Novel mouse models of late-onset Alzheimer's disease based on GWAS

Michael Sasner[2] on behalf of the IU/JAX MODEL-AD consortium [1,2,3]

[1]Stark Neuroscience Research Institute, Indianapolis, Indiana, USA; [2]The Jackson Laboratory, Bar Harbor, Maine USA; [3]Sage Bionetworks, Seattle, Washington, USA

MODEL-AD
MODEL ORGANISM DEVELOPMENT AND EVALUATION FOR LATE-ONSET ALZHEIMER'S DISEASE

The Jackson Laboratory
Leading the search for tomorrow's cures

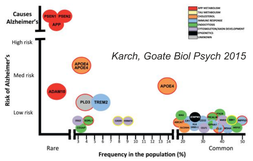
Sage BIONETWORKS

Abstract

The Alzheimer's Disease (AD) patient population consists almost entirely (~98%) of the late-onset form of AD (LOAD); however, most mouse models currently used to develop therapies for AD are based on familial AD (fAD) mutations in *APP*, *PSEN1* or *PSEN2*. This may contribute to failures moving potential therapies from preclinical models into the clinic. The Model Organism Development and Evaluation for Late-onset AD (MODEL-AD) Center was created to develop, characterize, and distribute more precise preclinical models for LOAD. Because the *APOE4* variant and variants at the *Trem2* locus are the strongest genetic risk factors for LOAD, we first created a homozygous model expressing humanized *APOE4* and the R47H allele of the *Trem2* gene. Additional genetic variants were prioritized in loci previously identified by GWAS using data from the ADNI and ADSP projects. These variants have been engineered into the APOE4/Trem*R47H model to increase the risk of developing AD-like phenotypes. We have created models including SNPs corresponding to risk variants in *ABCA7* and *PLCG2*, and knockouts of mouse *Il1rap* and *Ceacam1*. We have also created a humanized *APP* model by altering the three amino acids that differ between human and mouse Abeta42. In addition, we have created *APOE3* and *APOE2* variants to serve as controls. We will present validation data including transcriptomics, pathology, and functional assays on the APOE4/Trem2 model; all new models are currently being aged for phenotypic studies, and results will be compared to fAD models and clinical data. We have created novel mouse models that express combinations of genetic variants identified in human LOAD patient populations. Our strategy closely integrates human and mouse data, with the aim that these new AD models will show a high degree of clinical translatability for preclinical testing of new therapeutic targets. All new models will be made available for both academic and for-profit use from The Jackson Laboratory (www.jax.org/alzheimers), and all validation data will be shared via the AMP-AD knowledge portal (www.synapse.org/alzheimers). We seek input and collaborations from the basic research and pharma/biotech communities. For more information see www.model-ad.org .

Modeling Strategy

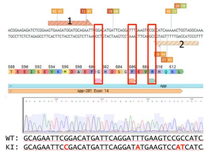
Existing animal models (over) express causative mutations in APP or PSEN1 or PSEN2; these model only a small percentage of the patient population. We aim to create more translational models of late-onset AD by combining relatively common, low risk alleles.



In order to test whether variants in previously identified GWAS are able to confer an AD-like phenotype in a mouse model, we first created a "sensitized" strain that expresses two of the strongest genetic risk factors for late-onset AD, the *APOE4* variant and the R47H mutation in *Trem2*. This model is described in poster P4-028, ***Characterizing the APOE4/Trem2*R47H mouse model for late-onset Alzheimer's disease***.

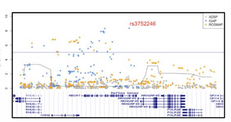
In recognition of the fact that mouse Aβ may not be as amyloidogenic as human Aβ, we then mutated the three amino acids that differ between human and mouse in the Aβ1-42 region. This "hAbeta KI" model is currently being analyzed and the humanized Aβ1-42 sequence will be incorporated into future models.

- **APOE4/Trem2*R47H (JAX #28709)**
- **hAbeta KI/APOE4/Trem2*R47H (JAX #30670)**
- **APOE4 KI (JAX #27894)**
- **APOE3 KI (JAX #29018)**
- **APOE2 KI (JAX #29017)**



Both rare and common *ABCA7* variants are associated with AD

Rare loss of function (LOF) variants in ABCA7 confer risk of AD (Steinberg et al, 2015). Haplotype analysis of the common AD variant rs3752246 revealed the absence of rare LOF variants on the same haplotype suggesting a different functional mechanism. This eQTL SNP encodes a glycine to alanine substitution at amino acid position 1527 in exon 32 of the canonical transcript, which is in the extracellular region of the transporter domain. Deletion of the *Abca7* gene in the J20 mouse model has been shown to increase cerebral Aβ accumulation (Kim et al., 2013). We can study the mechanisms of these distinct pathways by comparing a mouse model expressing a knock-in of the common human SNP to a mouse KO, resembling rare human LOF variants.



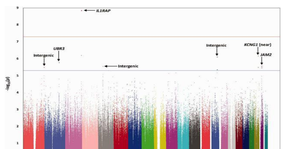
Study	Population	Design	OR	P-value
Steinberg et el. 2015	Iceland, Europe, U.S	Replication study: Stage 1: 3,419 AD cases; 151,805 controls Stage 2: 2,365 AD cases; 4,316 controls	2.03	6.8×10^{-15}
Cuyvers et al. 2015	Belgium	Genetic burden analysis of predicted loss-of function variants 772 AD cases; 757 controls	4.03	2.0×10^{-4}
Le Guennec et al. 2016	French	Meta Analysis of 1,256 familial AD cases; 1,347 controls	2.81	3.6×10^{-7}

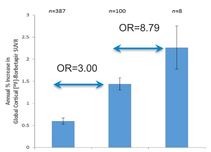
We used CRISPR to engineer the common SNP into the mouse gene to humanize this variant, as well as to generate a knockout in exon 32.

- **Abca7*A1527G/APOE4/Trem2*R47H (JAX #30283)**
- **Abca7 KO/APOE4/Trem2*R47Hf (JAX # 30320)**

A common *IL1RAP* variant is associated with amyloid accumulation in the ADNI cohort

The IL1RAP intronic variant rs12053868 is associated with amyloid accumulation in the ADNI imaging cohort. IL1RAP (7.1%) and APOE4 (3.4%) together explain 10.5% of the phenotypic variance in amyloid accumulation; age and gender alone explain ~1% of this variance (*Ramanan et al, Brain 2015*).





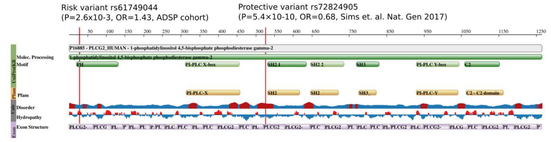
IL1RAP is highly conserved and intolerant to common functional variants (ExAC loss-of-function probability = 0.51. Targeted *IL1RAP* sequencing revealed an absence of coding variants in a subset of 435 patients.

We used CRISPR to target the first coding exon. A founder with a deletion of 387bp was selected. This mouse *Il1rap* knockout model will help us to elucidate the role of microglia in amyloid accumulation during aging.

- **Il1rap KO/APOE4/Trem2*R47H (JAX # 30304)**

A rare *PLCG2* missense variant is associated with AD in the ADSP cohort

Rare variants in *PLCG2* have previously been associated with AD risk (*Sims et al, Nature Genetics 2017*). PLCG2 is is part of a protein interaction network containing AD risk genes and is highly expressed in microglia. Analysis of ADSP whole-exome data revealed a novel *PLCG2* risk variant rs61749044, which is highly conserved across species and predicted to be deleterious by Polyphen and CADD.

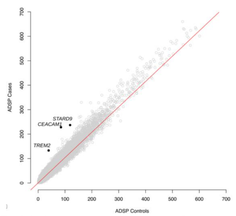


We used CRISPR to engineer this mutation into the homologous region of the mouse gene (along with a silent mutation to prevent re-cutting). A CRISPR-generated Plcg2 KO was also created.

- **Plcg2*M28L/APOE4/Trem2*R47H (JAX #30674)**
- **Plcg2 KO (JAX #29910)**

CEACAM1 shows significant rare, deleterious variant burden in the ADSP cohort

Rare variant burden was assessed in the ADSP exome cohort in 4,840 cases and 4,924 controls. Genetic burden testing revealed multiple loci harboring a significant excess of rare and deleterious variants in ADSP cases when compared to controls (adjusted p-value < 0.05).



ADSP rare variant burden analysis				
Gene	Number of variants tested	Cases with at least two deleterious variants (n=4840)	Controls with at least two deleterious variants (n=4294)	SKAT-O p-value Bonferroni adjusted
TREM2	19	133	39	2.22E-07
CEACAM1	17	228	85	7.47E-07
STARD9	129	237	119	0.047

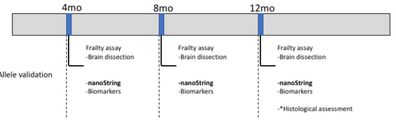
CEACAM1 is a potential novel AD locus linked to blood-brain barrier permeability and inflammation. The mouse paralog of *CEACAM1* lies in a QTL associated with cognitive resilience to AD. The mouse genome has identical exons in *Ceacam1* and *Ceacam2*, making it impractical to engineer a specific SNP into a mouse model. We therefore chose the create a knock-out model. CRISPR guides were targeted upstream and downstream of exon1. A founder with deletion of 601bp was selected.

- **Ceacam1 KO/APOE4/Trem2*R47H (JAX #30673)**

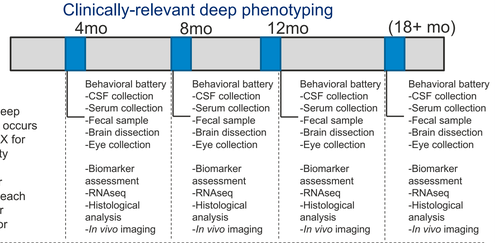
Phenotyping of new models

These models are undergoing a primary phenotyping screen as homozygotes for all three alleles (including APOE4 and Trem2*R47H). A new nanoString panel has been developed to assay mouse models of AD based on human AMP-AD gene modules. Those models that exhibit a transcriptomic profile similar to clinical late-onset AD will move on to a deep phenotyping pipeline, in order to validate the models by comparing them to clinical measures and to stage disease progression to define the therapeutic window. The most clinically-relevant models will then move to the MODEL-AD Preclinical Testing Core.

Primary screen to prioritize models for deep phenotyping



Clinically-relevant deep phenotyping



Histology:
Gross morphology/white matter: Luxol fast blue and Cresol Violet
Neurons: NeuN and CTIP
Plaques, dystropic neurites and myeloid cells: X34, LAMP1 and IBA1
TAU: AT8 and H&E
Neuroinflammation: IBA1 and GFAP
Vascular health: CD31, Fibrin and IBA1

***In vivo* imaging by MR/PET**:
Amyloid: 18F-AV45
Tau: 18F-1451
Glucose: 18F-FDG
Blood flow: 64Cu-PTSM

Biomarkers:
AB, Tau
Nfl
Neurogranin
sTREM2

Ongoing pilot projects:
Proteomic and metabolomics profiling
Future plans: Microbiome profiling to compare by genotype, age/disease progression, sex and site/health status.

Further information

- MODEL AD: www.modelad.org
- AMP-AD Knowledge portal: http://www.synapse.org/ampad
- JAX AD models: https://www.jax.org/alzheimers
- AlzForum research models: http://www.alzforum.org/research-models

Acknowledgements

MODEL-AD was established with funding from The National Institute on Aging (U54 AG054345-01). Aging studies are also supported by the Nathan Shock Center of Excellence in the Basic Biology of Aging (NIH P30 AG0380770).