Initial Characterization of Novel Mouse Models of Late-Onset Alzheimer's Disease Based on Human Genetic Associations

Gareth Howell for the MODEL-AD Consortium

The Jackson Laboratory, Bar Harbor, Maine; Indiana University, Indianapolis, IN; Sage Bionetworks, Seattle, WA

Abstract

Background:

The lack of predictive animal models has hindered the development of treatments for Alzheimer's disease (AD). 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.

Method:

Novel models are being created by genetically engineering AD GWAS variants selected 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.

Results:

On the C57BL/6J (B6J) background we have generated allelic series of humanized *APOE* (*APOE2*, *APOE3*, and *APOE4*) and *Trem2* (R47H, Y38C, KO and floxed KO). Using a B6J.*APOE4/Trem2*R47H* strain as the genetic background, we have engineered a humanized *App* (human Ab1-42) model and have edited human AD risk variants into the corresponding mouse genes for *Abca7*, *Clasp2*, *Kif21b*, *Mthfr*, *Plcg2*, *Snx1*, *CR1*, and *Sorl1*. Knockouts of *Abca7*, *Ceacam1*, *Il1rap*, 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.

Conclusion:

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. This model is described in the adjacent poster P2-131: ***NOVEL APOE4/Trem*R47H MOUSE MODEL: A better tool 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 in our deep phenotyping pipeline and the humanized Aβ1-42 sequence will be incorporated into future models.

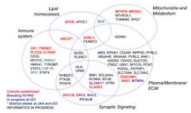
The models presented here have all been made on the B6J.APOE4/Trem2*R47H background. Models are being made on the B6J.hAPP/APOE4/Trem2*R47H in 2019. In the future we plan to also include a humanized MAPT allele.

Prioritization of novel models for detailed phenotyping is based on transcriptomic analysis using a newly developed mouse panel. This is described in the adjacent poster P2-105: ***A novel systems biology approach to evaluate mouse models of late-onset Alzheimer's disease: nCounter mouse AD panel.***

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

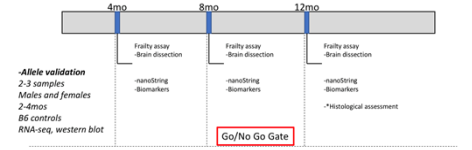


Variants and models

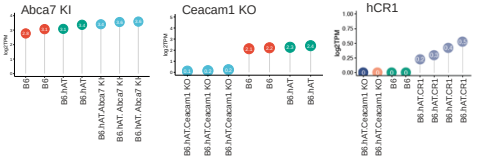
Locus	Allele (Human)	Allele (Mouse)	SNP	Type	Is allele conserved in mouse?	JAX model #
ABCA7	A1527G	A1541G	rs3752246	missense	yes	30283
APOE	APOE4	---	---	humanized locus	no	27894
CEACAM1	LOF rare variants	KO	---	KO	---	30673
CLASP2	L163P	L163P	rs61738888	missense	yes	31944
CR1	---	---	---	humanized 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 validation and Primary Screen

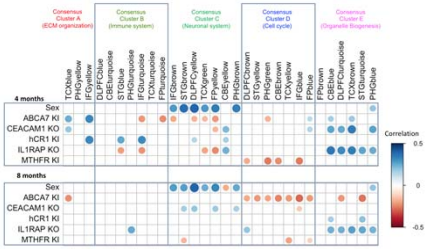
- GWAS variants have been engineered by CRISPR into the B6J.APOE4/Trem2*R47H model (JAX#28709). The genomic sequence of this background was confirmed not to include any novel mutations. All new mutations were confirmed by genomic sequencing of the engineered locus prior to primary screening.



- **Allele validation:** For each new model, RNA-seq was used to assay levels of expression and splice forms in the brain and confirm expression of the mutant allele. Where appropriate antibodies are available, we also confirmed protein expression.



- **Primary Screen:** Each new model is assessed for relevance to human AD by gene profiling of brain tissue using a custom Nanostring nCounter® Mouse AD panel that was designed to identify correlations to key human disease processes and pathways. Based on these results strains will be prioritized for deep phenotyping.



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)

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.**
- **All models are available from the Jackson Lab with no restrictions for use by for-profit companies.**

- **For more information on the Mthfr KI see Poster #P4-087 being presented on 7/17/19**

For futher information, please see

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

Acknowledgements

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