Model-AD: Standardized characterization of familial Alzheimer's disease models (5xFAD, 3xTg-AD, APP/PS1, and hTau)

Gareth Howell for the MODEL-AD Consortium

Stark Neurosciences Research Institute, Indianapolis, IN; The Jackson Laboratory, Bar Harbor, ME; Sage Bionetworks, Seattle, WA

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

ABSTRACT

To date, Alzheimer's disease (AD) is the most common form of dementia that has no effective treatment. Over the past 20 years researchers have used animal models to further understand the etiology of AD, and determine the pathways involved in this neurogenerative disease. While these animal models have proved fruitful in the understanding of some of the hallmark features associated with AD, they have not demonstrated predictive translatability for AD. The Model Organism Development and Evaluation of Late-onset Alzheimer's Disease (MODEL-AD) Center was established to develop, validate, and distribute novel mouse models for LOAD, with the aim to aid in the development of novel therapeutics for AD. MODEL-AD is a consortium involving Indiana University, The Jackson Laboratory, University California-Irvine, and Sage Bionetworks. To validate our characterization paradigm and preclinical pipeline for new models, we have utilized familial AD (fAD) models. Our phenotyping pipeline includes functional assays, in vivo MRI and PET imaging, transcriptomics, biochemistry, and neuropathology. Mice were assessed using a battery of functional assays and in vivoimaging performed at a variety of ages ranging from 4 mos to 14 mos (the timeframe when the majority of reported phenotypes occur in these strains). Tissue was then harvested and transcriptional profiling and neuropathology of the brain assessed in both male and female mice as follows. One brain hemisphere was frozen, and the other hemisphere fixed. Transcriptional profiling was performed on the frozen portion, while biochemical and neuropathological analysis was carried out on the fixed hemisphere. To assess AD relevant changes, we used antibodies against the following proteins: 22C11 (APP); 6E10, 4G8 (Aβ); Tau-46 (pan-tau); CP13, PHF1 (phospho-tau); NeuN, NFL, MBP, SYN and PSD-95 (neurons); GFAP, COL4 (astrocytes); IBA1, CD68, TREM2 (microglia, monocytes); PDGFRB (pericytes); and Fibrin, Albumin (vascular integrity). Transcriptional profiling was used to stage the progression of AD in fAD models and to compare the differentially expressed genes/pathways to those observed in human AD. This allows us to directly determine human relevance in each of these models. MODEL-AD is making all mouse models, protocols, and data sets widely available through a variety of resources including JAX mice, clnical and research services and the AMP-AD knowledge portal (Synapse). We welcome input and collaboration from the scientific community. For more information see www.model-ad.org.

METHODS

Mice will be assessed for biochemistry, histology, transcriptional profiling and *in vivo* imaging. For biochemistry/histology, mice will be sacrificed at designated time points, perfused, and tissue harvested. Half of the brain will be frozen and the other half fixed. For biochemistry, we will assess AD relevant changes in tissue samples using antibodies against the following proteins: 22C11 (APP); 6E10, 4G8 (Aβ antibodies); Tau-46 (pan-tau); CP13, PHF1 (phospho-tau); NeuN, NFL, MBP, SYN and PSD-95 (neurons); GFAP, COL4 (astrocytes); IBA1, CD68, TREM2 (microglia, monocytes); PDGFRB (pericytes); and Fibrin, Albumin (vascular integrity). We will use RNA-seq to stage AD in existing models. Multiple RNA profiling datasets exist for human AD and therefore RNA-seq of mouse models will provide a valuable tool to directly assess human relevance. Models will undergo in vivo MRI and PET scanning as well as secondary validation with autoradiography studies.

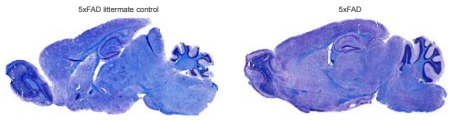
STRAINS ASSESSED

Common Name	Strain Nomenclature	JAX ID
5xFAD	B6.Cg-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax	8730
APP/PS1	B6.Cg-Tg(APPswe,PSEN1dE9)85Dbo/Mmjax	5864
3xTG-AD	B6;129-Tg(APPSwe,tauP301L)1Lfa Psen1^{tm1Mpm}/Mmjax	4807
hTau	B6.Cg-Mapt$^{tm1(EGFP)Klt}$Tg(MAPT)8cPdav/J	5491

5xFAD model

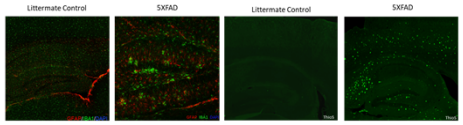
5XFAD transgenic mice overexpress both mutant human APP(695) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) Familial Alzheimer's Disease (FAD) mutations and human PS1 harboring two FAD mutations, M146L and L286V. Expression of both transgenes is regulated by neural-specific elements of the mouse Thy1 promoter to drive overexpression in the brain.

Cresyl Violet and Luxol Fast Blue



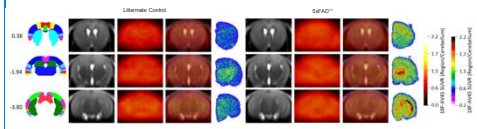
Tissue sections are stained with cresyl violet and counterstained with luxol fast blue to observe the white matter. Sections were then scanned using a Leica Aperio Versa system and uploaded to an eSlide manager for analysis.

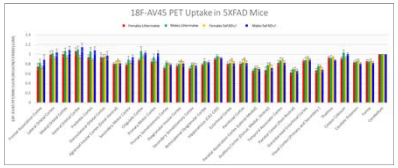
Immunohistochemistry

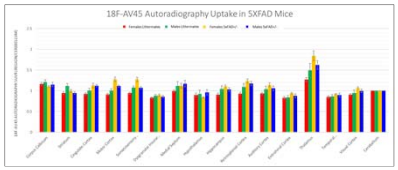


Immunohistochemical staining from 8 month old mice. GFAP (red) stains reactive astrocytes; IBA1 (green) stains microglia and DAPI (blue) stains nuclei, comparing neuroinflammation between 5xFAD and C57BL/6J controls. Thio S (green) stains amyloid beta plaques in 5xFAD and littermate controls.

MRI, PET and Autoradiography



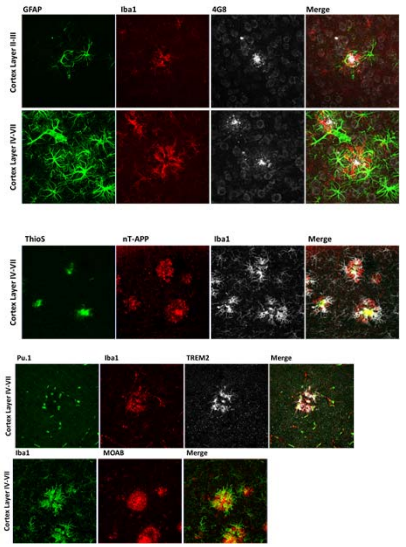




All PET scanning (15 min/ea.) was performed on the IndyPET3 scanner and post mortem brains were extracted and frozen for autoradiography (Autorad). MRIs were acquired (10 min/ea.) on a Siemens 3T Prisma scanner outfitted with a 4 channel phased array head coil. PET/MRI images were co-registered to Paxinos-Franklin atlas and 27 brain regions were extracted. For Autoradiography, frozen brains were sectioned at 20 um in sextuplet at 3 bregma targets, exposed on phosphor plates, scanned and manually segmented for 16 brain regions. Increased uptake is observed in the 5xFAD+/- mice compared to littermate controls.

APP/PS1 model

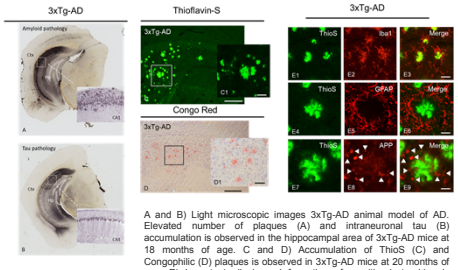
Double transgenic mice express a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9) both directed to CNS neurons. The "humanized" Mo/HuAPP695swe transgene allows the mice to secrete a human A-beta peptide.



Immunohistochemistry from 4 month old mice.
Top: Immunofluorescence staining for astroglia (GFAP), microglia, and A-beta plaques. Note the differential expression of these cells types in the superficial vs. deep layers.
Middle: Immunofluorescence staining for A-beta plaques and microglial cells.
Bottom: Immunofluorescence staining for IgG, microglia and Trem2.

3xTg-AD model

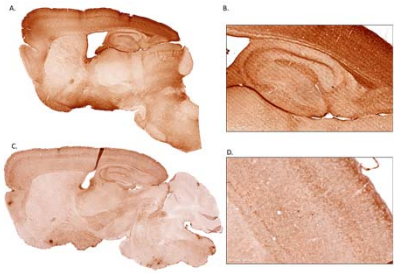
Mice are homozygous for all three mutant alleles (3xTg-AD; homozygous for the Psen1 mutation and homozygous for the co-injected APPSwe and TauP301L transgenes (Tg(APPSwe,tauP301L)1Lfa)) display no initial gross physical or behavioral abnormalities. Characterization of this mouse indicates a progressive increase in amyloid beta peptide deposition, with intracellular immunoreactivity as early as 3-4 months. Synaptic transmission and long-term potentiation are impaired at 6 months of age. Between 12-15 months aggregates of conformationally altered and hyperphosphorylated tau are detected in the hippocampus.



A and B) Light microscopic images 3xTg-AD animal model of AD. Elevated number of plaques (A) and intraneuronal tau (B) accumulation is observed in the hippocampal area of 3xTg-AD mice at 18 months of age. C and D) Accumulation of ThioS (C) and Congophilic (D) plaques is observed in 3xTg-AD mice at 20 months of age. E) Important gliosis and formation of neuritic dystrophies is observed in the hippocampus of 3xTg-AD at 20 month of age. Hp: Hippocampus; Ctx: Cortex. Scale bar: 200 μm (C and D), 50 μm (C1 and D1), 22.62 μm (E1 and E9).

hTau model

Mice express no endogenous mouse MAPT and all six isoforms (including both 3R and 4R forms) of human MAPT are expressed. Hyperphosphorylated MAPT is detected in cell bodies and dendrites by three months of age. Paired helical filaments of aggregated insoluble MAPT can be isolated from brain tissue as early as two months of age.



Immunohistochemistry from 6 month old mice.
A: Immunohistochemistry staining for AT8 in an hTau mouse at 12 mo of age. Panel shows the distribution of hyperphosphorylated tau at Ser202/Thr205.
B: Hyperphosphorylated Tau throughout the hippocampus and dentate gyrus.
C: Immunohistochemistry staining for AT180 in ah hTau mouse at 12 mo of age. Panel shows the distribution of hyperphosphorylated Tau at Thr231.
D: Hyperphosphorylated tau is observed throughout the cortex, but is more pronounced in cortical layers II/III and VI.

CONCLUSIONS

- All models, protocols, and data sets will be made widely available to researchers. We seek input and collaborations from research and pharma/biotech communities. For more information see www.model-ad.org.

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

ACKNOWLEDGEMENT

MODEL-AD Centers were established with funding from The National Institute on Aging (U54 AG054345; U54 AG054349).

NIH National Institute on Aging