473.05

Multi-phenotype comparison of an *APOE4.Trem2*^{R47H} mouse model and human late-onset Alzheimer's disease

Gregory W. Carter for the MODEL-AD Consortium

Indiana University, Indianapolis, IN; The Jackson Laboratory, Bar Harbor, Maine 04609; University of Pittsburgh, Pittsburgh, PA; Sage Bionetworks, Seattle, WA

MODEL-AD

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

ABSTRACT

Current animal models do not fully recapitulate late-onset Alzheimer's Disease (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. Characterization of this model at young and advanced ages will indicate more appropriate disease mechanisms useful in the treatment of LOAD.

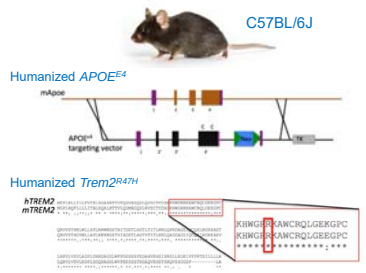
Humanized *APOEe4* and the *Trem2*^{R47H} mutation, two prominent genetic risk factors for LOAD, were inserted into the genome of C57BL/6J mice to create the B6.APOEe4.Trem2^{R47H} (B6J.hAT) model. Cohorts of B6J.hAT (with controls) were established at JAX and IU and aged to 2, 6, 12, and 24 months. At JAX, in vivo behavior and wellness assays measured activity, frailty, grip strength, coordination, and cognitive ability. At IU, MRI and PET studies using [18F]-FDG and [64Cu]-PTSM were completed. Post-mortem analyses at both sites included blood chemistry, brain immunohistochemistry, transcriptomics, metabolomics, and proteomics. Data from 'omics experiments were systematically compared to analogous data from the Accelerating Medicines Partnership for Alzheimer's Disease (AMP-AD) human disease cohorts.

By 12 months, female B6J.hAT mice displayed reduced frailty index scores, a measure of aging and vulnerability, suggesting a protective phenotype. B6J.hAT and B6.APOEe4 mice showed decreased levels of total cholesterol, LDL, and HDL at multiple time points - an effect of the *APOEe4* allele that has been reported previously. We also observed a novel splicing event in mice expressing the *Trem2*^{R47H} variant and approximately 50% reduction in *TREM2* expression. Global transcriptomics revealed age-related changes in B6J.hAT brain samples compared to controls. Differentially expressed genes were enriched in multiple AD-related pathways including immune response, splicesome, and osteoclast differentiation. Proteomic and metabolomics assays further align effects in the mouse models with a subset of changes observed in human post-mortem samples. We observed sex-specific effects from the *APOEe4* allele that generally aligned with LOAD in women.

The MODEL-AD consortium has established a new mouse strain to study the effects of two strong risk factors for LOAD that affect systems-level measures associated with human disease. This model serves 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.

MODELING THE GENETICS OF LOAD

Humanized *APOEe4* 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.APOEe4.Trem2^{R47H} (**B6J.hAT**) model.

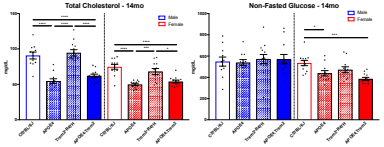


DEEP PHENOTYPING TO CHARACTERIZE NEW MOUSE MODELS

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.

BLOOD CHEMISTRY: B6.hAT MICE HAVE REDUCED CHOLESTEROL

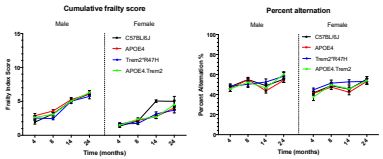
B6J.hAT and B6J.APOEe4 mice showed decreased levels of total cholesterol, LDL, and HDL at multiple time points compared to control mice - an effect of human APOE alleles reported previously.



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)

BEHAVIOR: B6.hAT MICE BECOME FRAIL BUT REMAIN CONGITIVELY INTACT

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.



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).

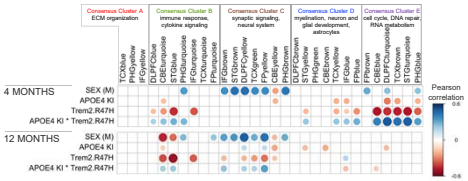
TRANSCRIPTOMICS: GENE EXPRESSION CHANGES ALIGNED WITH HUMAN LOAD

Nanostring Mouse AD Panel analysis (Pandey, *et al*., bioRiv 682856) and RNA sequencing revealed consistent age- and sex-related changes in B6J.hAT brain samples compared to B6 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, see adjacent **Poster 473.06**.

Gene expression differences were systematically compared to the 30 AMP-AD modules derived from multi-cohort meta-analysis (Logsdon, *et al.*, bioRxiv 510420). For mouse cohorts aged 4 and 12 months, we performed linear regression analysis to estimate effects from sex, *APOEe4* genotype, *Trem2*^{R47H} genotype, and the interaction between these two alleles. For each gene, we fit the model:

$$\log(expr) = \beta_0 + \beta_{male} + \beta_{APOEe4} + \beta_{Trem2R47H} + \beta_{A*T} + \varepsilon$$

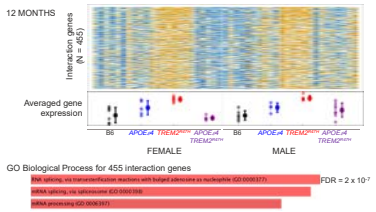
where $A * T$ denotes the interaction term. To determine similarity in expression with the 30 LOAD modules, we computed the correlation of (i) human log fold change for AD cases versus controls, and (ii) the β coefficient from the linear model, across all resident module genes.



Mouse-human transcriptome alignment. Pearson correlation between linear model factors (above) and log fold change for human cases versus controls, across all module genes. Blue circles denote positive correlation, red circles negative, and only correlations with p < 0.05 are shown.

GENETIC INTERACTION AFFECTS SPLISEOSOME GENES

We found 455 genes with significant interaction ($p < 0.05$) between *APOEe4* and *Trem2*^{R47H}, in which up-regulation by *Trem2*^{R47H} was suppressed by the *APOEe4* genotype.



This gene set was enriched in spliceosome components, suggesting broad differential splicing for *Trem2*^{R47H} that is *APOE*-dependent.

TRANSCRIPTOMIC CHANGES ARE ASSOCIATED WITH IMAGING PHENOTYPES

For data integration with cerebral blood flow and glucose metabolism in these mice, see adjacent **Poster 473.04**.

FUTURE PLANS

B6.APOE4, B6.TREM2^{R47H}, and B6.hAT mice are being assayed for brain and serum metabolomics and proteomics to identify multi-omic signatures potentially informative for LOAD.

We are currently generating additional late-onset AD risk variants on the B6J.hAβ/hAPOE4/Trem2*R47H background. In the near future we expect to add a humanized Tau (*MAPT*) allele.

In an attempt to shift phenotypes toward a neurodegenerative phenotype, some models will be assayed after aging on a high fat diet (45% fat as compared to standard 6% fat chow).

We will correlate disease phenotypes to differences in microbiome by comparing microbiomes across models/genotypes; ages; sexes; and sites.

Mice are now aging to 24 months for late phenotyping.

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.

- **APOEe4 allele mice showed expected aberrant cholesterol levels**
- **Age-dependent increases in frailty across all strains, however no significant differences in working memory**
- **Gene expression modifications in multiple AD-related pathways**
- **Genetic interaction of *APOEe4* and *Trem2*^{R47H} affecting spliceosome genes**
- **In vivo imaging, transcriptomic, histological, and biochemical analysis continues on all time points**

FURTHER INFORMATION

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

ACKNOWLEDGEMENTS

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