NOVEL APOE4.TREM2*R47H MOUSE MODEL: BUILDING A BETTER TOOL FOR LATE-ONSET ALZHEIMER'S DISEASE

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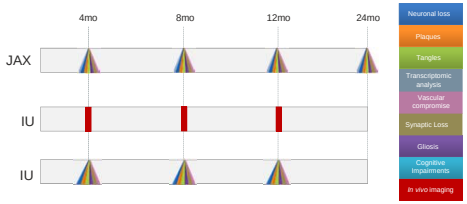
MODEL-AD
Model Organism Development & Evaluation for Late-Onset Alzheimer's Disease

ABSTRACT

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder. More than 46 million people are affected worldwide, with no effective treatment currently available. Of this AD population, those suffering from late-onset Alzheimer's Disease (LOAD) represent the largest subset of dementia patients. Unfortunately, current animal models do not fully recapitulate LOAD, thus are not ideal for therapy development. To address this, the Model Organism Development and Evaluation for Late-onset AD consortium (MODEL-AD) has created a novel LOAD mouse carrying two common risk alleles, human *APOEε4*, and the R47H allele of *Trem2*. Characterization of this model at young and advanced ages will reveal more appropriate disease mechanisms useful in the treatment of LOAD. This model will also serve as a backbone strain for the further addition of risk alleles identified from human LOAD patients to more properly represent the heterogeneity of the disease and better align phenotypes in the mouse to outcomes observed in the human patient population.

METHODS

Humanized *APOEε4* and the *Trem2*R47H* mutation, two prominent genetic risk factors for LOAD, were inserted into the genome of C57BL/6J mice to create the B6.APOEε4.Trem2*R47H (B6J.hAT) model. Cohorts of B6J.hAT (with controls) were established at JAX and IU and aged to 2, 6, 12, and 22 months prior to behavioral testing. At the JAX campus, *in vivo* behavior and wellness assays measured activity (open field), frailty, grip strength, coordination (rotarod), and cognitive ability (spontaneous alternation). Repeated congruently at Indiana University (IU) facilities were all behavior and physical phenotyping. Additionally, *in vivo* MRI and PET studies using [18F]-FDG and [64Cu]-PTSM were completed at IU. Post-mortem analyses at both sites included blood chemistry, brain immunohistochemistry, transcriptomics, and proteomics. Analyses of subsequent RNA sequencing data in combination with behavioral and molecular phenotypes has yielded additional insight into the transcriptional differences influencing animal health.



RESULTS

Over 24 months, B6J.hAT mice displayed progressively increasing frailty index scores, a measure of aging and vulnerability, and decreased survival. Similarly, decreases over time were observed in coordination (rotarod) and locomotion (open field) assays, however working memory (spontaneous alternation) appeared intact. B6J.hAT and B6J.APOEε4 mice showed decreased levels of total cholesterol, LDL, and HDL at multiple time points compared to control mice - an effect of human *APOE* alleles reported previously. PET data showed changes in glucose uptake over time in B6J.hAT mice, but MRI showed no gross abnormalities of the brain. RNA sequencing revealed age- and sex-related changes in B6J.hAT brain samples compared to controls. Differentially expressed genes were present in pathways central to AD pathogenesis, including metabolism, immunity, and mRNA/protein processing. These expression changes appear to be most prominent in mice expressing *Trem2*R47H*. We also observed a novel splicing event in *Trem2* due the R47H mutation.

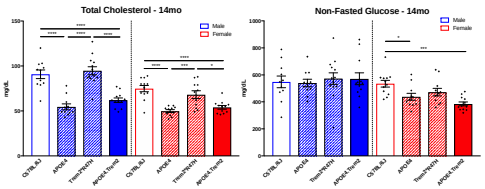


Figure 1. Blood biochemistry profiling. Non-fasted blood was taken at harvest from mice receiving normal diet. Serum was separated and profiled for glucose and various cholesterol fractions. (*p>0.05; **p>0.01; ***p>0.001; ***p>0.0001)

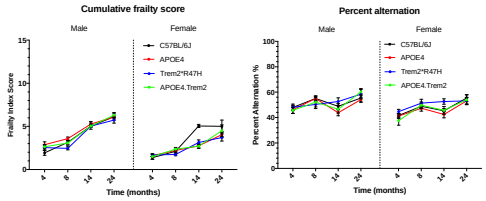


Figure 2. Assessment of frailty and working memory. Health assessment, measured by frailty assay (left), and hippocampal working memory, as measured by percent spontaneous alternations in a continuous alternation y-maze task (right).

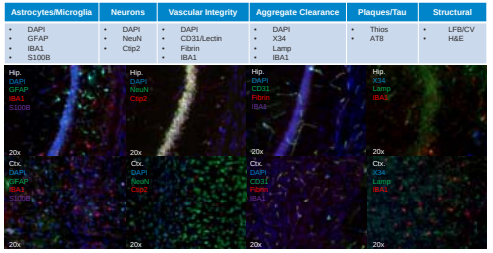


Figure 3. Immunohistochemistry pipeline. Brain hemisphere from a 9 month-old APOE4.Trem2*R47H female sectioned in series at 25µm was stained with various antibody combinations, as outlined in the table above. (Hip.:hippocampus; Ctx.:cortex)

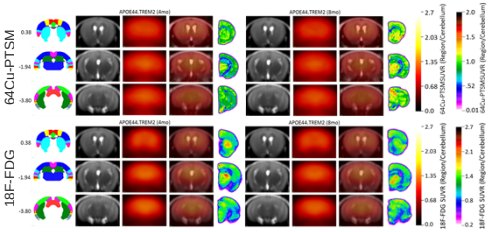


Figure 4. Patterns of cerebral perfusion and glycolytic metabolism in the APOE4, Trem2*R47H, and APOE4.Trem2 mouse models. PET/MRI images were co-registered to Paxinos-Franklin atlas and 27 brain regions were extracted. Representative autoradiography and MRI images, with regional heatmaps shown.

	Male						Female					
	4 months		8 months		14 months		4 months		8 months		14 months	
	UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
APOEε4	0	2	41	99	0	1	0	1	9	12	1	1
Trem2*R47H	11	49	4	25	125	69	6	18	4	9	60	58
APOE4.Trem2	12	7	5	26	0	2	2	2	2	2	2	11

Table 1. Differentially expressed genes across strains. RNAseq analysis of brains from 4-, 8-, and 14-month-old APOEε4, Trem2*R47H, and APOE4.Trem2 mice. Total number of genes significantly different from C57BL/6J control mice shown.

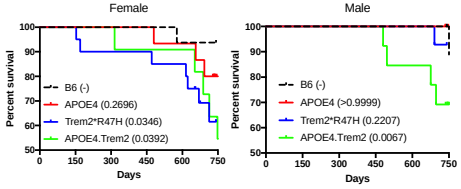


Figure 5. Differences in strain survival to 24 months. Ethical endpoints included for individuals meeting IACUC euthanasia criteria. Statistical p values represented in legend, relative to C57BL/6J.

CONCLUSIONS

The MODEL-AD consortium has established a new mouse strain to study the effects of two strong risk factors for LOAD. Characterization of this model is ongoing and will serve as a backbone strain for the further addition of LOAD risk alleles to more closely align phenotypes in the mouse to outcomes observed in human AD (see poster #P2-133, "Initial Characterization of Novel Mouse Models of Late Onset Alzheimer's Disease Based on Human Genetic Associations." South Hall GH, July 15th, 2019).

- ***APOEε4* allele mice showed expected aberrant cholesterol levels.**
- **Age-dependent increases in frailty across all strains, however no significant differences in working memory.**
- **Phenotypic changes in both brain perfusion and metabolism by 8 months**
- **Gene enrichment of multiple AD-related pathways**
- **Decreased survival of B6J.hAT mice at 24 months**
- ***In vivo* imaging, transcriptomic, histological, and biochemical analysis continues on all time points**

For further 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
- Poster #P4-091, "The Trem2*R47H Variant Alters Expression and Function in Mouse Models of Alzheimer's Disease." South Hall GH, July 17th, 2019
- Poster #P4-098, "Transcriptomic Alterations Driven By the Trem2*R47H Allele Vary across Different Transgenic Mouse Models." South Hall GH, July 17th, 2019

Acknowledgements

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