyloid-beta”, “beta-secretase”, and “Morris Water Maze”. Searches also included synonyms, possible variations in terms or phrases, or abbreviations. Treatments included substances that inhibited BACE, whether a traditional pharmacological BACE inhibitor or a natural, biologically active substance like Vitamin D. Included studies must have provided quantifiable data for A $\beta$  levels and quantifiable data for MWM escape latency on day 1 and either day 4 or day 5. Day 1 was used as the baseline performance and day 4 or day 5 was used to assess differences in cognition. Day 4 or day 5 was previously determined by prior work [18–20] to be optimal for measuring differences in cognition without risk of overtraining

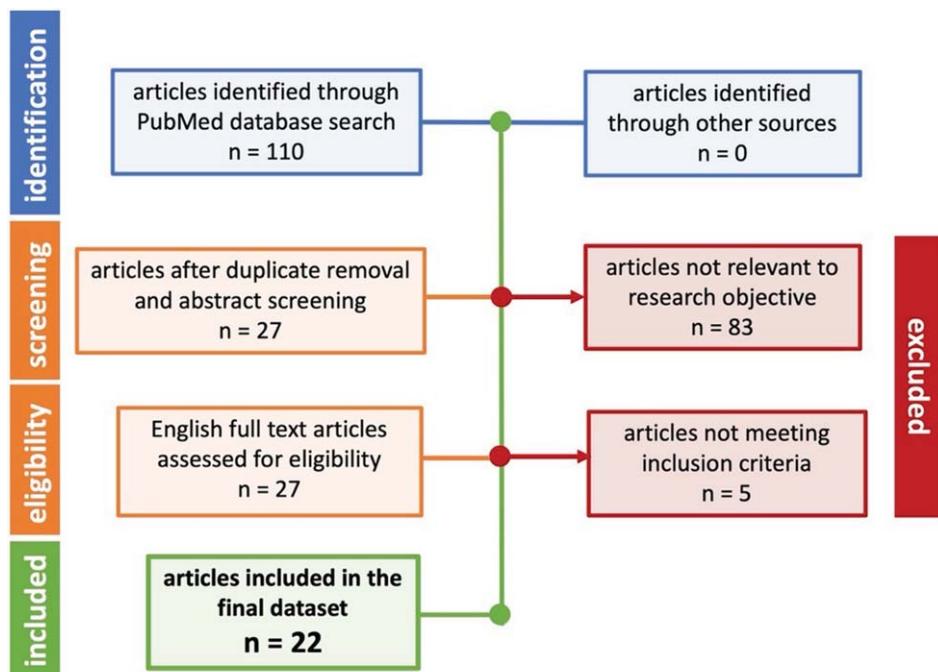


Fig. 1. PRISMA flow diagram for the systematic review of articles related to AD BACE. Once included, data was carefully curated into a relational database.

or undertraining the mice on the MWM task. To maximize overall included sample size, MWM escape latency from either day 4 or day 5 in each study was an acceptable measure for study inclusion. Only full text published articles in English were considered for inclusion. Figure 1 illustrates the PRISMA diagram for article inclusion. Table 1 illustrates the full list of included articles and extracted data.

#### *Data curation*

Data curation protocols with follow-up quality control by trained curators were as previously described [21]. Briefly, curators were trained to collect quantitative mouse model data and descriptive methods from full text articles downloaded from PubMedCentral or through online e-journal subscriptions available from Georgia Institute of Technology or Emory University. A minimum of 3 curators separately entered the values for all features and marked missing values as null within a relational database. Then, a project-specific curation quality control manager checked all data to ensure veracity. Tabular data was directly extracted from the original experimental studies and entered into the corresponding relational database fields. Quantitative data from graphs was extracted from the original experimental studies using WebPlot Digitizer and exported to the relational database. The final pooled dataset was exported into a standard spreadsheet conducive for follow-up analysis in Microsoft Excel and Python SciKitLearn.

The fields of the relational database and corresponding features of the dataset utilized by this study were guided by our prior work analyzing transgenic AD mouse data [18–20]. Feature definitions are briefly described here with comprehensive definitions provided in the Results. Continuous features included A $\beta$ <sub>42</sub> level, A $\beta$ <sub>40</sub> level, ratio of A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub>, BACE level, MWM experimental latency (on day 4 and/or day 5), MWM original latency (on day 1). Continuous features were also calculated as percentage of untreated control in each original study. Categorical features include mouse genotype, mouse treatment status (control or treated), significance of BACE level as reported in the original study, significance of A $\beta$ <sub>42</sub> as reported by the original study, and solubility status of the curated A $\beta$  measurement (soluble or insoluble).

#### *Data processing*

The primary analysis was to assess MWM as a function of A $\beta$  reduction. As noted in the Inclusion

Criteria, MWM escapes latency was captured for day 4 and/or day 5 and compared to day 1. Specifically, MWM escape latency on day 4 was calculated as percent of MWM escape latency on day 1. Likewise, MWM escape latency on day 5 was calculated as a percent of MWM escape latency on day 1. In studies where both day 4 and day 5 data were available, the average of the day 4 and day 5 measurement was utilized such that there was a single MWM escape latency measurement from each study. Due to a lack of measured feature uniformity across original studies, the A $\beta$  and BACE metrics were converted to percent of control on a per-study basis. All MWM measurements were first converted into units of seconds before being standardized into percentage-based features to normalize for any experimental set-up differences across studies [18–20].

There were no missing values for features related to A $\beta$ <sub>42</sub> level, MWM escape latency, or BACE level. However, about 20% of the samples were missing values for either A $\beta$ <sub>40</sub> and/or the A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> ratio. Missing features were imputed using the k-nearest algorithm (kNN). The kNN algorithm used the five nearest neighbors to impute the missing value. Prior to clustering features were processed to remove skew, which is otherwise known to adversely impact machine learning algorithms. Finally, a 5% outlier detection and removal were performed in Python using the isolation forest algorithm.

#### *Statistical analysis*

Statistical analysis was performed to compare features between clusters identified from unsupervised learning. When comparing two or more continuous features, a two-tailed student's *t*-test was performed using an alpha of 0.05 to identify significant differences between groups. When comparing two or more categorical features, a Chi Square analysis was performed at an alpha of 0.05. In the case of multiple comparisons for either *t*-test or Chi-Square, a Bonferroni correction with a family-wise alpha of 0.05 was utilized to prevent a Type I error. Statistical analysis was performed in Microsoft Excel.

#### *Unsupervised learning*

Unsupervised learning enables the elucidation of patterns from unlabeled data. Here two different forms of unsupervised learning were performed together, dimensionality reduction and clustering, to determine the best underlying grouping or “thresholds” of A $\beta$  reduction in transgenic AD mice treated

with BACE inhibitors. Dimensionality reduction algorithms minimize the number of features utilized to explain the preponderance of variance. Clustering algorithms take unlabeled data and use the measured features to cluster the samples into groups that are most similar. Prior work has shown that utilizing dimensional reduction prior to clustering can improve the clustering patterns and improve separability of clusters [22]. Here we perform PCA followed by k-means clustering. Silhouette analysis was performed to determine the optimal number and separability of clusters [23]. All unsupervised learning was performed in Python SciKitLearn using both continuous and categorical features.

#### Supervised machine learning

Supervised machine learning was used to assess how well the identified thresholds from unsupervised learning could be used to predict MWM escape

latency. The data was analyzed with and without feature selection. As is standard in machine learning, a set of traditional supervised algorithms were utilized: linear regression, ridge regression, polynomial ridge regression, decision tree, and tuned decision tree. Synthetic minority oversampling (SMOTE) was used to balance the data set. The data was randomly partitioned into 80% and 20% splits. The 80% split was used for model training and the 20% split was used as held-out test set. Model performance was measured using  $R^2$ . All supervised learning was performed in Python SciKitLearn.

## RESULTS

A combination of statistical analysis and machine learning was utilized to quantify the mean reduction of A $\beta$  necessary to achieve significant and meaningful improvements in MWM escape latency. Figure 2

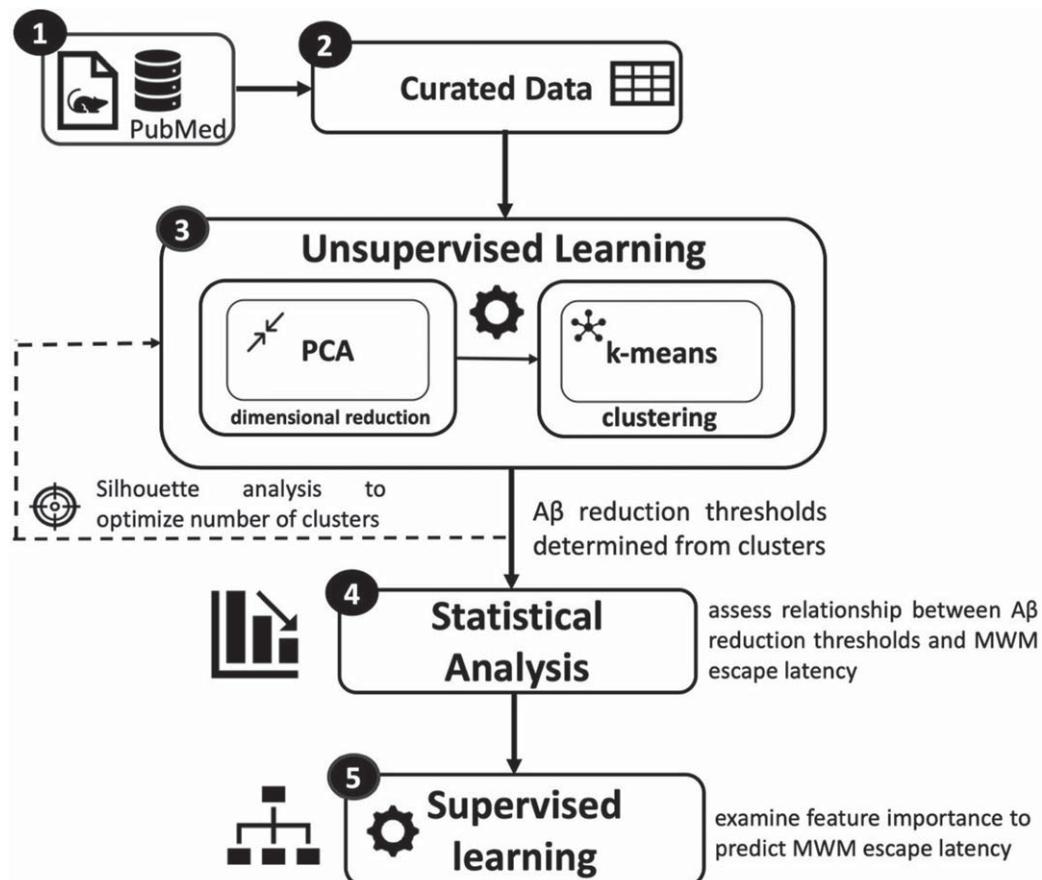


Fig. 2. Overview of data pipeline and analysis. Continuous and categorical features were curated from 22 journal articles. A combination of machine learning and statistical analysis techniques were utilized to quantify A $\beta$  reduction thresholds necessary to meaningfully improve AD transgenic mouse cognition measured via the Morris Water Maze escape latency.

Table 1

List of included mouse type, mouse type sample size, and included study citations

Mouse type	Sample size	Citations
3xTg-AD	116	[29–32]
A $\beta$ 1–42	50	[33]
APP/PS1	200	[24, 34–40]
LPS	30	[26, 41]
SAMP8	30	[27]
SAMR1	10	[27]
Tg2576	112	[25, 28, 42–45]
Wild Type	46	[24, 28, 39, 42, 45]
TOTAL	594	

illustrates the analysis pipeline. First, a total of 22 journal articles with transgenic AD mouse data met the study's inclusion criteria. Second, the data from the original articles ( $n=594$  mice) were curated to obtain a set of continuous and categorical features necessary for analysis. Third, unsupervised learning identified the clusters used to infer potential mean A $\beta$  reduction thresholds necessary for meaningful improvement of MWM escape latency. Fourth, statistical analysis was performed to determine if the identified A $\beta$  reduction thresholds significantly improved MWM escape latency. Finally, supervised learning algorithms were utilized to assess feature importance in predicting MWM escape latency.

A summary of the aggregated sample sizes is shown in Table 1. There was a total of 594 mice from 7 mouse types: 3xTg-AD, Tg2576, APP/PS1, A $\beta$ 1–42, APP/PS1, LPS, SAMP8, SAMR1, and wild type. The largest samples sizes were from APP/PS1 ( $n=200$ ), 3xTg-AD ( $n=116$ ), and Tg2576 ( $n=112$ ) transgenic AD mouse genotypes. Each mouse model was split into control or treated. The treated mice consisted of approximately 65% of the total samples. Treatments for directly or indirectly reducing or inhibiting BACE and/or A $\beta$  varied with examples including traditional BACE inhibitors like 2,2,4-Trihydroxychalcone [24] or GRL-8234 [25], a plant extract like 4-O-methylhonokiol [26], a nutritional supplement combination of vitamin D and resveratrol [27], and behavior modifying treatments like cognitive stimulation [28].

The following primary metrics were either directly curated from the original published studies or derived using the curated data. The naming convention of the features matches those shown in Fig. 3.

- Unique\_ID – this feature assessed the potential for study-specific biases in the aggregated data.

- N\_value – the sample size of the feature was collected to perform aggregate statistical analysis and to assess for potential sample size related biases.
- Mouse\_Model – this categorical feature included the various types of mouse model genotypes included for analysis. Genotypes included wild type, APP/PS1, A $\beta$ 1–42, Tg2576, LPS, 3xTg-AD, SAMP8, SAMR1.
- Control\_Treated – this categorical feature stated whether the sample was treated or untreated with the intention of decreasing A $\beta$ .
- Solubility – this categorical feature stated whether the measured A $\beta$  was soluble or insoluble.
- Abeta40\_pg\_g – this feature measured the quantitative level of A $\beta$ 40 in units of pg per gram. If a study had other units, it was converted to pg\_g for this feature.
- Units\_Abeta40 – this categorical feature stated the original units of the A $\beta$ 40 measurement prior to normalization. This feature was included only as a sanity check to the normalization process.
- Significant\_Abeta40 – this categorical feature stated whether the measured A $\beta$ 40 was significant compared to control in the original data source.
- Abeta40\_percent\_of\_control – this continuous feature measured the A $\beta$ 40 as a percentage relative to untreated transgenic control within the same original study.
- Abeta42\_pg\_g – this feature measured the quantitative level of A $\beta$ 42 in units of pg per gram. If a study had other units, it was converted to pg\_g for this feature.
- Units\_Abeta40 – this categorical feature stated the original units of the A $\beta$ 40 measurement prior to normalization. This feature was included only as a sanity check to the normalization process.
- Significant\_Abeta42 – this categorical feature stated whether the measured A $\beta$ 42 was significant compared to control in the original data source.
- Abeta42\_percent\_of\_control – this continuous feature measured the A $\beta$ 42 as a percentage relative to untreated transgenic control within the same original study.
- 42\_40\_ratio – this continuous feature measured the ratio of A $\beta$ 42 to A $\beta$ 40 within a given study for each mouse type sample.
- Ratio\_42\_40\_ratio – this continuous feature measured the ratio of A $\beta$ 42 to A $\beta$ 40 in BACE

inhibitor treated mice to the ratio of A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> in non-treated mice for a given mouse sample type within the same original study.

- Overall\_Abeta\_percent\_of\_control – calculated overall average of A $\beta$  relative to untreated transgenic control for a given mouse type within the same original study.
- MWM\_N\_value - the sample size was collected to perform aggregate statistical analysis and to assess for potential sample size related biases.
- Original latency – the MWM escape latency for a given mouse type within a given study prior to training. It is essentially a baseline measurement of innate swimming ability and/or differences in maze set-up and used to normalize Experiment\_Latency.
- Experiment\_Latency – this continuous feature measured the MWM latency at day 4 and/or day 5. Once normalized, it was the measure of cognition used to compare mouse types and the efficacy of amyloid-reducing BACE inhibitor treatment(s).
- BACE\_level\_percent\_of\_control – this continuous feature measured the level of BACE

compared to untreated transgenic control mice within the same study.

- BACE\_Significant – this categorical feature measured whether there was a significant difference in BACE for a given mouse type within a given study.

A correlation matrix was produced (Fig. 3) to illustrate the association(s) between the features. The correlations with Experiment\_Latency were deemed most relevant to the present study's goals. The features with the strongest correlations to Experiment\_Latency were: Original\_Latency, 0.62; Abeta42\_percent\_of\_control, 0.44; Control\_Treated, 0.38; Abeta40\_percent\_of\_control, 0.34; Ratio\_42\_40\_Ratio, 0.32. There was a relatively lower correlation with Mouse\_Model, 0.17.

Input features for unsupervised learning included both continuous and categorical data. Subsequently, PCA was performed followed by clustering with the k-means algorithm (Fig. 4). The different clustering patterns for  $k = 3$ ,  $k = 4$ , or  $k = 5$  clusters is shown in Fig. 4a-c, respectively. The corresponding silhouette plots (left side of each panel in Fig. 4) measured how

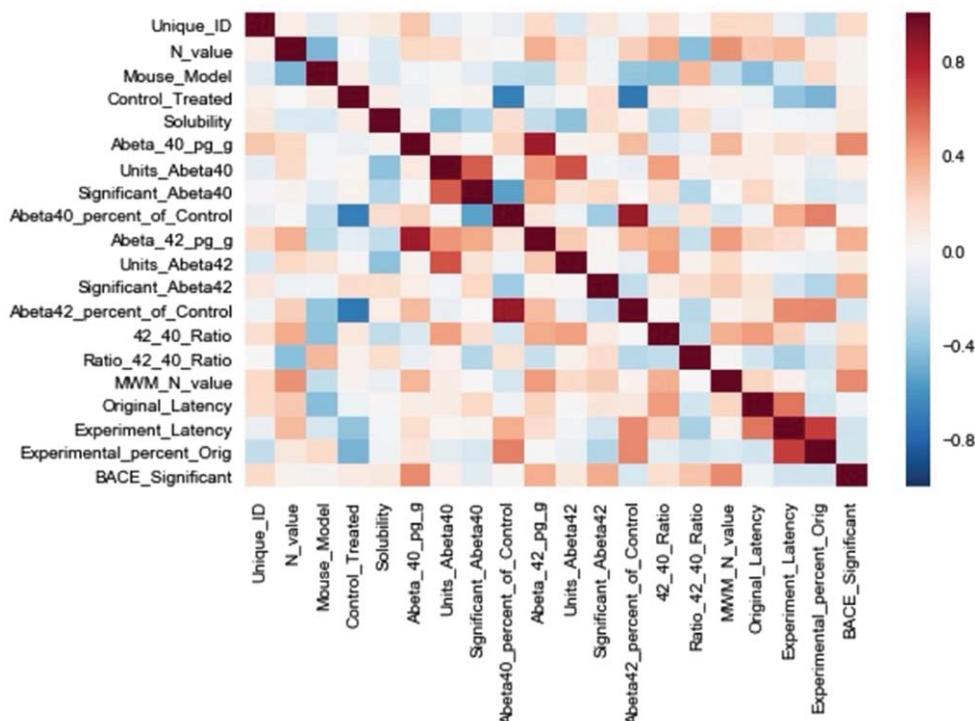


Fig. 3. Pearson cross-correlation matrix of extracted features. Feature definitions are defined in the main article text.

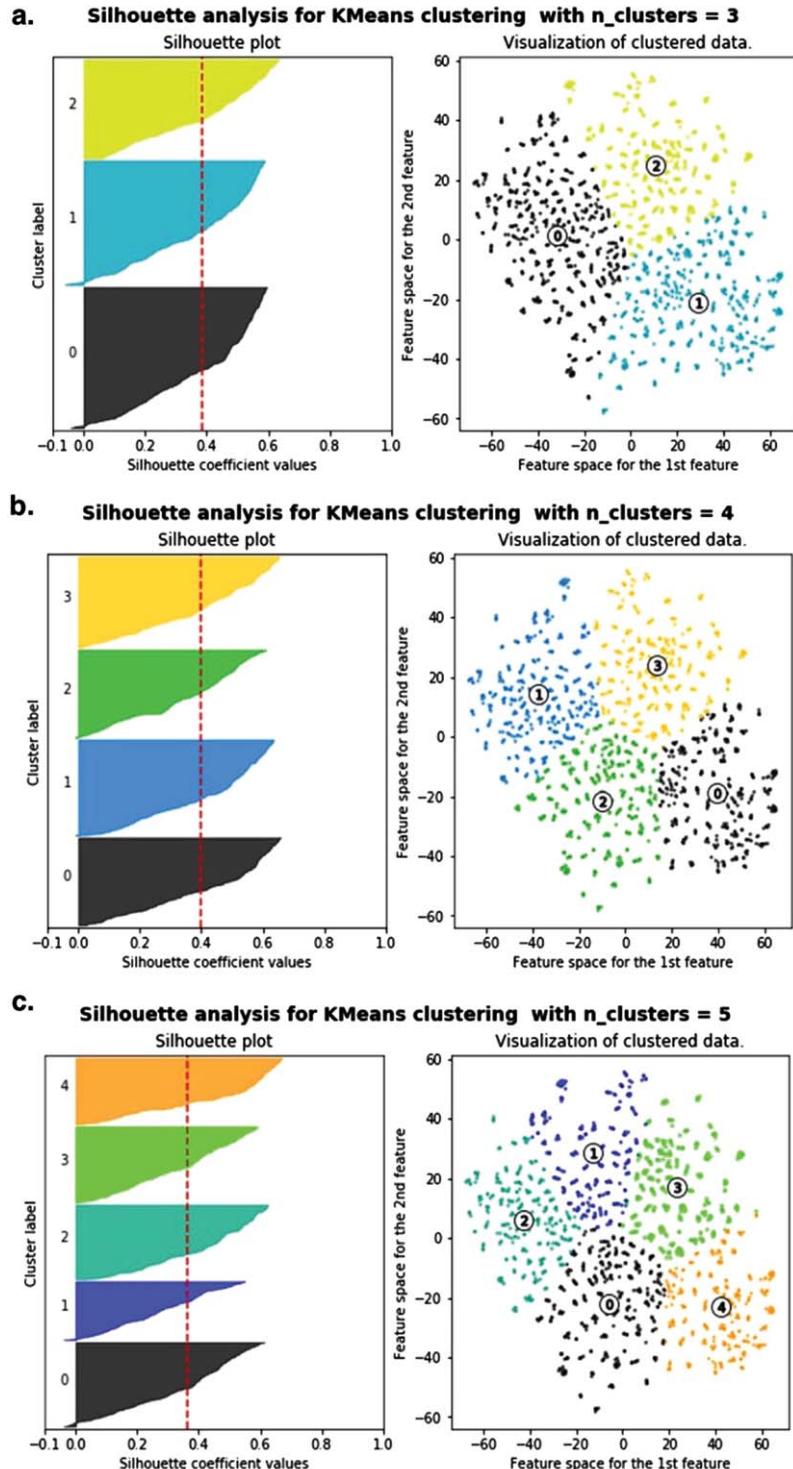


Fig. 4. Unsupervised clustering of transgenic AD mouse data to determine optimal amyloid- $\beta_{40}$  and amyloid- $\beta_{42}$  reduction thresholds using PCA followed by k-means clustering. Silhouette analysis (left) confirmed 4 clusters as the optimal number of clusters. The clustered data is visualized by plotting the second PCA feature by the first PCA feature.

Table 2

The cluster mean and standard deviation of key continuous features. The four clusters were determined by k-means clustering using both continuous and categorical features. Cluster 0 was primarily untreated transgenic control mice; cluster 1 was primarily BACE inhibitor treated transgenic mice characterized as having a “medium” reduction in A $\beta$  relative to control; cluster 2 was primarily wild type mice; and cluster 3 was primarily BACE inhibitor treated transgenic mice characterized as having a “high” reduction in A $\beta$  relative to control

Feature	Cluster 0	Cluster 1	Cluster 2	Cluster 3
Abeta40_percent_of_control	100%	78% $\pm$ 13%	27% $\pm$ 15%	51% $\pm$ 15%
Abeta42_percent_of_control	100%	76% $\pm$ 13%	10% $\pm$ 14%	50% $\pm$ 14%
Overall_Abeta_percent_of_control	100%	75% $\pm$ 13%	19% $\pm$ 14%	50% $\pm$ 14%
Ratio_42_to_40_ratio	1.0	1.09 $\pm$ 0.3	2.3 $\pm$ 1.8	1.2 $\pm$ 0.9
Escape_Latency	77 $\pm$ 14	58 $\pm$ 15	51 $\pm$ 16	56 $\pm$ 14
BACE_level_percent_of_control	100%	82% $\pm$ 17	56% $\pm$ 23	61% $\pm$ 24

close each point within a cluster was to the points in the neighboring clusters. Ultimately, Silhouette analysis and visual inspection confirmed that  $k=4$  was optimal, resulting in four clusters labeled in Fig. 4b as cluster 0, cluster 1, cluster 2, and cluster 3.

Unsupervised learning identified patterns from unlabeled data. That is, clusters were not predetermined based on original study experimental labels like “wild type”, “untreated transgenic control”, or “BACE inhibitor treated transgenic control”. Rather, the clustering algorithm examined data-driven patterns that best explained the separability of data points based on all included explanatory features.

The continuous feature patterns of untreated transgenic control mice and wild type mice were relatively easy to deduce via manual visual inspection (see Table 2). Briefly, all untreated transgenic mice had relatively high A $\beta$  levels and increased MWM escape latency. Conversely, all wild type mice had relatively low A $\beta$  levels and decreased MWM escape latency. Thus, it was not unexpected that the unsupervised clustering algorithm assigned all untreated transgenic mice to one cluster (labeled Cluster 0 in Fig. 4b) and all wild type mice were assigned to another cluster (labeled Cluster 2 in Fig. 4b). What was not obvious prior to unsupervised clustering was how many separable clusters were needed to optimally explain the features of the BACE inhibitor treated transgenic AD mice.

Ultimately, two of the four clusters explained the features of the AD mice treated with substances to lower BACE (labeled as cluster 1 and cluster 3 in Fig. 4b). The assignment of treated transgenic AD mouse data points to either cluster 1 or cluster 3 was predominantly based on differences in A $\beta$  reduction. The mean overall level of A $\beta$  reduction (e.g., Overall\_Abeta\_percent\_of\_control) best explained the variance and separability of the treated transgenic mice data assigned to either clusters 1 or 3. The mean Overall\_Abeta\_percent\_of\_control lev-

els of cluster 1 was 75%, which corresponded to a mean 25% reduction in A $\beta$  level compared to untreated transgenic control mice. The mean Overall\_Abeta\_percent\_of\_control of cluster 3 was 50%, which corresponded to a mean 50% reduction in A $\beta$  level compared to untreated transgenic control mice. The resultant mean A $\beta$  reduction seen in cluster 1 was henceforth named “medium A $\beta$  reduction” and the A $\beta$  reduction seen in cluster 3 was henceforth named “high A $\beta$  reduction”, respectively.

Follow-up *t*-tests with Bonferroni correction were performed to examine the presence of significant differences in overall A $\beta$  between clusters. Indeed, there were significantly different levels of Overall\_Abeta\_percent\_of\_control between all clusters ( $p < 0.05$ ). Collectively, the k-means clustering and statistical results indicate that the medium A $\beta$  reduction threshold determined by the mean of Cluster 1 and the high A $\beta$  reduction threshold determined by mean of Cluster 3 were both separable and significantly different ( $p < 0.05$ ).

The separate A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> levels as a percent of untreated transgenic control were also analyzed for each cluster. The cluster mean Abeta\_40\_percent\_of\_control was 100% for cluster 0; 78% for cluster 1; 27% for cluster 2; and 51% for cluster 3. The cluster mean Abeta42\_percent\_of\_control was 100% for cluster 0; 76% for cluster 1; 10% for cluster 2; and 50% for cluster 3. *T*-tests with Bonferroni correction found significantly different levels of A $\beta$ <sub>40</sub> or A $\beta$ <sub>42</sub> between all clusters ( $p < 0.05$ ).

Notably, the absolute doses and types of BACE inhibiting or reducing substances varied widely among the included studies. As such, there were no pre-defined experimental labels for the quantitative dose of BACE inhibitor administered. However, this did not prevent the assessment of the association between BACE level and A $\beta$  level. The continuous BACE levels as a percentage of matched untreated transgenic control groups were assessed for

all data points assigned to each cluster. The mean and standard deviation of BACE\_percent\_of\_control in each cluster was as follows: cluster 0 was 100% of untreated transgenic control; cluster 1 was  $82\% \pm 17\%$  of untreated transgenic control; cluster 2 was  $61\% \pm 24\%$  of untreated transgenic control; and cluster 3 was  $56\% \pm 23\%$  of untreated transgenic control. The BACE means were significantly different between all clusters ( $p < 0.05$ ). Nonetheless, the corresponding cluster standard deviations indicate that the levels of BACE inhibitor therapy required to get either a medium or high reduction of A $\beta$  vary substantially.

The ratio of A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> has been considered an important biomarker for predicting the diagnosis and long-term outcome in clinical AD [46]. Interestingly, there was not a significant difference in the ratio of A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> (e.g., ratio<sub>42-40\_ratio</sub>) between cluster 1 compared to cluster 3 ( $p > 0.05$ ). The ratio<sub>42-40\_ratio</sub> =  $1.09 \pm 0.3$  in cluster 1, which was primarily treated mice with a medium A $\beta$  reduction. The ratio<sub>42-40\_ratio</sub> =  $1.2 \pm 0.9$  in cluster 3, which was primarily treated mice with a high A $\beta$  reduction. The lack of significance appears to largely be driven by the large standard deviations in cluster 1 and 3. However, there was a significant difference in ratio<sub>42-40\_ratio</sub> between wild type (cluster 2) and the other three clusters ( $p < 0.05$ ).

K-means clustering resulted in the identification of two thresholds of A $\beta$  reduction in treated transgenic mice: a medium reduction threshold corresponding to 25% A $\beta$  reduction (e.g., cluster 1) and a high reduction threshold corresponding to a 50% A $\beta$  reduction (e.g., cluster 3). Next, statistical analysis was performed to determine if there were significant differences between clusters. *T*-tests with Bonferroni correction identified significant differences ( $p < 0.05$ ) in MWM escape latency between medium and high reduction A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub>. Finally, Chi-square analysis was performed to test the independence of A $\beta$  reduction with MWM latency reduction. The significant results ( $p < 0.05$ ) indicated there was indeed a significant association between A $\beta$  being reduced and MWM latency being reduced (Fig. 5). A 25% reduction in A $\beta$  using BACE inhibitors resulted in a significant 28% improvement in MWM escape latency compared to untreated transgenic control mice ( $p < 0.05$ ). Likewise, a 50% reduction in A $\beta$  using BACE inhibitors resulted in a significant 32% improvement in MWM escape latency compared to untreated transgenic control mice ( $p < 0.05$ ).

Table 3 illustrates the categorical feature composition for each of the four clusters. Categorical features were primarily analyzed by what percent of data points with the relevant feature label were present in a given cluster. Categorical features primarily included the mouse genotypes (APP/PS1, wild

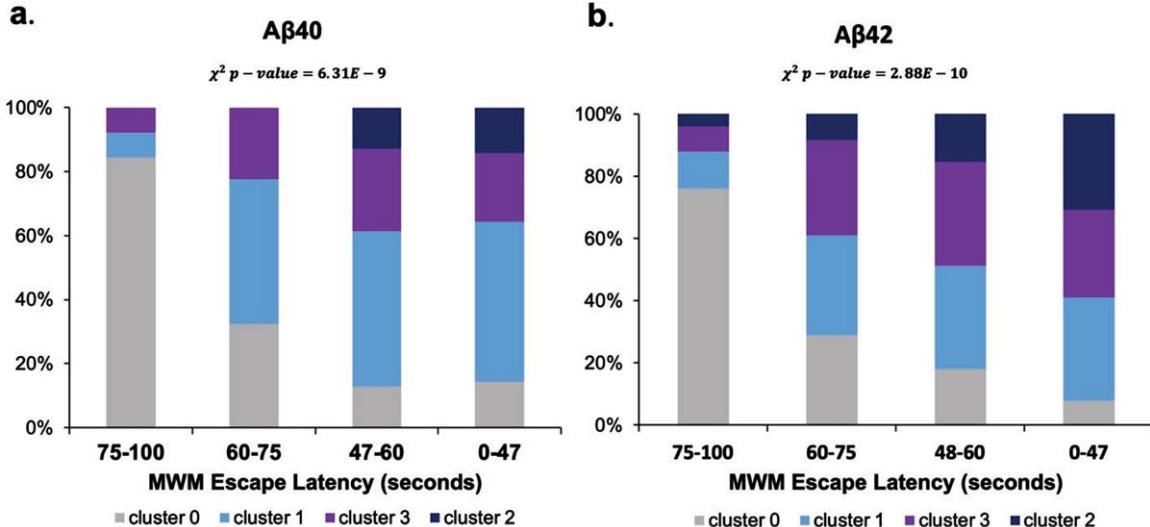


Fig. 5. Assessment of A $\beta$  reduction thresholds and association with MWM escape latency. Normalized MWM escape latencies are shown in seconds. Cluster 0 corresponded to untreated transgenic AD mice. Cluster 1 (light blue) corresponded to treated transgenic AD mice that had a mean 25% overall A $\beta$  reduction compared to control. Cluster 3 (purple) corresponded to treated transgenic AD mice that had a mean 50% A $\beta$  reduction compared to untreated transgenic control. Cluster 2 (navy) corresponded to wild type mice, which had on average 19% of the overall A $\beta$  levels compared to control.

Table 3

Categorical features of data points assigned to each of the four clusters. The four clusters were determined by k-means clustering using both continuous and categorical features. Cluster 0 was primarily untreated transgenic control mice; cluster 1 was BACE inhibitor treated transgenic mice with a medium reduction in A $\beta$  relative to control; cluster 2 was primarily wild type mice; and cluster 3 was primarily BACE inhibitor treated transgenic mice with a high reduction in A $\beta$  relative to control

Feature	Cluster 0	Cluster 1	Cluster 2	Cluster 3
APP/PS1	37.9%	17.2%	0%	44.8%
Wild Type	0.0%	0.0%	100.0%	0.0%
Tg2576	50.0%	27.8%	0.0%	22.2%
3xTg-AD	40.0%	10.0%	33.3%	50.0%
LPS	33.3%	0.0%	0.0%	66.7%
SAMR1	50%	50%	0%	0%
SAMP8	50%	50%	0%	0%
Control	100.0%	0.0%	0.0%	0.0%
Treated	0.0%	25.0%	23.1%	51.9%
Insoluble A $\beta$	33.4%	15.6%	18.8%	31.3%
Soluble A $\beta$	37.0%	18.5%	14.8%	29.6%
Significant A $\beta_{42}$	0%	0%	23%	77%
Significant BACE	0.0%	12.8%	28.2%	59.0%

type, Tg2575, 3xTg-Ad, LPS, SAMR1, SAMP8); the treatment label, including control or treated [with a BACE reducing substance]; whether the A $\beta$  was soluble or insoluble; and whether the data point had a significant level of BACE reduction [compared to the control group in its corresponding original study]. The treated transgenic mice were spread across clusters 1 and 3, which indicated that mouse genotype alone was not driving cluster separability. The only treated mice assigned to cluster 2 were the wild type mice treated with a BACE inhibiting substance. All treated transgenic AD mice were assigned to either cluster 1 or cluster 3.

Finally, supervised machine learning was performed to assess how well explanatory features predict MWM escape latency. As traditional in machine learning, a set of models was constructed to determine which model performed best for the application. Models used the continuous and categorical features of the dataset to predict MWM escape latency (Fig. 5). The polynomial ridge regression model performed the best and had a training R<sup>2</sup> of 0.95 and a test set R<sup>2</sup> of 0.80. Given the relatively small sample size, it was expected that there would be a decrease in R<sup>2</sup> in the independent test set compared to the training set. The learning curves (i.e., model accuracy versus training sample size) for the supervised model(s) did not illustrate overt overfitting or underfitting. Across all ML models, the two most important features for predicting MWM escape latency consistently comprised Abeta42\_percent\_of\_control

Table 4

Supervised machine learning regression models assessed how well the explanatory continuous and categorical features predicted MWM escape latency. The most important explanatory feature was A $\beta$  level

Supervised Learning Models	Training R2	Testing R2
Linear regression	0.81	0.65
Ridge Regression	0.79	0.74
Polynomial ridge regression	0.95	0.80
Decision Tree	0.96	0.70
Tuned Decision Tree	0.92	0.76

and Abeta40\_percent\_of\_control. Moderately important features included the BACE\_significance and ratio\_42\_to\_40\_ratio. Categorical mouse type was not a feature of consensus high importance across models.

## DISCUSSION

Prior studies that examined the impact of amyloid-reducing BACE inhibitors or BACE reducing substances in transgenic AD mice had a wide distribution of results. The current study provided new quantitative insight into the average amount of A $\beta$  reduction via reduction of BACE necessary to obtain a meaningful improvement in transgenic AD mouse cognition. Unsupervised learning identified 4 clusters that best explained MWM escape latency: cluster 0, A $\beta$  levels in untreated transgenic AD control mice (100% Abeta\_percent\_of\_control); cluster 1, medium A $\beta$  reduction in treated transgenic AD mice (25% Overall\_Abeta\_percent\_of\_control); cluster 3, high A $\beta$  reduction in treated transgenic AD mice treated; cluster 2, A $\beta$  levels in wild type mice treated with BACE inhibitors (19% Overall\_Abeta\_percent\_of\_control). Interestingly, similar clustering algorithms had also previously identified 4 clusters in clinical AD patients [23]. K-means clustering showed a 25% mean decrease in A $\beta$  led to a 28% mean decrease in MWM escape latency in transgenic AD mice treated with BACE inhibitors. Further, a 50% decrease in A $\beta$  led to a 32% decrease in MWM escape latency. For reference, wild type mice had a mean escape latency 41% lower than AD mice. There was a significant difference in mean MWM escape latency between the medium and high A $\beta$  reduction groups. Statistical analysis revealed there was a significant association between A $\beta$  being reduced and MWM latency being reduced. This finding was in line with previous research, which has shown that BACE proteases are central to the formation of A $\beta$  and, hence, the development of AD [8, 47, 48].

Multiple pre-clinical and clinical trials have measured the effectiveness of BACE inhibitors and other amyloid-reducing therapies for decreasing the rate of cognitive decline due to AD [49]. Mouse BACE inhibitor trials have generally shown good safety profiles with few adverse effects. However, human BACE inhibitor trials have raised concerns about potential side effects, particularly liver toxicity [49]. During previous clinical trials, BACE1 inhibitors were able to achieve up to 80–90% A $\beta$  reductions in cerebrospinal fluid; yet, these therapies failed to significantly demonstrate slowing of the cognitive decline [50]. More than seventeen different BACE1 inhibitors have failed in double-blind, placebo-controlled clinical trials in patients with AD [51]. The reasoning behind these results is unclear. One hypothesis is that the functional AD pathology is too advanced by the time BACE inhibitor therapy has begun.

One promising finding shown in this study is that a supplemental combination of the nutritional molecules, Vitamin D and resveratrol [27], fell into cluster 1, which was the medium A $\beta$  reduction cluster. Additionally, the cognitive stimulation treated Tg2576 mice fell into cluster 3, which was the high A $\beta$  reduction cluster. Thus, non-traditional means of reducing BACE and A $\beta$  via diet supplementation and behavior modification could be potential considerations for asymptomatic patients who have been identified as “at risk” for AD based on family history or their own biomarker profiles.

One interesting finding from the present study was that the ratio of A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> was not found to be as important of a feature as the absolute reduction of A $\beta$ <sub>42</sub> compared to control (e.g., Abeta42\_percent\_of\_control) in predicting treatment-related improvements in MWM escape latency. Researchers have contended that the ratio of A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> is superior to using the A $\beta$ <sub>42</sub> level when making a clinical diagnosis of AD [52]. In particular, the ratio of A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> was found to be more specific for measuring cognitive decline due to AD compare to other neurological disorders. Particularly, pooled evidence found that mild cognitive impairment patients with lower ratios of A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> were more likely to convert to AD [46]. Notably, our prior work examining the pooled Tg2576 transgenic AD mouse data found that there was only a weak qualitative trend with MWM escape latency [18]. It is possible that the present study’s findings were influenced by the mixed population of transgenic mice. Prior reports have concluded that some transgenic mice have pathology primarily

driven by a change in A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> ratio while others do not [7]. In short, the functional impact of the A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> ratio in mice compared to humans remains inconclusive.

Future longitudinal assessment will be necessary to determine the long-term benefits and safety profile of BACE inhibitor, amyloid-reducing therapies, or other non-pharmacologic therapies to reduce A $\beta$ . Ideally, patients at high risk of acquiring dementia should be identified as early as possible. Early identification can be made possible with cerebrospinal fluid, genetic, and/or blood-based biomarkers combined with machine learning-based clinical recommendation systems, which use personalized data to calculate a specific dementia risk, dementia onset date, and confidence interval on the prediction(s) [53, 54]. With such upfront quantitative forecasting available in the future, a physician could best inform at-risk asymptomatic patients of the pros and cons of prophylactic therapies aimed at preventing symptomatic AD. Note that, at the time of this writing, lecanemab is only FDA-approved in the US for symptomatic dementia patients rather than at-risk asymptomatic patients.

#### *Limitations and future directions*

The present study had a few limitations that were primarily tied to the inherent limitations of the underlying dataset. The learning curves (i.e., model accuracy versus training sample size) for the supervised model(s) did not illustrate overt overfitting or underfitting. Nonetheless, additional sample sizes would help to further confirm results. Additionally, the sex and age of the mice was not included due to too few studies including the variable. Our prior work examining the aggregate effect of sex as a feature in experimental AD mouse found that it was mostly insignificant except in APOE transgenic models [20]. However, APOE transgenic mice were not included in the present work. Due to its frequent usage in the literature, the MWM escape latency was used as the primary assessment of mouse cognitive function in this pooled analysis. The addition of other cognitive tests, such as the novel object recognition, Y-maze, Barnes maze, or other tests was considered but not possible due to sample size limitations with respect to the other required study inclusion criteria. Another possible limitation is the fact that not all included studies sampled A $\beta$  from the same brain region, and some simply did not state from which brain the A $\beta$  was sampled. Nonetheless, the statistical and machine learning models found no overt or study-

specific biases that would indicate brain region or another unincluded feature was substantively impacting the aggregate results. Notably, PCA determined that >90% of the variance was explained by the continuous and categorical features highlighted in the study.

Key future directions include the addition greater sample size, more mouse model types and more detailed features like mouse sex, age, brain region, and inclusion of additional cognitive tests beyond MWM escape latency. However, the most important work moving forward is the reproducible conversion of amyloid reduction thresholds that are applicable to mouse transgenic AD models to amyloid reduction thresholds necessary for safe and effective anti-amyloid human therapies.

### Conclusions

The key research question posed by the present work was: What level of A $\beta$  reduction is necessary to meaningfully improve cognitive function? Experimental data curated from 22 original studies was used to address this question using a combination of machine learning and statistical analysis. A “medium” or 25% reduction of A $\beta$  was necessary to achieve a 28% improvement in transgenic AD mouse cognition as measured via MWM escape latency, and a “high” or 50% reduction resulted in a 32% improvement. Comparatively, wild type mice had a mean 41% MWM latency improvement over untreated transgenic control. These results suggest that a 25% threshold in overall A $\beta$  reduction was necessary for reproducible, meaningful, and significant improvements in transgenic mouse cognition.

### AUTHOR CONTRIBUTIONS

Anita Singh (Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing – original draft); Matthew Maker (Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing – original draft); Jayant Prakash (Conceptualization; Formal analysis; Methodology; Software; Visualization; Writing – original draft); Raghav Tandon (Formal analysis; Methodology; Software; Validation; Visualization; Writing – review & editing); Cassie S Mitchell, Ph.D. (Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation;

Visualization; Writing – original draft; Writing – review & editing).

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### CONFLICT OF INTEREST

The authors have no conflict of interest to report.

### DATA AVAILABILITY

This study utilized data curated from previously published data sources, which are available in PubMed. Citations to access the original data sources is provided in Table 1.

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