

Exploring Relationships in Alzheimer's APOE Transgenic Mice

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Introduction

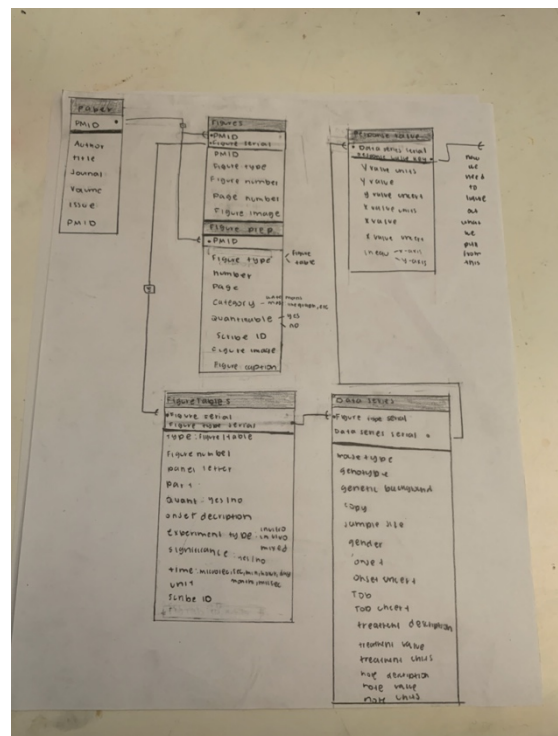
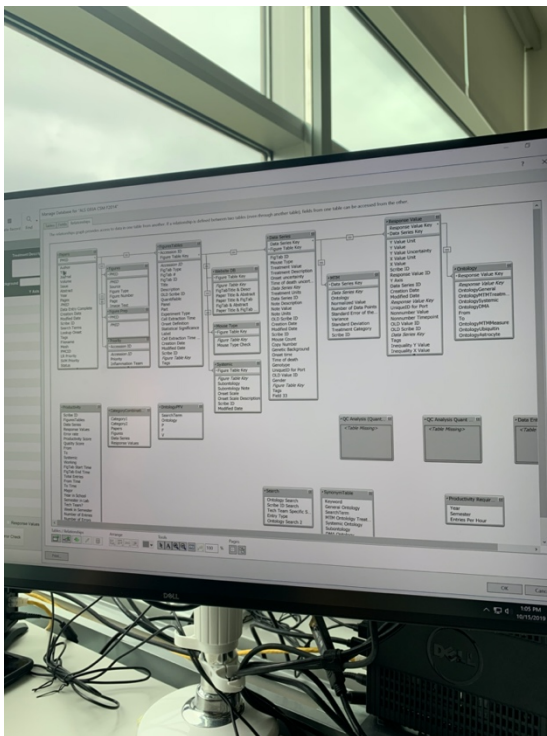
Associated with impairment of memory, comprehension, attention, reasoning, and judgement, Alzheimer's disease is the largest cause of decline in cognition in those over the age of sixty-five. Two-thirds of those suffering from dementia are from cases of Alzheimer's. It is characterized by accumulation of neuritic plaques and neurofibrillary tangles in abnormal amounts. This neurodegenerative disorder has no known cure despite risk factors being investigated; treatment has been found but only improves symptoms.

ApoE2, apoE3, and apoE4 have been connected to the accumulation of amyloid plaque; it was found that apoE4 is correlated with a greater risk for AD neurotic plaque formation. This risk is not definitive due to lack of knowledge if it is a gain of a toxic function in the system or if it is due to loss of protective function. Sex as well have been investigated as females are at a greater risk of developing AD than males. This has been shown as apoE4 increases the risk of AD onset predominantly in female mouse models. Another factor includes age. ApoE4 has an onset recorded at fifty-six years of age while non-carriers had it at seventy-six years. Age may show the biggest risk factor for AD; however, there is not much information on how age and gender and the various treatments all tie in together.

Risk factors have been investigated but no cure has been made despite knowledge of these variables. The relationship between the different factors is complicated and have not been ranked. This study will discover clarity for predictors of cognition in AD APOE mice. Understanding the relationship of risk factors can aid in finding possible targets for drug discovery. If risk factors are not clear, broad treatment will be difficult to produce and be effective.

Specific Contributions

To start off, I did an extensive literature review to loosely quantify the distribution of transgenic mouse types for Alzheimer's. All peer-reviewed published work came from PubMed. Research was done using keywords searched in PubMed such as "Alzheimer's", "mouse types", etc. The goal was to breakdown how many specific types of AD mice could be investigated in curation to devise a curation program. An excel sheet was made that included many different transgenic mouse types: PDAPP, Tg2576, APP23, TgCRND8, htau, 5XFAD, etc. It included the model, the number of articles found for each type, the pros, cons, and the symptoms. Focusing on ApoE's data, the next step I was involved in was creating the database.



Using Filemaker Pro, a database had to be organized to curate data needed for the meta-analysis. The right picture showed a rough draft I created, and the left showed the finalized version on Filemaker Pro. Once the database was finalized, search criteria were set to pull peer-

reviewed papers from PubMed. Pulling over one-hundred and ninety-one papers, I went over a large part of those to pick those that met the requirements for the study. After the reviewed papers were found, it was divided among the team to curate them. I was involved in training other teammates on curation protocol. I also had to curate my set of peer-reviewed papers. I then went back and checked other technical team members work to provide quality control. An example of one of the papers I curated is shown below:

VW - G31A Training Database (MITCH-WIN@neuro.gatech.edu)

File Edit View Insert Format Records Scripts Window Help

Records 6 / 83 Found (Sorted)

Layout: 3. Data Series Entry View As: Preview

FigTab ID: 18973555-F2B Panel: B

Quantifiable: Yes Paper Title: GABA(A) receptors present higher affinity and modified subunit composition in spinal motor

Significance: No Exp. Type: InVtro Cell Extract Time: Onset Definition: No onset defined in paper

Mouse Type	Genotype	Genetic Background	Copy	Sample Size	Gender	Onset	Onset Uncert	TOD	TOD Uncert	Treatment Description	Trtmnt Value	Trtmnt Units	Note Description	Note Value	Note Units	Scribe ID
Nontrans				9				15		GABA water	30	Mic				V
WT +		B6.JL		12				15		GABA water	30	Mic				V
G93A +		B6.JL		28				15		GABA water	30	Mic				V

After the data was collected, I helped run statistical tests to normalize the data. All the data was pulled from the database and transferred to an Excel sheet. Working with other members in the lab, I used a program to do so. The data set then was used in random forest modeling. I did not have any experience with machine learning but was able to get an understanding of how it worked with the guidance of Dr. Mitchell and other students in the lab.

PostUncovering Aggregate Trends in Alzheimer's Disease Through Meta-Analysis and Feature Selection of Pre-clinical APOE Literature



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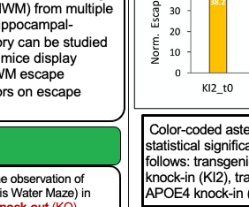
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Introduction

Associated with impairment of memory, comprehension, attention, reasoning, and judgement, Alzheimer's disease is the largest cause of decline in cognition in those over the age of sixty-five. Due to the complex relationship present between age, APOE genotype, gender, treatment, and cognition, a broadly effective treatment for Alzheimer's Disease has not been developed. **The objective of this present study was to utilize statistical and machine learning methodologies to investigate these factors on the cognition of transgenic APOE knockout (KO), APOE knock-in (KI), and wild type mice.** Cognition was measured by escape latency from the Morris Water Maze (MWM) from multiple peer-reviewed studies. As a way to measure hippocampal-dependent learning, spatial learning and memory can be studied in transgenic model mice through the task. AD mice display spatial memory dysfunction shown through MWM escape latencies; however, the importance of risk factors on escape latency studied from the MWM is not known.

Data Analysis of Treatment and Mouse Type

Figure 1: Normalized Mean Morris Water Maze Escape Latency between untreated wild type and transgenic APOE genotypes



Color-coded asterisk (*) indicates Bonferroni-corrected pairwise statistical significance between groups (p<0.002). Groups are as follows: transgenic APOE knockout (KO), transgenic APOE2 knock-in (KI2), transgenic APOE3 knock-in (KI3), transgenic APOE4 knock-in (KI4), wild type (WT), all mice in the study (all).

Random Forest Modeling

Model Predictions

Feature	Importance
APOE3 knock-in (KI3)	0.270
Age	0.094
Male Gender	0.092
APOE4 knock-in (KI4)	0.088
Treated	0.077
Positive treatment	0.074
APOE knock-out (KO)	0.069
Wild Type	0.061
Mixed or Unknown Gender	0.058
Negative Treatment	0.054
Female Gender	0.038
APOE2 knock-in (KI2)	0.023

Methodology

→ Assessing AD APOE impact on cognition through the observation of escape latency (assessment performed through Morris Water Maze) in three mouse types and three subgenotypes: **APOE knock-out (KO), APOE2 knock-in (KI2), APOE3 knock-in (KI3), APOE4 knock-in (KI4), wild type (WT)** mice and produce a Random Forest model to predict cognitive.

Path towards developing Meta-data Analysis

AD APOE Papers searched and identified through Pubmed

→

Data curation of WT, APOE KI (2,3,4) and APOE KO escape latency

→

T-test constructed to examine escape latency between mouse types

→

Random forest modeling utilized to predict weighted importance of various mouse features on cognition

Data Curation

Search Criteria

Three search terms were used:

- "Alzheimer's Disease"
- "APOE"
- "Morris Water Maze"

Inclusion Criteria

Each paper contains:

- APOE knockin (KI), wild type (WT) genotype
- Morris Water Maze escape latency at baseline along with data from 4 or 5 days of training

191 reviewed papers met all the requirements

31 reviewed papers met all the requirements

Data Set for Random Forest Modeling

Data Sets

Training Set	Testing Set
80% of the dataset	20% of the dataset
In training, we give the model both the features and the labels so it can learn to classify points based on the features	In testing, we only give the model the features and have it make predictions about the labels
89% Model Accuracy	82% Model Accuracy

Published Work

This work was very recently accepted for publication into the Journal of Alzheimer's Disease. Please view the full open access article for more details. Watson, Yassin et al. 'Aggregate Trends of Apolipoprotein E on Cognition in Transgenic Alzheimer's Disease Mice'. 1 Jan. 2021 : 435 - 450.

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This work was very recently accepted for publication into the Journal of Alzheimer's Disease. It has an open access copyright and is reformatted from the originally published work to meet thesis guidelines: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8461675/>

Watson, Yassin et al. 'Aggregate Trends of Apolipoprotein E on Cognition in Transgenic Alzheimer's Disease Mice'. 1 Jan. 2021 : 435 – 450.

INTRODUCTION

Alzheimer's disease and the Apolipoprotein E gene

Alzheimer's disease (AD) is a neurodegenerative disorder that gradually impairs cognition in patients suffering from the condition [1] and is considered to be a polygenic disease [2]. The Apolipoprotein E (*APOE*) gene synthesizes various isoforms of apoE, a biomarker strongly hypothesized to be implicated in AD, as initially observed by researchers at the Duke Alzheimer's Disease Research Center in 1993 [3–5]. The biochemical exhibits three common isoforms in humans: apoE2, apoE3, and apoE4, of which apoE2 [6] and apoE3 [7] have been shown to confer protective effects, while apoE4 has been documented to increase cognitive impairment [7–10]. The protein apoE primarily functions in brain lipid transport, glucose metabolism, and neuronal signaling, among other roles [11]. Its biochemical pathway involves binding to surface apoE receptors on cells throughout the body to initiate further metabolism, transport, and signaling, but the single amino acid differences between apoE2, E3, and E4 result in a diversity of protein structures that, therefore, affect receptor binding in different ways respective to each isoform [12]. For instance, apoE3 and E4 display 50-fold greater binding affinity when compared to apoE2 [12].

Moreover, through yet-uncovered mechanisms, *APOE* isoforms are also believed to accentuate or attenuate pathways involving other well-established AD biomarkers, such as amyloid- β ($A\beta$) [13] and p-tau [14]. The amyloid cascade hypothesis posits that the deposition of $A\beta$ plaques from improper cleavage of amyloid- β protein precursor is the most important molecule in AD pathogenesis, but heterogeneity of results in the literature has challenged this notion [15, 16]. For example, Foley et al. showed that mice with increased $A\beta$ levels do not perform significantly worse on cognitive assessments than mice with lower levels of $A\beta$, suggesting that the plaques could instead be a side effect of AD pathogenesis [13]. The interaction between *APOE* isoforms and amyloid metabolism was examined to show that *APOE4* was associated with elevated $A\beta$ secretion and defective $A\beta$ uptake compared to isogenic *APOE3* human brain cells [8]. This interplay of *APOE* isoforms with the amyloid cascade further strengthens the literature trend that *APOE4* confers negative effects on cognition since conversion of apoE4 to E3 repeatedly mitigated various pathologies related to AD [8]. Lastly, meta-analysis work revealed that p-tauopathy is a greater predictor of cognitive decline than $A\beta$ in mouse models [14].

AD APOE mouse model

Endogenous mouse apoE is structurally different from human isoforms, so the most prominent *APOE* mouse models use human isoforms for study [13, 17]. The selection of appropriate promoters and other regulatory elements is important to note since *APOE4* expressed in neurons have demonstrated more detrimental effects compared to its expression in astrocytes [18]. Furthermore, mouse *APOE4* knock-in (KI) groups display neural plasticity defects that appear to be absent in *APOE3* KI groups [19–21].

Genotype effect on AD

Extensive research has been conducted on transgenic mouse models to investigate the effects of various features, such as genotype, treatment, age, and gender on AD pathogenesis. Because of the high-dimensional nature of AD diagnosis, there is heterogeneity within the literature about the importance of such features [13]. For example, Shi et al. demonstrated that *APOE4* KI mice exert a gain of toxic function while *APOE* knock-out (KO) mice instead displayed protective effects [22]. However, others have found conflicting results among the same genotypic groups. Zerbi et al. found that both *APOE4* KI and *APOE* KO mice demonstrate a comparable magnitude of brain functional connectivity deficiencies in progressing from adulthood (12 months) to old age (18 months) [23]. Thus, uncovering aggregate trends is pivotal to AD *APOE* research. But genotype is not the only feature whose effect on AD is still under debate.

Treatment effect on AD

The effects of various forms of treatment have shown similarly unclear findings. While prior studies have tested treatment regimens that may be promising avenues for remediating AD progression in transgenic mouse models [24], the overall efficacy of treatment is still undetermined. For instance, pharmacological targeting of the apoE/A β interaction pathway significantly reduced aggregation of both apoE and A β in *APOE2* and *APOE4* KI mice, preventing memory decline in both groups [24]. However, Cramer et al. demonstrated that oral administration of bexarotene, a retinoid X receptor agonist, reversed various cognitive deficiencies observed in WT mice but without significant effect on *APOE* KO groups [25]. The complicated interplay between *APOE* and other AD biomarkers makes it difficult to discern whether *APOE* modulatory therapy could successfully lessen cognitive decline in AD.

Furthermore, treatments that have been investigated in transgenic AD mice have not translated well to human trials. Such difficulty in translating *APOE* therapies from mice to humans is likely due to inherent differences in *APOE* between species. Humans express three major *APOE* isoforms, while only one is naturally observed in wild type mice [11].

Gender and age effect on AD

Female gender and old age are frequently cited in human studies as probable AD risk factors. However, it is difficult to assess whether gender has a significant effect on cognition due to confounding variables such as life expectancy, innate differences in performance on cognitive tasks, and difference in hormone levels [26]. Although many studies have shown that AD may occur more often and more severely in women [27–29], the underlying mechanism by which women are more predisposed than men remains undiscovered [26, 30–33]. Meanwhile, incidence of AD increasing with age is perhaps the most substantiated pattern documented in the literature [34–37]. However, even this trend is still under scrutiny due to a lack of understanding of how the effects of natural aging can be compounded by other non-age-related features to result in increased AD risk for certain populations [37].

Scope of present study

A complex and difficult to interpret relationship exists between age, gender, treatment, and genotype on cognition in AD, making it challenging to develop broadly effective treatments through diet, vitamin supplementation, exercise, or other specific apoE modulatory drugs. Though age has most clearly and consistently been shown to be the biggest risk factor for developing AD [37], the relationships between both age and cognition [34–37] as well as gender

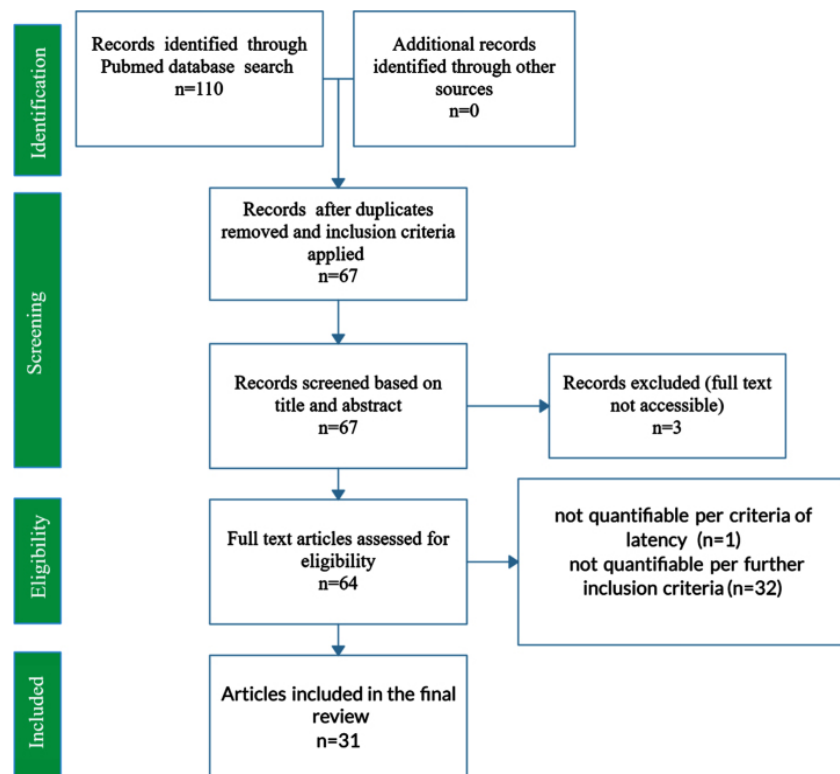
and cognition [26, 30–33] are less well known. The effects of various *APOE* treatments are also similarly disputed [24, 25, 38]. Likewise, categorizing genotypes by the effect they have on cognition has yielded inconsistent results [19–23]. Therefore, there is a need for a deeper understanding of the relationship between various features that contribute to AD pathogenesis across a large, aggregate set of data from multiple published *APOE* transgenic mouse experimental studies. The objective of the present study was to ascertain aggregate trends in AD *APOE* mouse literature by performing statistical analysis machine learning feature importance modeling to ascertain the extent that cognition in AD *APOE* mice is affected by *APOE* genotype, *APOE* modulatory treatment, mouse gender, and mouse age.

METHODS

The goal of this work was to perform an aggregate analysis of cognition in *APOE* transgenic AD mice to determine the importance specific features, namely mouse genotype, type of *APOE* modulatory treatment, mouse age, and mouse gender, in predicting cognition as measured via Morris water maze (MWM) escape latency. PubMed database (<http://www.pubmed.gov>) searches identified peer-reviewed publications that were manually reviewed to determine if their data met criteria for inclusion into the present study. All quantifiable experimental data from tables and figures were transcribed into a relational database with resultant transcription accuracy in excess of 98.8% under consistent oversight by meticulous quality control using a published protocol [39].

Inclusion criteria

PubMed was queried using the key search terms “Alzheimer’s Disease”, “APOE”, and “Morris Water Maze” in addition to various synonymous combinations and abbreviations. To be included in the present study, each publication must have included MWM escape latency results at baseline and after 4 or 5 days of training; inclusion of a control and treatment group; wild type genotype, *APOE* KI genotype (namely *APOE2*, *APOE3*, or *APOE4* knock-in), or *APOE* KO genotype; demarcation of mouse gender; demarcation of mouse age at time of MWM testing. A total of 31 peer-reviewed papers met all requirements for inclusion. [Figure 1](#) displays a PRISMA Flow Diagram for the present study.



[Fig. 1](#)

PRISMA Flow Diagram for the systematic review of PubMed articles related to AD *APOE*. The PRISMA flow diagram represents the systematic review of PubMed articles and the compilation and curation of journal article data into a manually constructed relational database. The final set

of included studies comprised 31 journal articles and 3,045 mice. Features assessed include mouse genotype (wild type, *APOE2* knock-in, *APOE3* knock-in, *APOE4* knock-in, *APOE* knock-out); type of external *APOE* modulatory treatment and its intended impact on the underlying etiology and corresponding cognition (positive treatment, negative treatment, untreated); mouse age (in days); and mouse gender (male, female, or mixed/unknown).

Morris water maze

The MWM is used to test spatial memory as a measure of cognitive functioning and was first developed by Richard Morris in the early 1980s [40]. MWM is a standard metric of cognitive performance in mouse models, and due to its widespread use in pre-clinical AD studies [40, 41], MWM escape latency was chosen as the outcome metric for mouse cognition in the present study. In the beginning of the MWM, a mouse is placed into a pool of cold water (13°C) that contains a visibly discernible platform [42, 43]. On the first day of training, the mouse learns to find the visible platform and, thus, escape the cold-water bath. Subsequently, the mouse is reintroduced to the maze with the platform hidden under the water, instead. As a result, the mouse must rely on visible cues outside of the maze to locate the platform. Training with the hidden platform usually takes place approximately four times daily over the course of four to seven days. The amount of time it takes for the mouse to find the hidden platform is measured as escape latency [41]. To prevent skewed results from over-training in the MWM, only the first 5 days of training trials were included for analysis. Overtraining due to either too many days of training or too many trials per day can cause all of the mice in the experiment, whether wild type or transgenic, to display similar escape latency values that inhibit quantitative assessment of learning differences [44]. Trials on the first day of training are used as a control group between

mice to account for individual swimming performance [42, 43]. [Supplementary Table 1](#) includes the specific description of the MWM used in each data source included in the present study.

Data sources

The C57BL/6 mouse model is the most widely utilized genetic background of altered mice for study of human pathology due to its ability to maximally express most mutations [45]. In addition to being relatively easy to breed, the mouse model is highly sensitive to cold temperatures, making it particularly useful for examining cognition through the MWM [46]. The C57BL/6 mouse model subline was originally isolated at the Bussey Institute and most extensively distributed by the Jackson Laboratory; however, as a result of its ubiquity in research, many groups have bred colonies in isolation from one another for many generations, potentially exacerbating the effects of genetic drift, the random change in allele frequency in a population over subsequent generations [47]. The studies included in the database range from years 1997 to 2020, with the majority from 2013 to 2020. [Table 1](#) details collected data segregated into the subpopulations of interest used in the present aggregate analysis. [Supplementary Table 1](#) displays all included studies, their respective publication years, available source information for mouse backcrossing, and detailed treatment descriptions.

Table 1

Mean normalized escape latency and standard deviation is the age-normalized Morris water maze escape latency in seconds. Mice n is the sample size of the group, whereas study n is the number of studies utilized for the group. Bracketed numbers in the sources column correspond to

full data source references. There were 1,430 female mice, 1,181 male mice, and 258 mixed/unknown gender mice

Group	Mean Norm. Latency (s)	Std. Dev. Norm. Latency (s)	Mice (<i>n</i>)	Data Sources (<i>n</i>)	Data Source References
KI3_t−	37.6	–	8	1	[48]
KI4_t−	30.9	11.0	66	3	[48–50]
KO_t−	47.7	6.2	62	4	[49, 51–53]
WT_t−	32.8	9.5	203	5	[49–53]
all_t−	35.3	11.0	339	6	[48–53]
KI2_t0	38.2	5.4	354	3	[34, 54, 55]
KI3_t0	30.6	9.0	677	16	[34, 48, 54–67]
KI4_t0	48.6	15.3	738	19	[34, 49, 54–57, 59–71]
KO_t0	40.9	15.3	337	10	[34, 49, 53, 58, 63, 72–76]
WT_t0	35.8	17.2	326	11	[49, 50, 53, 63, 64, 68–70, 72, 74, 75]
all_t0	39.3	14.9	2432	28	[34, 48–50, 53–76]
KI3_t+	29.2	5.2	61	5	[56, 61, 64–66]
KI4_t+	33.5	8.9	115	9	[56, 61, 64–66, 68, 70, 71, 77]

Group	Mean Norm.	Std. Dev. Norm.	Mice	Data	Data Source References
	Latency (s)	Latency (s)	(<i>n</i>)	Sources (<i>n</i>)	
KO_t+	28.3	6.0	72	3	[58, 74, 75]
WT_t+	25.8	1.6	26	2	[68, 74]
all_t+	30.4	7.9	274	12	[56, 58, 61, 64–66, 68, 70, 71, 74, 75, 77]

Assessed features

Twelve possible categorical or continuous features of each study were curated in the present work as follows: transgenic *APOE* knockout (KO), transgenic *APOE2* knock-in (KI2), transgenic *APOE3* knock-in (KI3), transgenic *APOE4* knock-in (KI4), wild type (WT), treatments meant to improve cognition or improve the AD etiology (t+), treatments meant to decrease cognition or worsen the AD etiology (t–), untreated mice (t0), female gender (g_f), male gender (g_m), mixed or unstated gender (g_mi), mouse age in days (age), and MWM escape latency in seconds (latency). Notably, mouse age was normalized and resampled such that treated and untreated groups had the same mean age within 0.05%. Unless stated otherwise, reported MWM escape latency is the normalized MWM escape latency in seconds.

Varied treatment methods were employed to directly or indirectly change the underlying neurodegenerative etiology and corresponding mouse cognition. To appropriately aggregate treatment effects, treatments were categorized based on their intended effect on cognition (positive or negative) using descriptions of the Methods in the original published works.

Examples of positive treatments include bexarotene administration [56], medical ganglionic eminence transplantation [64], and acetyl-L-carnitine and dexlipotam dietary supplementation [70]. Examples of negative treatments include repetitive traumatic brain injury [50], intraperitoneal injection of EtOH and AcH [53], and a high-fat diet [49]. Detailed treatment descriptions and treatment category labels for each included data source are listed in [Supplementary Table 1](#).

Statistical analysis

Age-normalized mean MWM escape latency and standard deviation was used to conduct all statistical analysis in the present study as noted under *Assessed Features* section above.

The reported aggregate mean and standard deviation for each group in [Table 1](#) was calculated using the frequency distribution, which weights the average and standard deviation for each group based on the sample size associated with each observation within the group. As such, the contributions of individual original data sources to the reported aggregate mean and standard deviation are a function of the original source's reported mouse sample size for each observed group. Equal weighting of all studies, which would disregard mouse sample size for each reported study when calculating the aggregate group mean and standard deviation, was not possible due to lack of statistical power.

Data distributions were assessed for normality using Shapiro-Wilk test and found to exhibit a sufficiently Gaussian distribution for assessment via ANOVA and *t*-tests. An ANOVA was performed to assess which groups should be further examined using *post-hoc* testing to identify

pairwise significant differences. To explore pairwise differences in cognitive performance as measured by mean normalized MWM escape latency, two-tailed *t*-tests were performed at an overall alpha of 0.05. Bonferroni correction was utilized to correct the *p*-value threshold for significance for multiple comparisons, resulting in a *p*-value threshold for significance of $p < 0.002$. All statistical analysis was performed in Microsoft Excel.

Random forest modeling

The MWM escape latency data in the present study was used to produce a supervised random forest machine learning model in Python version 3.8.3 (with Pandas version 1.0.4, NumPy version 1.18.0, and Scikit-learn version 0.23.2 python packages) to predict the importance of twelve binary features: transgenic KO, KI2, KI3, KI4, WT, treatments meant to improve cognition or improve the AD etiology (t+), treatments meant to decrease cognition or worsen the AD etiology (t-), untreated mice (t0), female gender (g_f), male gender (g_m), mixed or unstated gender (g_mi), and mouse age (age). The model was set to classify either superior or inferior normalized MWM escape latency performance using a threshold obtained via data standardization. Curated data was standardized and converted into binomial features (feature present=1, feature not present=0). The continuous features of normalized escape latency and age were also standardized using a z-score. For example, normalized escape latency is standardized using the following procedure: [(normalized escape latency for each mouse – mean normalized escape latency for all mice)/ standard deviation of normalized mean escape latency for all mice]. Standardized age was calculated using the same z-score method. A standardized normalized escape latency less than or equal to the mean was binomially classified as “superior” performance, whereas inferior performance was binomially classified as “inferior” performance.

Likewise, standardized mouse age less than the mean was binomially classified as “young”, whereas standardized mouse age greater than the mean was classified as “old”.

Random forest models have been widely used in AD literature to assess the high dimensional nature of diagnosis [78]. A random forest is composed of a set of decision trees that each consist of split nodes and leaf nodes. The decision tree is fed a predictor, or target variable, as well as corresponding features used to predict the target variable [79]. MWM escape latency was used as the labelled predictor in this study. Each sample passed to the model is assessed by the split node before being passed to its left or right child, depending on the sample’s features. The decision trees are fed random samples (with replacement) from a subset of the training data or testing data. Beginning with the top split node, or root node, each subsequent node in the tree is trained to continuously split until it achieves stop criteria such as maximal tree length or minimal number of samples. The mean and variance of the target group in the data subset of each decision tree is stored in leaf nodes for future forecasting [79].

While in the training stage, the random forest model can see the labels on the target variable so that it can recognize when a mistake is made by incorrectly predicting the target variable based on the corresponding features for a particular observation. The process of training is how the model learns. During the independent testing stage, the model is exposed to data that was not seen during the training phase, so it does not know the true value of the target variable [79].

RESULTS

This systematic review and meta-analysis with adjunctive machine learning compares cognitive function as empirically measured through escape latency from the MWM test in various WT, *APOE* KI, and *APOE* KO mice. A total of 3,045 mice from 31 peer-reviewed scientific papers [34, 48–77] were included in the analysis. The included peer-reviewed studies consisted of MWM escape latency results at baseline and after 4 or 5 days of training; inclusion of a control and treatment group; WT or *APOE* transgenic mouse type; mouse age in days; and mouse gender (female, male, mixed/unknown). There were 1,430 female mice, 1,181 male mice, and 258 mixed/unknown mice.

[Table 1](#) displays the study citation references for each aggregated data pool utilized for standard statistical analysis to compare the normalized escape latency between groups. Groups are categorized by transgenic mouse type and treatment with an underscore “_” in between. Mouse genotypes include: KO, KI2, KI3, KI4, WT, and all mice in the study (all). Treatment types include those meant to directly or indirectly improve the AD etiology and thus improve cognition (t+), treatments meant to perturb or assess the worsening of AD etiology and thus diminish cognition (t–), or untreated mice (t0) for which no additional treatments or procedures were performed. For standard statistical analysis, the age was normalized such that there was less than a 0.05% difference between groups (see Methods). To maintain statistical power, gender was not considered in the statistical analysis comparing normalized escape latency between groups, but gender was considered in the supervised machine learning model. Unless stated otherwise, reported MWM escape latency is the normalized MWM escape latency in seconds.

Comparison of cognition in untreated mice

Standard statistical analysis with two-tailed *t*-tests and overall alpha of 0.05 was utilized to compare the impact of mouse genotype on cognition as measured via the normalized MWM escape latency. To prevent skew of data results from over-training in the MWM, only the first 5 days of training trials were included for analysis. The threshold for significance was adjusted using a Bonferonni correction for multiple comparisons (with $p < 0.002$ required for significance), which greatly reduced the likelihood of a false positive.

First, mean normalized MWM escape latencies were examined for all included mouse genotypes without any additional modulatory treatments. [Figure 2](#) illustrates the mean escape latency in seconds for each untreated group (t0) with the error bars denoting the corresponding positive standard deviation. Untreated transgenic *APOE3* KI mice (KI3_t0) was the only group to have a significantly lower normalized mean escape latency compared to WT. Untreated KI2 mean normalized MWM escape latency was not significantly different than WT. However, KI4 and KO had significantly longer MWM escape latencies compared to WT. Moreover, untreated KI4 mice had the highest normalized mean escape latency, meaning they had significantly worse cognitive performance compared to the other untreated groups. The colored * above each bar in [Fig. 2](#) represents a significant pairwise comparison between the group corresponding to the labeled bar and the group corresponding to the color of the asterisk. For example, untreated transgenic *APOE3* KI (KI3_t0) had a significantly different normalized mean escape latency than every other untreated group (KI2, KI4, KO, WT, and all).

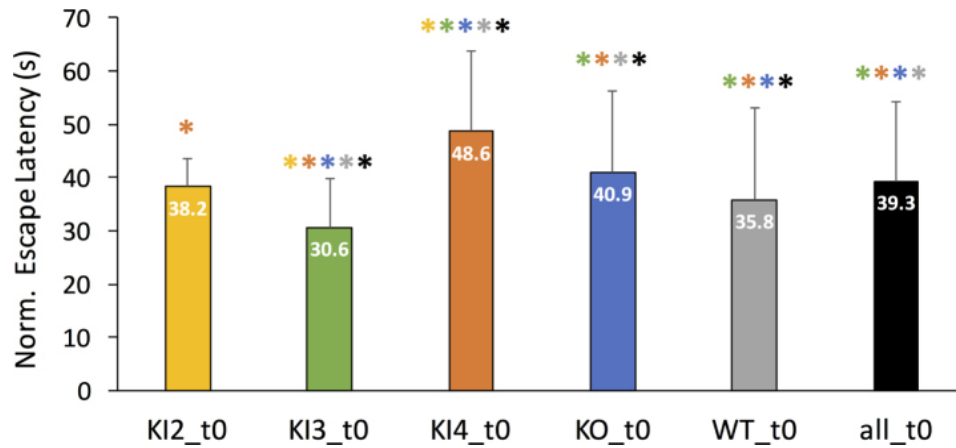


Fig. 2

Comparison of mean normalized Morris Water Maze (MWM) mean escape latency (in seconds) between untreated (t0) wild type and transgenic *APOE* genotypes. MWM escape latency is normalized for age but not gender. Mean normalized escape latency comprises MWM escape latency measurements for baseline through day 4 or 5 of training. The error bar corresponds to the positive standard deviation for the corresponding group. Groups are as follows: transgenic *APOE* knockout (KO), transgenic *APOE2* knock-in (KI2), transgenic *APOE3* knock-in (KI3), transgenic *APOE4* knock-in (KI4), wild type (WT), all mice in the study (all). The color-coded asterisk (*) indicates Bonferonni-corrected pairwise statistical significance between groups ($p < 0.002$).

Treatment effect on cognition in *APOE* mice

The normalized mean MWM escape latency was analyzed for mice that were treated with *APOE* modulatory treatments. Such treatments could include either direct or indirect modulation of underlying etiology beyond the original *APOE* genotype modifications listed in [Table 1](#). Treatments were separated based on their intended effect. Positive treatments (t+)

were meant to lessen the AD etiology and thus improve cognition, whereas negative treatments (t-) were meant to perturb or worsen the AD etiology, and thus, further impair cognition. A full description of treatments for each study is given in [Supplementary Table 1](#). The untreated group (t0) for each mouse genotype is shown in each panel of [Fig. 3](#) for visual comparison. Again, to maintain statistical power, the statistical analysis was not segregated by mouse gender. However, all groups were normalized for age to better isolate the impact of the treatment and mouse genotype.

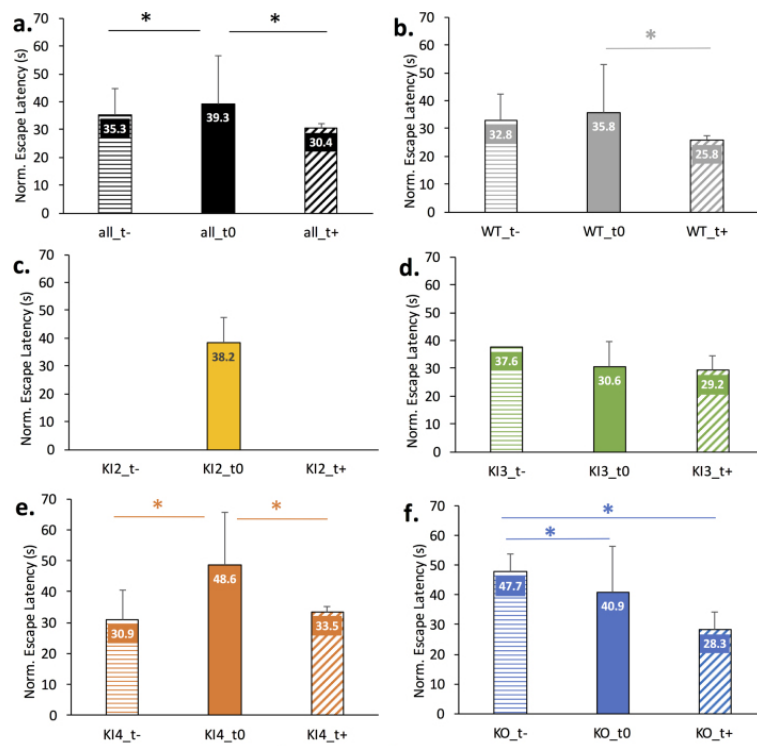


Fig. 3

Comparison of modulatory treatments on normalized Morris Water Maze (MWM) escape latency across wild type (WT) or *APOE* mouse genotypes. Data is normalized for age but not gender. Mean normalized escape latency comprises MWM escape latency measurements for baseline through day 4 or 5 of training. The error bar corresponds to the positive standard

deviation for the corresponding group. Positive treatments (t+) were meant to lessen the AD etiology and/or improve cognition; negative treatments (t-) were meant to worsen the AD etiology and/or worsen cognition; untreated mice (t0) of the same genotype are shown for comparison. A full description of the treatments for each study is given in [Supplementary Table 1](#). The asterisk (*) indicates Bonferonni-corrected pairwise statistical significance between groups ($p < 0.002$). a.) All mice genotypes; b.) Wild type mice; c.) Transgenic *APOE2* knock-in (KI2) mice; d.) Transgenic *APOE3* knock-in (KI3) mice; e.) Transgenic *APOE4* knock-in (KI4) mice; f.) Transgenic *APOE* knockout (KO) mice.

[Figure 3](#) illustrates the impact of positive treatment (t+), negative treatment (t-), or no treatment (t0) for each mouse genotype. A couple of groups had extremely limited data. Namely, only untreated data was available for KI2 and only one study was available for negatively treated transgenic *APOE3* KI (KI3_t-). For the remaining groups, the mean normalized escape latency is shown with the error bar visually denoting standard deviation. Pairwise significance is denoted by the * and corresponds to significance less than the Bonferonni-corrected threshold of $p < 0.002$. [Figure 3a](#) shows a significant pairwise difference between the normalized escape latencies of all untreated mice (all_t0) and all negatively treated mice (all_t-) as well as between all untreated mice (all_t0) and all positively treated mice (all_t+). [Figure 3b](#) illustrates a significant difference in normalized escape latency between untreated WT (WT_t0) and positively treated WT (WT_t+) mice. [Figure 3c](#) illustrates the normalized escape latency of untreated transgenic *APOE2* KI (KI2_t0) given treatment data was absent for this genotype. [Figure 3d](#) illustrates no significant difference in normalized escape latency between treated and untreated transgenic *APOE3* KI mice. [Figure 3e](#) illustrates significant pairwise

differences in normalized escape latency between negatively treated transgenic *APOE4* KI (KI4_t-) and untreated (KI4_t0) as well as between untreated and positively treated *APOE4* KI (KI4_t+). [Figure 3f](#) illustrates a significant pairwise difference in normalized escape latency between negatively treated transgenic *APOE* KO (KO_t-) and untreated (KO_t0) and between KO_t- and positively treated (KO_t+).

Evaluation of feature importance with random forest modeling

A supervised machine learning technique, random forest modeling, was performed to assess the impact of twelve specific binary features on the prediction of normalized MWM escape latency, including gender (g_m=male; g_f=female, g_mi=mixed/unknown), which was not analyzed in the pairwise statistical analysis presented above. Data from the curated relational database was randomly partitioned into a training set (80% of data) and an independent testing set (20% of data). A supervised random forest model was performed using a binomial standardized normalized MWM escape latency as the predictor where latency was classified as either “superior” or “inferior” (see Methods). Model accuracy on independent test data was 82%. Random forest modeling yielded the following feature importance for the prediction of superior cognition as measured via standardized and normalized MWM escape latency: [KI3, age, g_m, KI4, t0, t+, KO, WT, g_mi, t-, g_f, KI2]=[0.270, 0.094, 0.092, 0.088, 0.077, 0.074, 0.069, 0.061, 0.058, 0.054, 0.038, 0.023] ([Fig. 4](#)). These results indicate KI3 is most important for predicting superior cognition. Mouse age, male gender, and KI4 comprise the next group of closely ranked features that was second-most important for predicting superior cognition. Untreated (t0), positively treated (t+), and KO comprise the third tier of features for predicting superior cognition. WT, mixed gender (g_mi), and negative treatment (t-) comprise the fourth

tier of features for predicting superior cognition. Female gender (g_f) and KI2 comprise the fifth and final tier of features for predicting superior cognition; as such, this final tier is least important for predicting superior cognition as measured by standardized and normalized MWM escape latency.

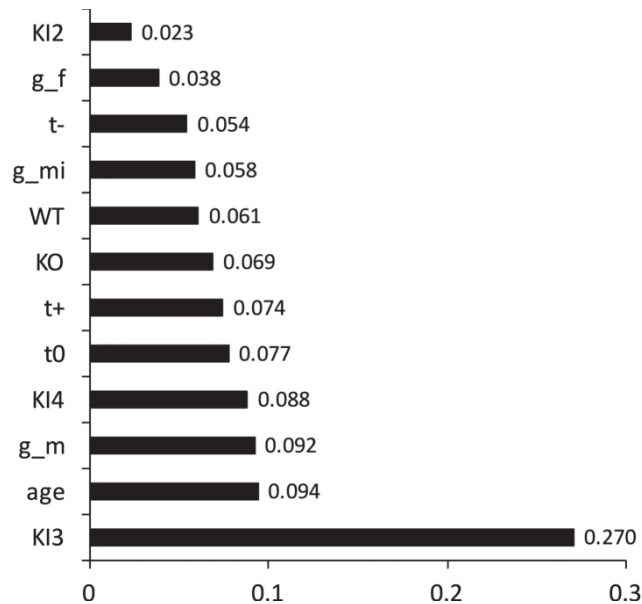


Fig. 4

Importance of binomial features on normalized and standardized Morris Water Maze (MWM) escape latency as predicted by supervised random forest modeling. Data was randomly partitioned into a training set (80%) and an independent test set (20%). Overall model accuracy during independent testing was 82%. Twelve binary features were utilized to predict escape latency; thus, each feature was marked for each mouse as being either present or absent. In descending order, the importance of the following binary features was predicted for classifying “superior” cognition: [KI3, age, g_m, KI4, t0, t+, KO, WT, g_mi, t-, g_f, KI2]=[0.270, 0.094, 0.092, 0.088, 0.077, 0.074, 0.069, 0.061, 0.058, 0.054, 0.038, 0.023]. Superior cognition was

defined as a standardized and normalized MWM escape latency less than or equal to the overall sample mean (see Methods). Feature legend is as follows: transgenic *APOE3* knock-in (KI3), male gender (g_m), transgenic *APOE4* knock-in (KI4), untreated (t0), positive treatment meant to enhance cognition (t+), transgenic *APOE* knockout (KO), wild type (WT), mixed or unknown gender (g_mi), negative treatment (t-), female gender (g_f), and transgenic *APOE2* knock-in (KI2). The overall sample size for the supervised random forest model was $n=3,045$ mice. There were 1,430 female mice, 1,181 male mice, and 258 mixed/unknown gender mice. The breakdown of mouse genotype sample sizes is given in [Table 1](#).

DISCUSSION

The statistical meta-analysis and machine learning prediction of MWM escape latencies in transgenic mice performed in the current study provided aggregate insight into the *APOE* etiology and therapeutic modulation of *APOE* to improve cognition in AD transgenic mice. Further perspective on the impact of *APOE* or AD modulatory treatment, mouse age, mouse gender, and mouse genotype on cognitive performance as measured by normalized MWM escape latency. The results are explored in the context of prior and common literature examining the role of *APOE* in experimental animal models and in clinical AD in humans.

Impact of APOE genotype on cognitive performance

The *APOE* gene is primarily involved in the synthesis of apolipoprotein E, and its expression has been documented in both the central and peripheral nervous system. However, the role of apoE in the body is potentially broader than just lipid metabolism, as its namesake implies. This may include maintaining healthy brain functioning, particularly as apoE acts as a carrier for various

cholesterols involved in neuronal activity and repair. As such, normal apoE is important for clearing of plaques and other wastes from dying cells [80], as well as in the initiation of regenerating neurons [81]. However, pathological apoE, due to alteration of copy number or the presence of an *APOE* mutation, contributes to degeneration resulting in decreased cognition [13, 82].

Among the three human isoforms of *APOE* (E2, E3, and E4), *APOE4* is commonly known to be the greatest genetic risk factor for onset of human AD, while *APOE2* has been demonstrated to decrease the risk of onset in humans [6]. The *APOE4* allele is carried by 3–41% of the global human population [83]. Moreover, there is increasing evidence that the *APOE4* isoform increases risk of AD in humans via pathways involved in loss of function or gain of toxic function. These include well-studied biomarkers of AD, such as tau pathology, tau-mediated neurodegeneration, and an expanding list of A β -dependent and A β -independent pathways that are conditionally affected by different apoE isoforms [13, 84]. In the case of human A β pathways, *APOE4* expression has been shown to catalyze the folding of A β peptides into higher order sheets and other conformations, which contributes to AD pathogenesis by facilitating plaque accumulation in the brain [85].

In the present study's aggregate data analysis, statistical comparison of mean normalized MWM escape latency data in untreated transgenic *APOE* AD mice indicated that untreated knock-in *APOE3* (KI3_t0, Fig. 2) mice out-performed untreated wild type mice (WT_t0, Fig. 2). Like results seen in human AD risk studies on the basis of *APOE* isoform, untreated *APOE2* knock-in (KI2_t0) transgenic AD mice had the closest normalized escape latency to untreated wild type

mice ([Fig. 2](#)). However, untreated *APOE4* knock-in (KI4_t0, [Fig. 2](#)) and *APOE* knock-out (KO_t0,) transgenic AD mice performed significantly worse than wild type ([Fig. 2](#)). These results illustrate that either loss of apoE function or gain of toxic apoE function can exacerbate AD pathology and corresponding decreased cognition in transgenic mice, which is consistent with human AD observational studies.

The strong association of *APOE4* with MWM escape latency in both the pairwise statistical analysis ([Figs. 2 and 3](#)) and the machine learning feature importance ([Fig. 4](#)) underscored its known prominence in the AD etiology. The fact that *APOE2* knock-in appeared less protective in the present study compared to observations noted in human studies could be explained by experimental differences in *APOE* in transgenic mice. Mice have fundamental differences in *APOE* compared to humans; even *APOE* knock-in mice that utilize human *APOE* isoforms have drastic quantitative differences in apoE content compared to WT mice [[86](#)]. While the presence of altered *APOE* genotype or copy number often garners the most attention in human studies, lack of *APOE* can also cause neurodegenerative etiology. As noted in the present study, *APOE* knock-out mice performed worse than WT ([Figs. 2 and 3](#)), a finding which is also supported by prior work [[58](#)].

The present study examined the role of *APOE* in preclinical data through transgenic mouse models. As such, it is important to note a few marked differences between *APOE* expression and functioning in mice when compared to humans. For instance, mice are shown to naturally express only one isoform of *APOE* [[11](#)], whereas humans exhibit three. Furthermore, mice and humans differ in their relative lipoprotein levels, which may alter respective clearance

mechanisms. Moreover, mice utilize HDL as a cholesterol shuttler as opposed to the primary use of LDL by humans. In particular, *APOE* KO mice have much higher plasma lipid levels, the effects of which could potentially contribute to synaptic dysfunction and resulting neurodegeneration via independent mechanisms. Overall, the immediate phenotype of deficient *APOE* levels may differ considerably in mice and humans [87].

Impact of age on cognition in the Morris water maze

Age has been widely cited as the single greatest risk factor for AD development in humans [37]. The process of aging is highly intricate and results from the aggregate contribution of various body systems being influenced at the cellular level. Many physiological pathways have been identified in the aging process, and those that most directly affect brain functioning and have been most strongly associated with AD risk include mitochondrial dysfunction, inflammatory reactions in the innate immune system, insufficient glucose metabolism, disruption of lipid homeostasis, worsening A β processing, and decreased regenerative ability [35, 36]. This age-linked neurodegeneration in transgenic AD mice has been demonstrated through downregulation of an NMDAR signaling pathway resulting in a loss of function [34].

Notably, the present aggregate study took extensive care to control for confounds introduced by differences in mouse age or differences in neurodegeneration at the time of MWM escape latency testing. In the present study, the mean escape latency of trials from baseline through day 4 or 5 MWM training was utilized to prevent skewed escape latencies, and mice ages are normalized and resampled to ensure a minimal standard deviation (see Methods). These procedures reduced experimental confounds over the timeline of MWM testing [72, 88, 89] that

would have otherwise clouded study results. Because of the normalization and resampling, assessment of the impact of mouse age on cognition with traditional statistics was not appropriate. However, the random forest machine learning model provided a means to assess the impact of age alongside other features, including *APOE* mouse genotypes, treatment types, and gender. The random forest feature importance ([Fig. 4](#)) illustrated that mouse age (in days) was the second-most important individual feature for predicting cognitive performance as measured by MWM escape latency. Specifically, mice with ages younger than the sample mean are more likely to have superior performance in the MWM.

***APOE*-targeted treatments have a significant impact on cognition independent of age**

Multiple potential treatments have been studied to alter levels of *APOE* expression to improve cognitive performance [38]. Most studies focus on “positive” treatments meant to indirectly or directly reduce the *APOE*-related pathology, and correspondingly, improve cognition. However, some studies apply “negative” treatment in an attempt to perturb the neurodegenerative process to allow further etiological assessment; such negative treatments worsen the cognition of the mice compared to their baseline or untreated genotype. For this reason, the present study divided modulatory treatments as positive (t+), negative (t–), or untreated (t0), to better elucidate the external modulation of *APOE* in WT or transgenic mice. A detailed description of modulatory treatments for each study is given in [Supplementary Table 1](#).

It is the positive treatments that are most important for translational development of future human AD treatments. Statistical analysis of modulatory treatments in the present study illustrated that positive treatments were able to significantly to improve cognition in wild type

(WT_t+, [Fig. 3b](#)), *APOE4* knock-in (KI4_t+, [Fig. 3e](#)), and *APOE* knock-out (KO_t+, [Fig. 3f](#)). Positively treated *APOE3* knock-in (KI3_t+, [Fig. 3d](#)) has a small, albeit insignificant improvement in MWM escape latency. No prior positively treated *APOE2* mouse model study data met criteria for inclusion in the present study; therefore, the impact of positive treatment on *APOE2* knock-in (KI2, [Fig. 3c](#)) was unable to be assessed.

Many AD treatment routes attempt to improve cognitive performance through the use of drug agonists for defined receptors in the brain. A commonly used agonist is bexarotene, which has been suggested to provide neuroprotection and synaptic plasticity. Another important connection is the inclusion of ABCA1-mediated lipidation in agonist treatments. ABCA1 typically causes better cognitive performance in both KO and WT. Looking into the subcategory of diet-related treatments, lower caloric intake was seen to have a positive impact on cognitive performance. However, a key issue with many treatments intended to positively modulate *APOE* is that they may be effective in WT mice but not in transgenic *APOE* KI or KO mice.

Negative treatments are less meaningful for human clinical treatment development, but still provide interesting perspective on the underlying AD etiology in transgenic AD mice experiments. In the present study, only negative treatments for *APOE4* knock-in (KI4_t–, [Fig. 3e](#)) and *APOE* knock-out (KO_t–, [Fig. 3f](#)) had significantly different normalized MWM escape latencies compared to untreated mice of the same genotype. However, the negatively treated *APOE4* knock-in (KI4_t–, [Fig. 3e](#)) ironically had improved normalized MWM escape latency compared to untreated *APOE4* knock-in (KI4_t0, [Fig. 3e](#)). The aforementioned finding with *APOE4* illustrates its heterogeneity and overall complexity in the AD etiology.

Challenges in translation of pre-clinical APOE treatments to human Alzheimer's disease

Many treatments that show promise in transgenic mice do not translate to human trials. This is likely due to the vast differences of *APOE* in mice, who naturally present one isoform, versus humans, which present three isoforms [90]. Another challenge is that *APOE* pathology is not isolated in human AD, as apoE interacts with multiple factors that contribute to neurodegeneration and corresponding cognitive decline [91]. Nonetheless, parenteral administration of citicoline, an intermediate in the anabolism of structural membrane phospholipids, has been one of the more promising treatments in humans with *APOE4* genetic risk who have early signs of cognitive decline [92]. Moreover, although the study was limited due to a relatively small sample size and uncontrolled design, supplementation of a drink containing antioxidants, omega-3 fatty acids, and resveratrol appeared to elicit positive immune and cognitive effects in *APOE* E3/E3 patients [93].

In a double-blind, placebo-controlled human clinical trial of bexarotene, an agonist of the retinoid X receptor, the overall results were negative. Bexarotene was shown to increase serum A β in *APOE4* noncarriers; as such, it increased associated risk factor for contracting cardiovascular disease without conferring any positive effects on cognition [94]. Similarly, in phase III trials of bapineuzumab, a humanized anti-A β antibody, results were negative in that no significant difference between the treatment and placebo groups was observed [95]. In summary, ongoing research is still required to successfully translate *APOE* modulatory treatments for human AD.

Impact of gender on cognition in AD

In the present aggregate data analysis, traditional pairwise statistical analysis could not separately address the impact of gender on each transgenic AD mouse model's mean normalized cognitive performance in the MWM due to a lack of statistical power. However, the supervised random forest machine learning model was able to examine the importance of gender in context with the other features that predict normalized MWM escape latency, including mouse genotype, mouse age, and *APOE* modulatory treatments. The random forest model calculated the feature importance for 12 binary features used to predict "superior" MWM performance, which was defined as a normalized MWM escape latency faster than the sample mean. The plot of random forest feature importance ([Fig. 4](#)) illustrated that the male gender (*g_m*) is the third-most important individual feature for predicting superior cognitive performance in the MWM. The male gender fell into the second of five tiers that grouped features of similar importance in predicting normalized MWM escape latency. Mixed gender (*g_mi*) and female gender (*g_f*) were not prominent features for predicting superior MWM escape latency. In fact, mixed gender ranked eighth (of twelve) as an individual feature and fell into the fourth (of five) tiers of grouped features to predict superior MWM escape latency. Likewise, female gender ranked eleventh (of twelve) as an individual feature and fell in the fifth or least important tier for predicting superior cognitive performance via MWM escape latency. Therefore, female gender was not a common feature present among mice that performed superiorly in the MWM.

The findings of gender in the present aggregate study do align with what has been seen with human AD patients in the clinic, as females tend to be at greater risk for AD. Regarding the role of gender, Mielke et al. propose that several possibilities could explain the discrepancy in AD incidence between men and women, including risk factors with equal frequency in each gender

but are stronger in one group, risk factors with the same effect but different frequency in each gender, risk factors that vary in effect and frequency in each gender, and risk factors limited to one gender [26]. An example of risk factor with the same effect but different frequency is smoking, of which rates are higher in men, and an example of a risk factor that varies in effect and frequency is that women appear to be more sensitive to head trauma despite the fact that men suffer from such injuries at higher rates [26].

Additionally, several studies have indicated that women have a higher risk for AD than men if they are *APOE4* carriers, but the reasoning behind this discrepancy is still disputed [27]. Moreover, prior studies have suggested that decreasing levels of estrogen as women age may lead to higher rates of cognitive decline due to the effects of menopause on mitochondrial pathways associated with AD risk [28]. Interestingly, previous work found that estrogen appears to upregulate the *APOE* gene [29], yet other studies did not arrive at the same conclusion, mainly due to the complications associated with isolating the effects of estrogen in multiple brain pathways [27].

Females have been documented as more susceptible to development of AD across epidemiological studies, as well, which have similarly attributed the phenomenon to declines in estrogen levels [26, 30–33]. Estrogen has a demonstrated involvement in $A\beta$ pathways associated with neurodegeneration. Mitochondria from young females confers protection against $A\beta$ toxicity that is eventually lost as females reach old age [31]. Additionally, multiple studies have shown that female transgenic AD mice express higher plaque load in comparison to male mice [30].

In an effort to improve outcomes in women, human estrogen replacement therapy trials have attempted to remediate the hormone-linked components of AD pathogenesis. However, there are mixed results in the literature. The most notable negative outcomes are increased risk of cancer and cardiovascular disease upon estradiol administration [31]. Unfortunately, there remains a deficiency of findings addressing the efficacy of pharmacotherapies aimed at preventing and treating AD in at-risk women [32].

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Future Work

The same procedure that went into this study can be applied to different proteins such as Tau or BACE that have been tied Alzheimer's. It will lead to a better understanding of the features that are importantly tied to the disease. They can be statistically analyzed to see their relative weight. Is Tau more important than Amyloid? Random forest modeling can be used once again to investigate feature importance. A decision tree model can determine how much a protein must vary from wild type to cause a certain amount of change in escape latency. It can be done with APOE protein data. Collaboration as well can be done with other technical teams in Dr. Mitchell's lab.