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

Background: The Model Organism Development and Evaluation for Late-onset AD (MODEL-AD) Consortium was established to generate and characterize more translatable animal models for late-onset Alzheimer's disease (LOAD) based on human genetic risk variants. While numerous genetic risk loci for LOAD have been identified, few have been experimentally verified *in vivo* and in many cases the risk variants have not been validated. The development of new models incorporating LOAD genetic risk should improve our understanding of disease mechanisms and improve preclinical testing of potential therapeutics.
Method: Coding and non-coding LOAD risk variants were prioritized based on human data sets, with the goal of targeting diverse pathways (e.g., neuroinflammation, vascular risk, metabolic function, lipid homeostasis). Risk variants in Abca7, Adamts4, Bin1, Erc2, Mthfr, Mtmr4, Plcg2, Ptk2b, Slc6a17, Snx1, Sorl1 and other loci were engineered into mouse models expressing humanized APOE4 and the Trem2*R47H risk variant; in more recent models, a humanized Abeta allele was also included. A novel transcriptomic panel, based on clinical LOAD samples (Preuss et al, 2020) was used to evaluate how well each model replicated clinical transcriptomic changes with disease. Models were aligned to ROSMAP clinical subtypes.
Result: We have identified specific human AD-related pathways disrupted in an age-dependent manner in these novel mouse models. Specifically, mouse models carrying human AD risk variants Abca7*A1527G, Mthfr*C677T, and Plcg2*M28L exhibited transcriptomics changes similar to those seen in LOAD patients. Most models could be readily matched to either the inflammatory or non-inflammatory subtype.
Conclusion: We have prioritized mouse models expressing LOAD risk variants in *Abca7*, *Mthfr*, and Plcg2 for comprehensive phenotyping using clinically relevant measures including transcriptomics and proteomics, biomarkers, neuropathology and in vivo imaging at advanced ages (24 months). Ongoing projects will use human data to guide how to combine alleles to create models that match multi-omic signatures of AD subtypes and test the effects of environmental risk factors such as high-fat diet. Through this effort we aim to develop improved models for testing targeted therapeutics.

Strategy to model late-onset AD

Late-onset AD risk variants were prioritized based on:

- Significance in multiple studies
- Predicted effect on function
- Human-mouse sequence conservation
- Differential expression in AD
- Diverse mechanisms of action
- Diverse allele types (SNP, KO, KI)

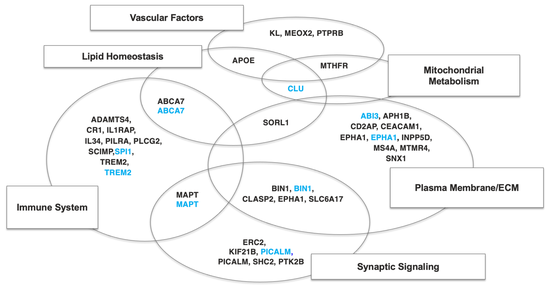
METHODS

Initially, alleles were created using CRISPR to knock-in risk variants to the mouse locus on a sensitized C57BL/6J genetic background expressing APOE4 and Trem2^{R47H} ("LOAD1"; see Kotredes et al, 2021, *PMID: 34707490*). Later models were made on the same background with a humanized Aβ1-42 region ("LOAD2").

Transcriptomic analysis was carried out on brain tissue at 4 and 12 months of age (see Preuss et al, 2020, PMID: 33172468).

Prioritization of genetic risk variants for late-onset AD

The majority of genetic risk for late-onset AD is found in non-coding regions of genes expressed in microglia. For practical reasons, we initially focused on coding variants in loci that are well conserved between human and mouse. More recently, we have created non-coding variants in conserved regions. We are currently developing mouse models with fully humanized loci to study risk variants found in non-conserved regions.



IU/JAX alleles in black; UC IRVINE alleles in blue

Generation of novel mouse models of late-onset AD

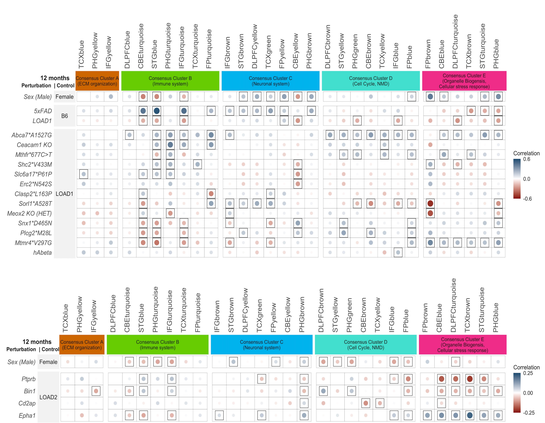
Novel knock-in mouse models were created using CRISPR to avoid issues with transgenic artifacts. All coding models were verified using RNA-seq to demonstrate the expression of AD risk variant.

Locus	Allele (Human)	Allele (Mouse)	SNP	Allele Type	JAX model #
MODELS ON LOAD 1 background (APOE4/Trem2*R47H)					
ABCA7	A1527G	A1541G	rs3752246	missense	30283
CEACAM1	LOF variants	KO	---	KO	30673
CLASP2	L163P	L163P	rs61738888	missense	31944
ERC2	N542S	N542S	rs12488237	missense	31946
MEOX2	LOF variants	HET KO	---	HET KO	33770
MTHFR	A222V (c677C>T)	A262V	rs1801133	missense	30922
MTMR4	V297G	V297G	rs2302189	missense	31950
PLCG2	M28L	M28L	rs61749044	missense	30674
SHC2	V577M	V433M	rs2298813	missense	31952
SLC6A17	P61P	P61P	rs41281364	silent mutation	31948
SNX1	D466N	D465N	rs1802376	missense	31942
SORL1	A528T	A528T	rs41281364	missense	31940
MODELS ON LOAD 2 background (hAβ/APOE4/Trem2*R47H)					
Bin1	non-coding		rs10194375	intron 1 SNP	33869
Cd2ap	non-coding		rs4715019	promoter SNP	33873
Epha1	non-coding		rs12703524	promoter SNP	33875
Ptprb	D57N		rs2584021	missense	33867

Transcriptomics shows an age-dependent alignment to AMP-AD consensus modules

Blue dots indicate differential gene expression correlated to AMP-AD clinical results for specific gene modules; red indicates anti-correlation.

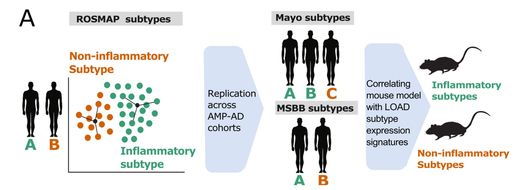
Boxes represent statistical significance at P<0.05.

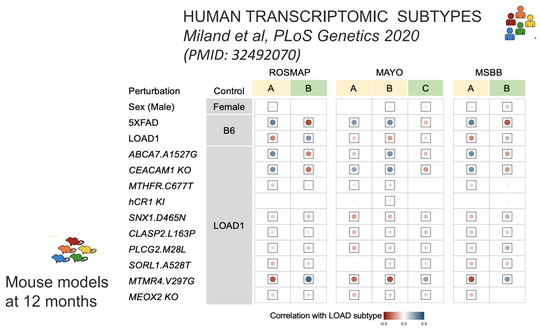


Diverse models are needed for LOAD subtypes

Age-dependent correlation of mouse models to late-onset AD molecular subtypes.

- Abca7*A1527G model shows strong correlation with inflammatory subtype A.
- Mtmr4*V297G model shows strong correlation with non-inflammatory subtype B.





CONCLUSIONS

- The MODEL-AD consortium is focused on generating novel mouse models of late-onset Alzheimer's disease, characterization of these models by clinically relevant measures, and providing them for preclinical testing.
- As a step in this process, we have tested AD risk variants identified in genetic association studies by creating *in vivo* models.
- Here we demonstrate that variants in *Plcg2*, *Mthfr*, and *Abca7* cause molecular changes consistent with those seen in AD patients. We have done deep phenotyping of the Plcg2*M28L (Oblak et al, 2022 PMID: 35813947) and Mthfr*C677T (Reagan et al, PMID: 36050860) models.
- Our results presented here support the use of mouse models to validate and prioritize disease risk variants identified in clinical studies, and to match models to AD sub-types. This is an essential step toward developing improved models of late-onset AD to be used for preclinical studies.
- Ongoing work includes: a new sensitized base model ("LOAD3") expressing APOE4, humanized Aβ, and a humanized MAPT locus; novel combinations of alleles (e.g. Plcg2 and Mthfr alleles) based on the analysis described here; and genetic models described here are being exposed to environmental risk factors (e.g. high-fat diet, heavy metals).
- All mouse models are made available without restriction from the JAX mouse repository.
- All data sets are made available from the AD Knowledge Portal; some data types can be interrogated using the AD Data Explorer.

Related AAIC 2023 presentations

- P2-03: An Integrative Multi-Omics Approach Reveals Molecular Signatures Associated with Age and High-Fat Diet in Mouse Models of Alzheimer's Disease
- P2-05: Assessing the Role of PLCG2*M28L Risk In A Novel Mouse Model Late-Onset Alzheimer's Disease
- P3-03: Elucidating the complex role of ABCA7 in late-onset Alzheimer's disease
- P4-03: Aging × Genetics × Environment: Characterization of Precision Disease Models for Preclinical Testing for Late-onset Alzheimer's disease

FURTHER INFORMATION

- MODEL AD: model-ad.org
- AD Knowledge portal: adknowledgeportal.org
- AD Data Explorer: modeladexplorer.org

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