Novel mouse models of late-onset Alzheimer's disease

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MODEL-AD
Model Organism Development & Evaluation for Late-Onset Alzheimer's Disease

ABSTRACT

The Alzheimer's Disease (AD) patient population consists almost entirely (~98%) of the late-onset form of AD (LOAD); however, most animal models currently used to develop therapies for AD are based on familial AD (FAD) mutations in *APP*, *PSEN1* or *PSEN2*. This may contribute to the lack of translation to the clinic for therapeutic agents being evaluated in preclinical studies. 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. Our approach is to engineer mouse models to express combinations of genetic variants identified in human LOAD patient populations. This strategy integrates human and mouse data, with the purpose of creating new AD models with a high degree of preclinical translatability for novel therapeutic targets. Since the *APOE4* allele and variants at the *TREM2* locus are among the strongest genetic risk factors for LOAD, we first created a homozygous model expressing humanized *APOE4* and the R47H allele of the *Trem2* gene. This model exhibits altered metabolic phenotypes and neurovascular deficits. We then generated a humanized *APP* model on this background by altering the three amino acids that differ between human and mouse Abeta42, as the human protein may be more prone to aggregate.
Additional genetic variants have been prioritized in loci previously identified by GWAS using data from the ADNI and ADSP projects. These variants have been engineered into the APOE4/Trem2*R47H model to increase the risk of developing AD-like phenotypes. We have created mouse models expressing SNPs corresponding to risk variants in *ABCA7*, *MTHFR and PLCG2*, knockouts of mouse *Il1rap* and *Ceacam1* and a knock-in model expressing human *CR1*. In addition, we have generated *APOE3* and *APOE2* variants to serve as controls. The new models are aging for phenotypic studies. We will present validation data including transcriptomics, pathology, and functional assays, with new models being compared to both fAD models and clinical samples. All new models will be made available for both academic and for-profit use from The Jackson Laboratory, 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 (LOAD) by combining relatively common, low risk alleles in a single model.

Karch, Goate Biol Psych 2015

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 G10 (467.03), ***MODEL-AD: Late-onset Alzheimer's disease models***.

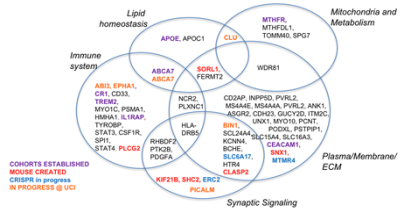
In recognition of the fact that mouse Aβ may not be as amyloidogenic as human Aβ, we then mutated the three amino acids that differ between human and mouse in the Aβ1-42 region. This "hAbeta KI" model is currently being analyzed in our deep phenotyping pipeline and the humanized Aβ1-42 sequence will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

APOE models	genetic background	JAX #
APOE4/Trem2*R47H	B6J	28709
APOE4 KI	B6J	27894
APOE3 KI	B6J	29018
APOE2 KI	B6J	29017
APP models		
hAbeta-loxP-KI	mixed B6J; B6N	30898
hAbeta-loxP-KI	B6NJ	32013
hAbeta KI	B6J-APOE4/Trem2*R47H	30670
App KO	B6J-APOE4/Trem2*R47H	31722
Trem2 models		
Trem2*R47H	B6J	27918
Trem2*Y38C	B6J	29725
Trem2 KO	B6J	27197
floxed Trem2	B6J	29853

PRIORITIZATION OF LOAD GENETIC VARIANTS

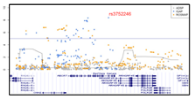
Variants were prioritized based on:

- **Replication across multiple studies**
- **Pathogenicity of SNP**
- **Allele frequency**
- **Conservation of mouse to human gene**
- **Expression in the brain**
- **Relevance of gene/pathway to Alzheimer's disease**
- **A distribution of variants in distinct pathways, as shown below**



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 knock-out, which resembles rare human loss-of-function variants.



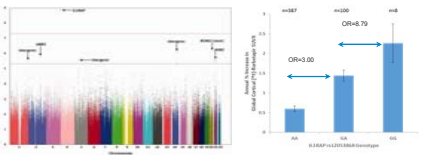
Study	Population	Design	OR	P-value
Steinberg et al. 2015	Iceland, Europe, U.S	Replication study: Stage 1: 3,410 AD cases; 151,805 controls Stage 2: 2,365 AD cases; 4,316 controls	2.03	6.8×10^{-16}
Cuyvers et al. 2015	Belgium	Genetic burden analysis of predicted loss-of function variants 772 AD cases; 757 controls	4.03	2.0×10^{-4}
Le Guennec et al. 2016	French	Meta Analysis of 1,266 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*R47H (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.

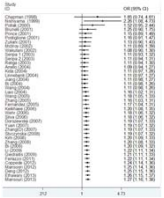
We used CRISPR to target the first coding exon. A founder with a deletion of 387bp was selected. This mouse *Il1rap* knockout model will help us to elucidate the role of microglia in amyloid accumulation during aging.

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

Mthfr variant C677T as a potential risk factor for LOAD

Association between the MTHFR missense variant C677T (rs1801133) and LOAD have been widely reported. The common variant is associated with LOAD risk in European & Asian populations and rs1801133 increases basal plasma homocysteine levels which are associated with cognitive impairment late in life. We used CRISPR to engineer the corresponding mutation A262V (GCC→GTC) into the mouse gene; this precise mouse model will allow testing of the impact of the functional variant on folate metabolism during aging.

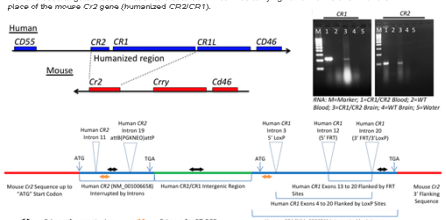
- **Mthfr*C677T APOE4/Trem2*R47H (JAX #30922)**



Forest plot of meta-analysis results for the C677T variant (Peng et al. 2016, Neuroscience Letters)

Humanizing CR1 and CR2 to model AD risk

Components of the complement cascade are strongly implicated in Alzheimer's disease (AD) both in increasing risk and in the underlying pathophysiology. Variations in complement receptor 1 (CR1) have been identified as risk factors for Late Onset AD (LOAD), but the mechanisms are not clear. To address this need, we have used targeted recombination to create C57BL/6J mice carrying human forms of *CR2* and *CR1* in place of the mouse *Cr2* gene (humanized *CR2/CR1*).

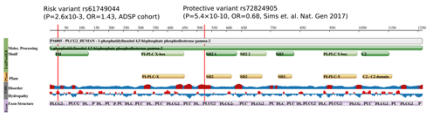


LoxP and FRT sites were included within the *CR1* gene to allow for the generation of multiple splice forms or *CR1* (CR1-S or long and CR1-F or short), and the ability to generate a CR1 knockout leaving human CR2 intact.

- **hCR1^{long} KI/APOE4/Trem2*R47H (JAX #31668)**
- **hCR1^{long} KI (JAX #27713)**

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 a 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 the M28L (ATG→TTG) 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)**

A common SORL1 missense variant is associated with increased Aβ42 secretion in LOAD

Rare loss of function variants in the retromer complex member *SORL1* have been associated with LOAD but the role of common deleterious variants remains largely unexplored. We identified a common, highly conserved missense variant (rs2298813) that is significantly associated with LOAD and has been shown to segregate with disease in 54 multiplex families. Overexpression of the missense variant rs2298813 leads to elevated Aβ42 secretion (Vardarajan et al. 2016, Ann Neurol). The SNP rs2298813 has been recently associated with increased risk of developing dementia in Parkinson's patients supporting a shared etiology across diseases (Grodem et al. 2018, Neurosci Lett.)

Cohort	Population	Design	SNP	OR	P-value
ADSP Whole Exomes	Caucasian	Stage 1: 4,840 AD cases; 4,294 controls	rs2298813 (A528T)	1.29	1×10^{-4}
Vardarajan et al. 2016	Caucasian	Targeted Analysis of SORL1 coding variants: 151 multiplex families, 498 controls	rs2298813 (A528T)	n.a	6×10^{-7}

We used CRISPR to engineer the A528T (GCC→ACC) mutation into the homologous region of the mouse gene, along with a silent R529R AGG→CGC mutation to disrupt a Bstn1 site and introduce a Faul site.

- **Sorl1*A528T/APOE4/Trem2*R47H (JAX #31940)**

A common, deleterious missense variant in SNX1 supports the role of the retromer complex in LOAD

Multiple recent studies have implicated members of the retromer complex, including *SNX1* and *SORL1*, in LOAD. We identified a common missense variant (rs1802376) in *SNX1* which has been associated with LOAD in multiple independent cohorts. This is the only variant in SNX1 predicted to be highly deleterious (CADD score: 28).

Cohort	Population	Design	SNP	OR	P-value
IGAP	Caucasian	Stage 1: 17,008 AD cases; 37,154 controls	rs1802376 (D465N)	1.22	6.08×10^{-7}
		Stage 2: 8,572 AD cases; 11,312 controls			
ADSP Whole Exomes	Caucasian	Stage 1: 4,840 AD cases; 4,294 controls	rs1802376 (D465N)	1.37	2.6×10^{-4}
Desikan et al. 2015	Caucasian	Meta Analysis of 29,054 AD cases; 114,824 controls	rs1802376 (D465N)	1.13	1.28×10^{-6}

This mutation is located in the highly conserved BAR domain of SNX1 that is important for self-assembly of the retromer complex and the formation of cargo-containing vesicular carriers.

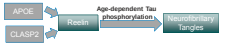
We used CRISPR to engineer the D465N mutation (GAC→AAC) into the homologous site of the mouse gene, as well as a silent AGG→GCT mutation just upstream to introduce a TaqI site. Note that human and mouse numbering differ by a single amino acid.

- **Snx1*D465N SNP/ APOE4/Trem2*R47H (JAX #31942)**

CLASP2 as a potent modifier of Reelin signaling

We identified a highly conserved missense variant (rs61738888) which is associated with LOAD in two independent cohorts. *CLASP2* is a microtubule-binding protein involved in the regulation of microtubule dynamics. *CLASP2* is a key cytoskeletal effector of the Reelin signaling pathway during brain development. Reelin signaling regulates synaptic transmission and depletion of Reelin signaling is an early feature of LOAD.

dbSNP variant ID	ADSP - Pvalue	ROSMAP- Pvalue	OR	CADD Score
rs61738888	4.9×10^{-3}	7.9×10^{-4}	1.14	27



We used CRISPR to engineer the corresponding L163P (CTG-->CCA) mutation, as well as a downstream GTC→GTG silent mutation to introduce a MboI site.

- **Clasp2*L163P APOE4/Trem2*R47H (JAX #31944)**

KIF21b as a novel susceptibility factor in LOAD

KIF21B has been previously implicated in several neurological disorders, including LOAD (Kreft et al. 2014). *KIF21B* belongs to the kinesin family, proteins involved in intracellular transport of proteins and organelles. Analysis of ADSP whole-exome data revealed a novel synonymous *KIF21B* risk variant rs75565610, which is highly conserved across species. We used CRISPR to introduce the ACA→ACG mutation at T82.

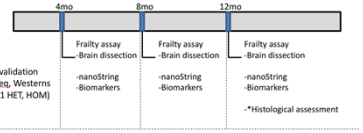
- **Kif21b*T82T (ACA>ACG) /APOE4/Trem2*R47H (JAX #31938)**

PHENOTYPING OF NOVEL 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 be used in the MODEL-AD Preclinical Testing Core.

PRIMARY SCREEN GOALS:

1. Assess impact of novel variants on AD outcomes
2. Prioritize new models for deep phenotyping



6M/6F per genotype at each time point

nanoString mouse AD panel:
-Newly developed with AMP-AD modules
-Half brains

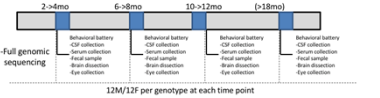
IF relevant transcriptomics changes, Neuropathology on other half brain:
Neurons: NeuN/Ctip2
Amyloid/microglia: X34/GFAP/IBA1
Tau: AT8/H&E

Nanostring Mouse AD Panel

Based on gene modules identified by the AMP-AD program, we have worked with NanoString to create and validate a 770 gene expression assay for the evaluation of mouse models of AD. The new nCounter® Mouse AD Panel provides an efficient, reproducible and cost-effective way to compare genetic models of AD. We have validated the panel by showing a strong correlation of results to RNA-seq run on the same samples of existing fAD models (5XFAD, APP/PS1). We are now using this to prioritize which models move to the deep phenotyping stage. For details see poster 467.05 (Board G12): *Translational genetic and genomic analysis of new mouse models of Alzheimer's disease* and Logsdon et al, *Heterogeneity across human AD coexpression modules identified by meta-analysis of the human brain transcriptome* on biorxiv.

DEEP PHENOTYPING GOALS:

1. Compare mouse models using translationally relevant measures
2. Stage models for preclinical testing



12M/12F per genotype at each time point

Metabolic:
Weight
Total Cholesterol, LDL, HDL
Triglycerides and Non-essential Fatty Acids
Glucose

Behavioral Battery:
Aging: Frailty index
Circadian Activity: Homecage wheel running
Exploratory and locomotor behavior: Open field
Cognition: Spontaneous Alternation
Motor Coordination: Rotarod
Neurophysiology: EEG

-Omics analyses
RNA-seq
Proteomics[1]
Metabolomics[1]
Microbiome[1]
[1]U54 Supplements

PET/MR imaging
Amyloid: AV45
Tau: AV1451
Blood flow: PTSM
Glucose metabolism: FDG

Biomarkers in Tissue/CSF/Blood:
Neurogranin
NF-L
Aβ
Tau

Neuropathology
Brain morphology: LFB/CV
Neurons: NeuN/Ctip2
Amyloid/microglia:X34/22C11/IBA1
Tau: AT8/H&E
Astrocyte/microglia: GFAP/IBA1
Vascular/microglia: CD31/Fibrin/IBA1

These assays have been chosen to closely model clinical assays, so that results from mouse models are clinically relevant, and ideally different models can be matched to stratified patient populations.

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

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NIH National Institute on Aging