Development and characterization of novel mouse models of late-onset Alzheimer's disease

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

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ABSTRACT

AD therapy development efforts have consistently failed, despite success in preclinical trials in animal models. This may have many causes, but more predictive animal models would certainly be beneficial. 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 late-onset AD (LOAD). Our approach is to engineer mouse models to express combinations of genetic variants identified in human LOAD populations in genome-wide association studies (GWAS). These are being generated into a model that predisposes to AD risk by expressing humanized APOE4 and mutant Trem2. In the future, we will also include humanized APP and MAPT (Tau). GWAS variants are prioritized to impact discrete pathways (e.g. neuroinflammation, lipid metabolism, etc.). Models are prioritized for comprehensive phenotyping based on a newly designed nanoString transcriptomics panel compiled from human post mortem co-expression data from the AMP-AD consortium. This approach enables us to correlate transcript profiles in the mouse models to key human disease processes and pathways.
We have edited human AD risk variants into the corresponding mouse genes for *Abca7, Clasp2, CR1, Kif21b, Mthfr, Mtmr4, Plcg2, Shc2, Slc6a17, Snx1* and *Sorl1*. Knockouts of *Abca7, Ceacam1, Il1rap*, Meox2 and *Plcg2* have also been created to model human loss of function variants in the mouse. Analysis at 4, 8, and 12 months shows that some of the AD-associated transcriptomic modules are disrupted in an age-dependent manner, including immune and DNA-repair pathways. The most promising models (as well as existing fAD mouse models) are being phenotyped to 24 months of age using clinically relevant measures including transcriptomics, proteomics, metabolomics, biomarkers in CSF and blood, neuropathology, *in vivo* imaging (for amyloid, tau, blood flow and glucose metabolism) and cognitive assays. This will determine key phenotypes and the therapeutic window for each model in a comprehensive manner. We plan to later combine variants that impact various AD-relevant pathways. Through introducing combinations of human AD risk variants into mice, we expect to create new models that provide more precise and relevant translational models for preclinical development. All models are 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. For more information see www.model-ad.org.

Strategy to model late-onset AD

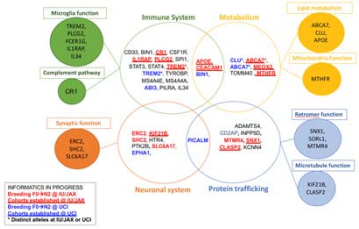
Most existing mouse models of AD (over) expressing causative mutations in APP, PSEN1 and/or PSEN2; these therefore model only the ~2% of the patient population that carry these familial mutations. The goal of the MODEL-AD program is to create, validate, and distribute more translationally relevant models of late-onset AD (LOAD) by combining relatively common, low risk alleles in a single model.
To test whether variants identified by 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 *Trem2**R47H variant.
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.
The models presented here have all been made on the B6J.APOE4/Trem2*R47H background. Models are being made on the B6J. hAβ /APOE4/Trem2*R47H in 2019. In the future we plan to also include a humanized MAPT allele.

Prioritization of genetic risk variants for late-onset AD

Variants identified in ROSMAP, MSBB and Mayo RNA-seq data sets 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

Variants and progress on models



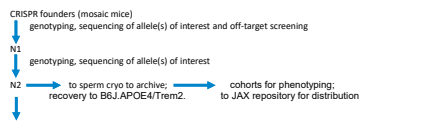
Variants and models

The models presented here have all been made on the late-onset sensitized B6J.APOE4/Trem2*R47H background. Models are being made on the B6J.**hAPP**/APOE4/Trem2*R47H background in 2019. In the future we plan to also include a humanized TAU (MAPT) allele.

Locus	Allele (Human)	Allele (Mouse)	SNP	Type	Is allele conserved in mouse?	JAX model #
ABCA7	A1527G	A1541G	rs3752246	missense	yes	30283
APOE	APOE4	---	---	humanized locus	no	27894
CEACAM1	LOF rare variants	KO	---	KO	---	30673
CLASP2	L163P	L163P	rs61738888	missense	yes	31944
CR1	---	---	---	humanized locus	no	31668
IL1RAP	intronic SNP	KO	rs12053868	KO	---	30304
KIF21B	T82 (ACG->ACA)	T82 (ACG->ACA)	rs7556510	synonomous	yes	31938
MEOX2	CNV	KO	---	KO	---	33770
MTHFR	A222V (c677C>T)	A262V	rs1801133	missense	yes	30922
MTMR4	V297G	V297G	rs2302189	missense	yes	31950
PLCG2	M28L	M28L	rs61749044	missense	yes	30674
PLCG2	P522R	P522R	rs72824905	missense	yes	30771*
SHC2	V577M	V433M	rs61749990	missense	yes	31952
SLC6A17	P61 (CCG>CCA)	P61 (CCA->CCG)	rs41281364	synonymous	yes	31948
SNX1	D466N	D465N	rs1802376	missense	yes	31942
SORL1	A528T	A528T	rs2298813	missense	yes	31940
TREM2	R47H	R47H	rs75932628	missense	yes	27918

Strain development and validation

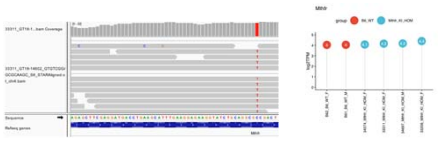
For each new model, RNA-seq on whole brain homogenate was used to assay levels of expression and expression of splice variants, as well as to confirm expression of the mutant alleles.



N2F1 as HOM, HET, WT: take brain tissue at 2-3 months of age

- RNA-seq on brain tissue to show expression of mutant allele and identify splice variants
- Western blot (when an antibody is available) to demonstrate expression of mutant protein/loss of expression with KO
- For key models (e.g. B6J.APOE4/Trem2*R47H; hAβ/APOE4/Trem2*R47H) full genomic sequencing is performed

Below left, RNA-seq shows engineered Mthfr T>C mutation.
Below right, transcript counts indicate normal levels of expression.



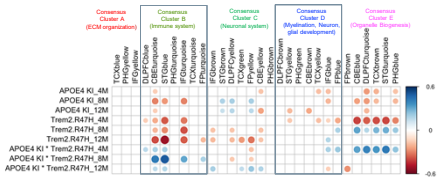
Primary screening

Each new model is assessed for relevance to human AD by gene profiling of mouse brain tissue using a custom Nanostring nCounter® Mouse AD panel that was designed to identify correlations to changes in gene expression specific to clinical late-onset AD*. Based on these results, strains are prioritized for deep phenotyping.

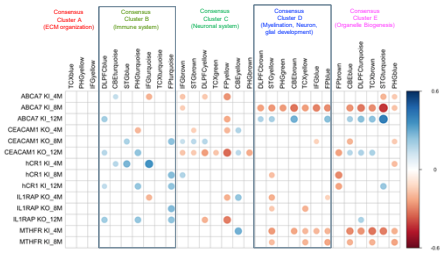
Homogenate from whole brain was assayed at 4, 8, and 12 months of age. Differential gene expression was determined based genotype, age, and sex.

STRAINS	Total	Male	Female	4M	8M	12M
C57BL/6J	35	18	17	12	12	11
B6J.APOE4	32	15	17	12	11	9
B6J.TREM2*R47H	35	18	17	12	11	12
B6J.APOE4.TREM2	35	18	17	11	11	13
A/T.ABCA7-KI	36	18	18	12	12	12
A/T.CEACAM1-KO	32	16	16	12	8	12
A/T.IL1RAP-KO	35	18	17	12	11	12
A/T.MTHFR-KI	20	10	10	10	10	tbd
A/T.CR1	34	17	17	10	12	12
A/T.MIXED-WT	24	12	12	7	9	8

Age-related changes in APOE4/Trem2*R47H homozygous mice
Circles denote effect of each factor significantly correlated (P-value < 0.05) with effects in LOAD cases. C57BL/6J female mice are baseline.



Age-related changes due to additional LOAD risk variant
Circles denote effect of each factor significantly correlated (P-value < 0.05) with effects in LOAD cases. B6J.APOE4/Trem2*R47H homozygous female mice are baseline.



The ABCA7 knock-in model was selected for deep phenotyping based on age-dependent late-onset AD-like gene expression changes in Consensus clusters D and E.

*Preuss et al, *A novel systems biology approach to evaluate mouse models of late-onset Alzheimer's disease.* bioRyiv 2019.

Deep phenotyping

Prioritized models will undergo deep phenotyping at 4, 8, 12 and 24 months to include:

- RNA-seq
- Proteomics
- Metabolomics
- Biomarkers (brain, blood, CSF): Ab, tau, NF-L, neurogranin
- Neuropathology for: amyloid, tau, neuronal number, microglia, astrocytes, vascular compromise
- Cognitive/Behavioral battery: Frailty index, spontaneous alternation, EEG, open field, wheel running
- PET/MR imaging for: Amyloid (AV45), Tau (AV1451), Blood flow (PTSM), Glucose metabolism (FDG)

Future plans

We are currently generating additional late-onset AD risk variants on the B6J.hAβ/hAPOE4/Trem2*R47H background. In the near future we expect to be able to add a humanized Tau (*MAPT*) allele as well.

To determine the effect of genetic backgrounds, we are moving APP alleles onto genetically diverse Collaborative Cross lines.

In an attempt to shift phenotypes toward a neurodegenerative phenotype, some models will be assayed after aging on a high fat diet (45% fat as compared to standard 6% fat chow).

We will correlate disease phenotypes to differences in microbiome by comparing microbiomes across models/genotypes; ages; sexes; and sites.

CONCLUSIONS

- **We are determining whether late-onset AD risk variants identified in GWAS are causative by engineering homologous mutations in mouse models. All mutations are being engineered on a sensitized genetic risk background that is homozygous for APOE4 and Trem2*R47H. Moving forward, this standard background will also include a humanized Aβ allele and eventually also a humanized MAPT (Tau) locus.**
- **All models are being characterized by transcriptomics and neuropathology for AD-related phenotypes.**
- **The most promising models are undergoing deep phenotyping out to 24 months of age.**
- **Phenotypic data and protocols are being made available from the AMP-AD knowledge portal.**
- **Model summaries will be made available at AlzForum research models database.**
- **All models are available from the Jackson Lab with no restrictions appled by MODEL-AD for use by for-profit companies.**

FURTHER INFORMATION

- MODEL AD: www.modelad.org
- AD Knowledge portal: www.ampadportal.org
- Jax AD models: www.jax.org/alzheimers
- AlzForum research models: www.alzforum.org/research-models

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