Characterizing the APOE4/Trem2^{R47H} Mouse Model for Late Onset Alzheimer's Disease

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

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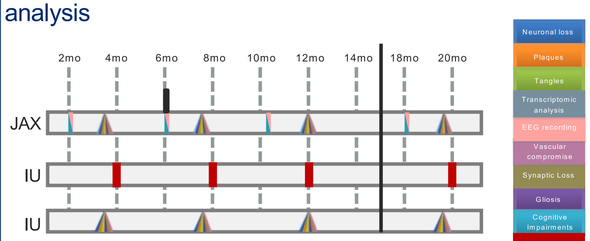
ABSTRACT

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder. More than 46 million people are affected worldwide, with no effective treatment currently available. Research into fully understanding the disease has been fueled by animal models with familial AD (fAD) mutations, which accounts for 2-5% of the AD population. While these models have proved fruitful in understanding some of the baseline endophenotypes of AD, they have not been adequate to develop therapeutics or fully elucidate the processes leading to AD. One component of this lack of an effective therapeutic is due to the inability to fully reproduce an AD phenotype in these animals.

Here, the MODEL-AD consortium presents a new mouse model for Late Onset Alzheimer's disease (LOAD). We have developed a model carrying the two highest genetic risk factors for LOAD, the most common and strongest risk factor, *APOE4*, and the R47H allele of *Trem2*. To characterize this model, we have looked at three time points to understand the aging phenotype of these *APOE4/Trem2**R47H mice.
At these three time points, mice were exposed to a battery of behavioral assays, consisting of continuous activity monitoring, frailty assessment, open field, grip strength, spontaneous alternation, rotarod, delayed spatial novelty, and episodic memory. Once completed, brains are removed and assessed. We are primarily looking for hallmark signs of AD, such as beta amyloid plaques and neurofibrillary tangles, as well as levels of neuroinflammation, vascular compromise, and neuronal morphology/counts.
The development of this new model will enable us to gain a deeper understanding into the two of the genetic factors contributing to LOAD. Our goal is that the new models will lead to better therapeutic testing as well as determining if any preventative actions can be taken to impede the onset of AD. For more information see www.model-ad.org .

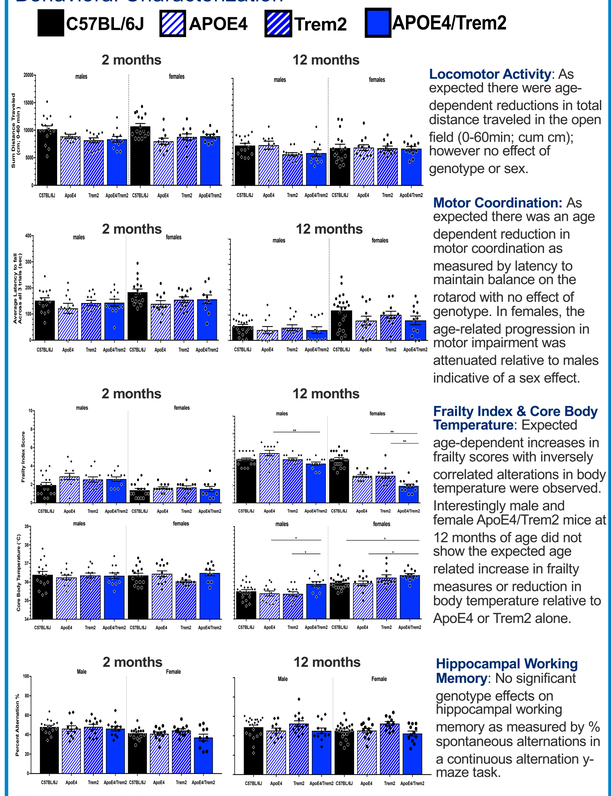
Deep phenotyping testing paradigm and histological analysis



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

Behavioral Characterization



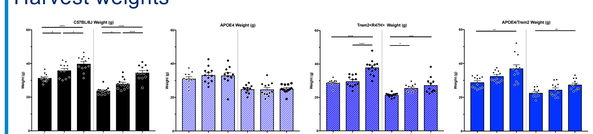
Locomotor Activity: As expected there were age-dependent reductions in total distance traveled in the open field (0-60min; cum cm); however no effect of genotype or sex.

Motor Coordination: As expected there was an age dependent reduction in motor coordination as measured by latency to maintain balance on the rotarod with no effect of genotype. In females, the age-related progression in motor impairment was attenuated relative to males indicative of a sex effect.

Frailty Index & Core Body Temperature: Expected age-dependent increases in frailty scores with inversely correlated alterations in body temperature were observed. Interestingly male and female ApoE4/Trem2 mice at 12 months of age did not show the expected age related increase in frailty measures or reduction in body temperature relative to ApoE4 or Trem2 alone.

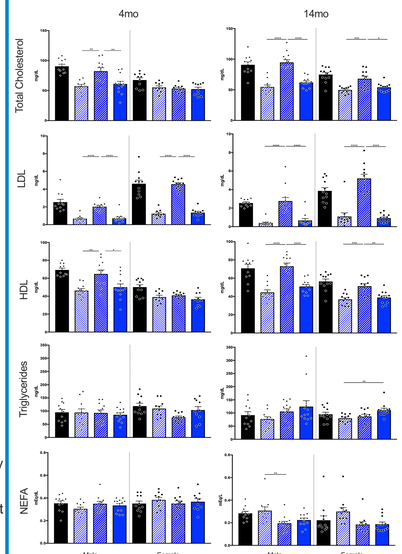
Hippocampal Working Memory: No significant genotype effects on hippocampal working memory as measured by % spontaneous alternations in a continuous alternation y-maze task.

Harvest weights



At harvest, each mouse is weighed. As expected, C57BL/6J mice gain weight as they age. Whereas the APOE4 mice do not appear to gain weight through age.

Blood biochemistry



Non-fasted blood was taken at harvest. Serum was separated and profiled for various cholesterol fractions.

Total cholesterol: Levels were seen to be significantly reduced in ApoE4 carrying mice in both males and females. This reductions was seen at 4 and 14mo.

LDL: LDL levels were significantly reduced in the ApoE4 carrying mice at both time points.

HDL: A significant reduction in HDL is seen in male mice at 4mo. At 14mo, a significant reduction of HDL was seen in both sexes.

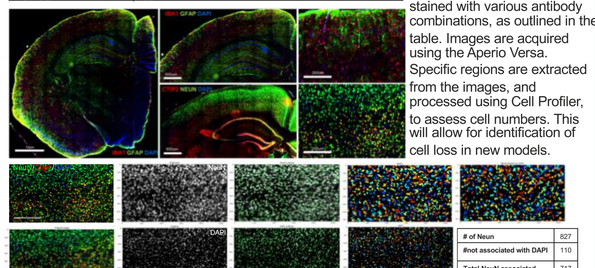
Triglycerides: No significant differences were seen in triglyceride levels at 4mo of age. At 14mo of age, triglyceride levels were significantly increased in comparison to ApoE4 in females.

Non-Essential Fatty Acids (NEFA): At 4mo of age levels remained consistent across all genotypes. At 14mo Trem2 males had significantly reduced NEFA levels in comparison to ApoE4 alone.

Histological imaging and analysis methods

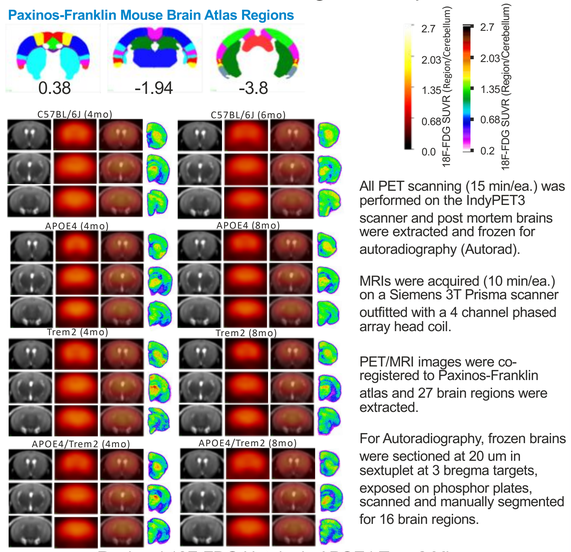
Marker	Target
Neun/Ctip2	**Neurons/Excitatory neurons**
GFAP/Iba1	Reactive astrocytes/microglia-monocytes/Astrocytes
X-34/Lamp1/Iba1	Plaques/Dystrophic neurites/Microglia-monocytes
CD31/Fibrin/Iba1	Vessels/Vascular leakage/Microglia-monocytes
LFB/Cresyl Violet	Gross morphology and myelin
AT8/H&E	pTau

Upon harvest, brains are removed, weighed, and hemispheres are separated. One hemisphere is fixed, cryopreserved, and sectioned in series at 20µm. Sections are stained with various antibody combinations, as outlined in the table. Images are acquired using the Aperio Versa. Specific regions are extracted from the images, and processed using Cell Profiler, to assess cell numbers. This will allow for identification of cell loss in new models.



# of Neun	827
#not associated with DAPI	110
Total NeuN associated with DAPI	717

MR/PET: 18F-FDG to assess glucose uptake

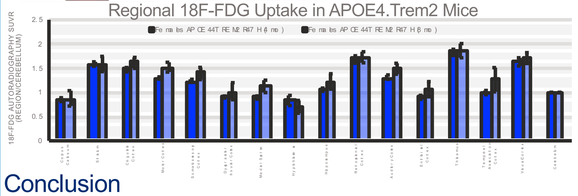


All PET scanning (15 min/ea.) was performed on the IndyPET3 scanner and post mortem brains were extracted and frozen for autoradiography (Autorad).

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.



Conclusion

- Behavioral characterization revealed the expected age-dependent alterations across phenotypes with no effect of genotype up to 12 months of age which would be anticipated in a model of LOAD. Further testing at 18mo of age is in progress.
- While there are no gross statistical significances between regions at, and between, 4 and 8mo in PET and AutoRad, further analysis continues for additional time points.
- *In vivo* imaging, transcriptomic, histological, and biochemical analysis continues on all time points.

Acknowledgments

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