MODEL-AD: The Disease Modeling Project 386.05

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

INTRODUCTION

The Alzheimer's Disease (AD) patient population consists almost entirely (~98%) of the late-onset form of AD (LOAD); however, most mouse models used to study AD are based on familial AD (fAD) mutations in *APP*, *PSEN1* or *PSEN2*. 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 AD.

AIMS of the DISEASE MODELING PROJECT

- Generate at least 40 novel mouse models of LOAD over 5 years.
- Validate a phenotyping pipeline using existing fAD models (APP/PS1, 5XFAD, hTau).
- Screen at least 24 models at a single time point ("high-throughput phenotyping").
- Validate up to 8 models at multiple ages to define disease staging ("deep phenotyping").
- Provide validated models, with defined therapeutic windows and biomarkers, to the MODEL-AD Preclinical Testing Core for compound testing.
- Make all models available for academic and for-profit use through the JAX mouse repository.
- Make all validation data, along with protocols, available through the AMP-AD Knowledge Portal. This will be compared to, and integrated with, clinical data sets.
- In preparation for future projects to potentially engineer variants that have been prioritized in mouse models into rat models, we are also setting up a pipeline to phenotypically characterize the F344 rat model fAD at 3 & 6 months. This will include blood and tissue biomarkers as well as traditional measures of pathology.

GENERATION OF NOVEL MODELS

Because *APOE4* and *TREM2* alleles are the strongest genetic risk factors for LOAD, we have created a novel model expressing both human *APOE4* and the R47H allele of *Trem2*. This model is being phenotypically characterized, and will serve as the standard background as we introduce additional LOAD genetic variants such as *ABCA7*, *IL1RAP* and *CR1*. We have also generated other *Trem2* variants and a humanized *APOE3* variant to serve as a control, with a humanized *APOE2* in development.

We plan to include humanized wild-type *APP* and *tau* (*MAPT*) alleles in the future. Additional LOAD-associated genetic variants are being prioritized by the MODEL-AD Bioinformatics and Data Management Core (see poster W3 for details).

All novel models (~8/year) will be distributed to the research community from the JAX mouse repository. ***All models will be available for academic and for-profit use.***

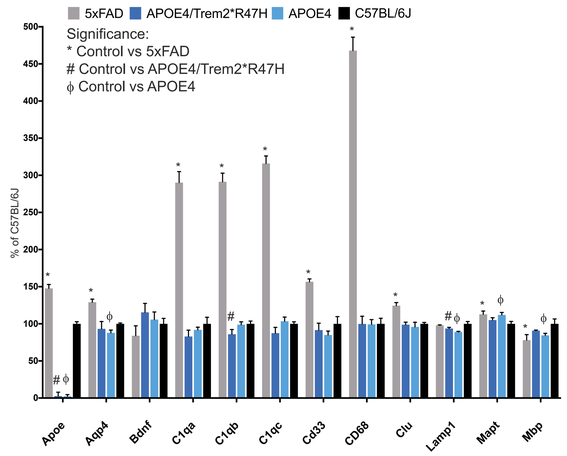
MODEL	JAX ID	STRAIN NOMENCLATURE
APOE4/Trem2^{*R47H}	28709	B6(SJL)-Apoe$^{tm1.1(APOE*4)Adiuj}$ Trem2^{em1Adiuj}/J
APOE4	27894	B6(SJL)-Apoe$^{tm1.1(APOE*4)Adiuj}$/J
APOE3	29018	B6(SJL)-Apoe$^{em2(APOE*)Adiuj}$/J
Trem2^{*R47H}	27918	C57BL/6J-Trem2^{em1Adiuj}/J
Trem2^{*Y38C}	29725	C57BL/6J-Trem2^{em3Adiuj}/J
Trem2 KO	27197	C57BL/6J-Trem2^{em2Adiuj}/J
Floxed Trem2	29853	B6(C3)-Trem2$^{tm1c(EUCOMM)Wtsi}$/AdiujJ

HIGH THROUGHPUT MODEL VALIDATION

Each year, 6 models will undergo phenotypic characterization at 12 months of age (3 models each at IU and JAX). This will include pathologic and biomarker assays, and transcriptomic analysis. Transcriptomics will be prioritized as we consider it to be a sensitive, non-biased method to validate animal models relative to clinical samples.

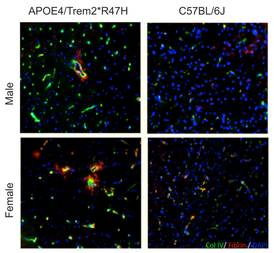
TRANSCRIPTOMICS

The nCounter Neuropathology panel (nanoString) was used to compare transcriptome changes in fAD (5XFAD) and LOAD (APOE4 and APOE4/Trem2^{*R47H}) models relative to a C57BL/6J control at a single age (8 months). This pilot data is from female mice only.

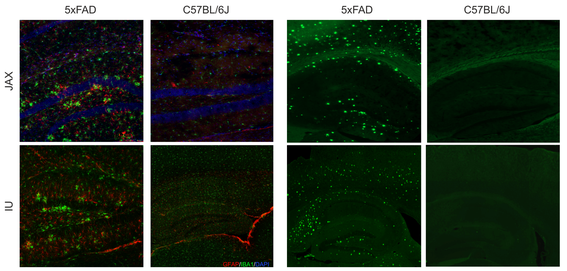


Significant differences between 5XFAD and control were detected in AD-related genes including *Apoe, Aqp4*, and *Mapt*, as well as neuroinflammation-related transcripts including *C1qa, C1qb, C1qc, Cd33,* and *Cd68*.

PATHOLOGY



Left: Representative images of APOE4/Trem2^{*R47H} tissue at 7-8 months of age. Col IV (green) staining of blood vessels; Fibrin (red) strains vascular leakage and DAPI (blue) as a nuclear counterstain. **Below**: Pipeline testing at two sites, using standardized protocols. **Below left**; GFAP (red) stains reactive astrocytes; IBA1 (green) stains microglia and DAPI (blue) stains nuclei, comparing neuroinflammation between 5xFAD and C57BL/6J controls. **Below right**: Thio S (green) stains amyloid beta plaques in 5xFAD and C57BL/6J controls.



COGNITIVE ASSAYS

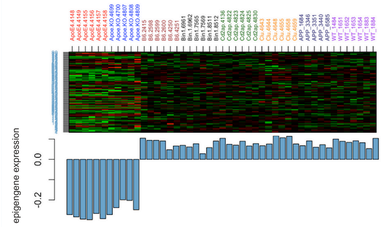
In our high-throughput screen, we will assess frailty, open field, and spontaneous alternation assays at 12 months of age.

DEEP PHENOTYPING

The most promising models, as based on the high-throughput screening at a single time point, will undergo a more detailed characterization pipeline. This will include second-site validation of the high-throughput assays and more detailed characterization at multiple ages (4, 8, and 12 months).

TRANSCRIPTOMICS

Pilot RNA-seq was performed on whole brain tissues from a panel of mice with AD-related genetic perturbations. Tissue from females at six months of age were collected from the following strains: APP/PS1, Apoe -/-, ApoE4 knock-in, Clu-/-, Bin1-/+, Cd2ap-/+, and two control cohorts (labeled B6 and WT). Data were co-normalized and corrected for batch effects with ComBat. Weighted gene correlation network analysis (WGCNA) was used to identify modules of co-expressed genes which were associated with strains that drive differential expression.

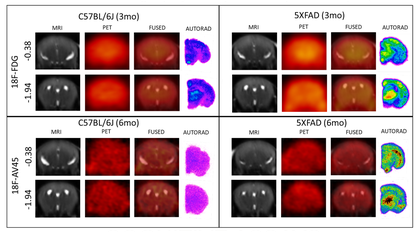


Heatmap and module eigengene expression values of the skyblue3 module
Skyblue3 module is a set of 90 genes that are differentially expressed in both ApoE-/- and ApoE4 strains. Other modules (not shown) identified additional effects with differential expression from a range of one to three strains.

We are now comparing these modules with homologous modules in the AMP-AD study of human cohorts. Although these samples were derived from relatively young mice, our results suggest AD-related molecular phenotypes will be readily observed in models derived from LOAD genetics.

IMAGING

Positron emission tomography (PET) and autoradiography validation studies in 5XFAD and wild-type control mice (C57BL/6J). The top two panels illustrate glucose uptake (2-^{18}fluoro-2-deoxyglucose; ^{18}F-FDG) in 3 month old wild-type (top left) and 5XFAD (top right) mice. Increased glucose metabolism in 5XFAD mice is observed compared to the age matched controls. Autoradiogrphy studies confirm increased glucose metabolism in 5XFAD mice. The bottom two panels are PET imaging studies using the radiolabeled tracer AV45 to label amyloid beta. Autoradiography studies were completed for validation. Increased AV45 binding is observed in the 5XFAD mice at 6 months of age compared to age-matched wild-type controls. (Resolution for PET imaging: 1 mm x 1 mm x 1 mm. Resolution for autoradiography: 25 µm x 25 µm x 20 µm)



COGNITIVE ASSAYS

In addition to assays run in the high-throughput screen, we also run prioritized models through episodic and novel spatial memory assays, as well as openfield and rotarod tests, and continuous activity monitoring.

PROTEOMICS & METABOLOMICS

Ongoing pilot studies are using proteomic and metabolomic assays to validate both existing fAD models and novel LOAD models.

In collaboration with an AMP-AD project led by Dr. Rima Kaddurah-Daouk at Duke, serum and brain metabolomes from both male and female fAD (5XFAD) and LOAD (APOE4/Trem2*R47H) models will be assayed at 12 months of age.

In collaboration with an AMP-AD project led by Dr. Nick Seyfried at Emory, brain proteomes from both male and female fAD (5XFAD) and LOAD (APOE4/Trem2*R47H) models will be assayed at 12 months of age.

The resulting data will be aligned to clinical data in order to identify expression modules specifically altered in LOAD.

HARMONIZATION OF MODEL AND CLINICAL RESULTS

Model validation assays have been designed to match to clinical assays where possible. This will enable direct comparison of models to the AMP-AD clinical populations that they are meant to represent, as both datasets are stored and analyzed in the AMP-AD knowledge portal.

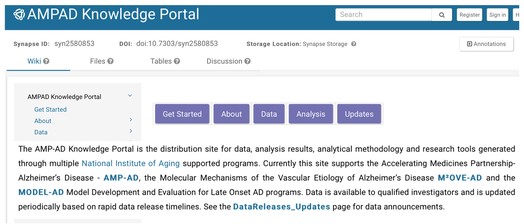
In the future, the pre-clinical testing core will use these same read-outs to determine efficacy of therapeutics, including some compounds that are currently in the clinic. In this manner we directly assay translational validity of models by comparing the same biomarkers in models and patients.

Assays	AMP-AD	MODEL-AD	MODEL-AD
		high throughput	deep phenotyping
		(12 months)	(4, 8, 12, 18 months)
Amyloid and tau pathology	✔	✔	✔
Neuroinflammation	✔	✔	✔
Synaptic and neuronal loss	✔	✔	✔
Biomarkers (Quanterix)	✔	✔	✔
Biomarkers	✔		✔
Transcriptomics (nanostring)	✔	✔	
Transcriptomics (unbiased)	✔		✔
Proteomics	✔		✔
Metabolomics	✔		✔
Imaging (FDG PET/MRI)	✔		✔
Imaging (Autorad/ISH)	✔		✔
Cognitive assays	✔	✔	✔
Single-cell transcriptomics	✔	pilot in progress	

Dark blue indicates identical assays; light blue indicates similar assays.

FOR FURTHER INFORMATION

- MODEL-AD: www.modelad.org
- JAX AD models: https://www.jax.org/alzheimers
- AMP-AD Knowledge Portal: www.synapse.org/ampad



Scan the QR code to visit the MODEL-AD website, which provides information on how to obtain mouse models, links to access model validation data along with accompanying protocols, along with news and updates.

ACKNOWLEDGEMENT

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