Aging × Genetics × Environment: Characterization of Precision Disease Models for Preclinical Testing for Late-onset Alzheimer's disease

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

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INTRODUCTION

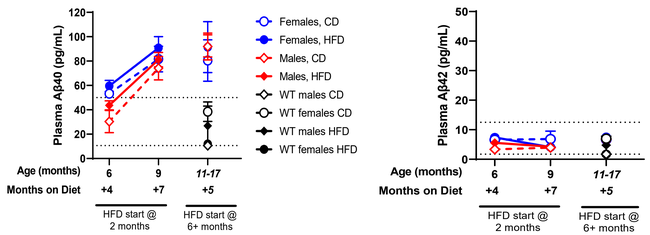
- The ability to effectively translate therapeutic efficacy from the bench to clinical success for Alzheimer's disease (AD) has been hampered in part due to limited recapitulation of the complexity of the disease in animal models. While analogous AD risk mutations have been engineered into animal models and have dominated the research field, these have primarily been familial, early onset risk alleles which do not capture the risk for AD for the majority of patients that present with sporadic late onset AD (LOAD).
- The IU/JAX/PITT MODEL-AD consortium is focused on developing mouse models with genetic risk variants associated with LOAD, in combination with environmental risk factors and aging to enable improved translation. The present studies aimed to characterize mice expressing humanized Aβ in combination with multiple genetic risk factors (APOE4 and the R47H risk variant in the Trem2 gene; LOAD2) and aged in the presence of a high-fat, high-sugar diet (HFD).

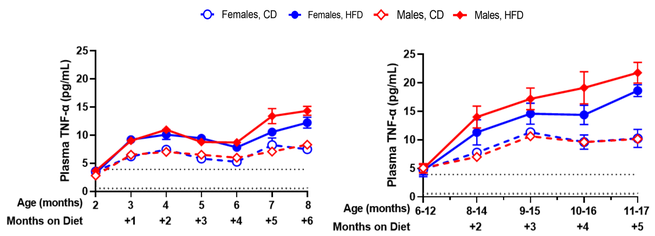
METHODS

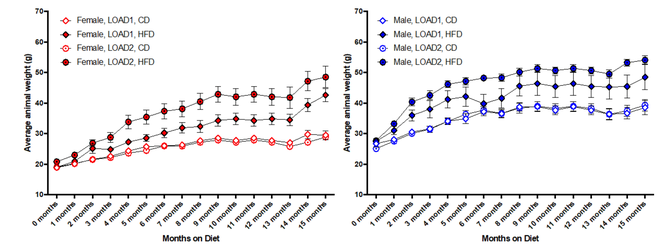
- Initially, alleles were created using CRISPR to knock-in risk variants to the mouse locus on a sensitized C57BL/6J genetic background expressing APOE4 and Trem2^{R47H} ("LOAD1"; see Kotredes et al, 2021, *PMID: 34707490*). Later models were made on the same background with a humanized Aβ1-42 region ("LOAD2"). The LOAD2 mice are congenic on a C57BL/6J background and available at The Jackson Laboratory without restrictions (JAX stock# 030670).
- To investigate the role of high fat diet (HFD) exposure on exacerbating the manifestation of AD related pathologies on aging and genetic risk, we provided HFD ad libitum (Research Diets, Inc. catalog# D12451i; 45% fat) using two separate paradigms: beginning at 2 months of age following weaning (adolescent HFD, aHFD) as well as beginning later in life (middle aged, at 6-12 months; mHFD).

RESULTS

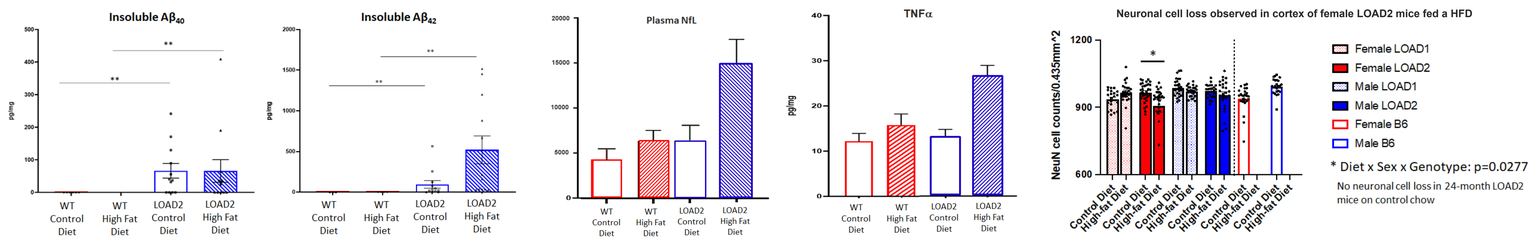
Trajectory of HFD × Aging in LOAD2 mice



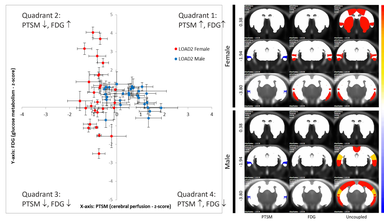




Characterization of LOAD2 mice exposed to a High Fat Diet from early adolescence reveals emerging AD-related pathologies from 12+ months of age



Vascular and Perfusion Changes

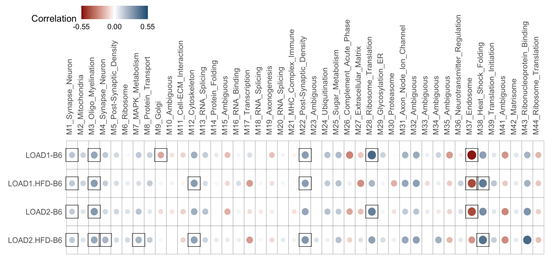


- Left: Uncoupling analysis of 28 brain (56 bilateral) regions in male (blue) and female (red) mice at 12 months control diet (reference) compared to HFD.
- Right: Univariate statistical p-value maps for females (top) and males (bottom) at bregma 0.38, -1.94, and -3.80 mm for 64Cu-PTSM (left column), 18F-FDG (middle column), and uncoupling (right column).
- For uncoupling plots, data are presented as mean±StDev; for p-value maps, color indicates the direction of the change for 64Cu-PTSM, 18F-FDG, and Uncoupling.

Touchscreen task sensitive to detecting Cognitive Changes

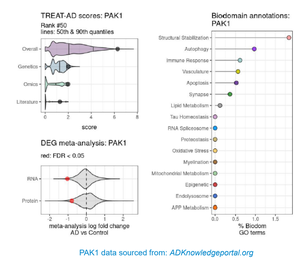
- The location discrimination task (LD) training and testing protocol was similar to the methods of *Oomen et al 2013 Nat Prot.*
- Mice began initial training at 12-18 months of age using Lafayette Instruments Touchscreen systems.
- Subjects were food restricted to 80% of free-feeding body weight. A 10% sucralose solution was used as the reinforcer.
- Technicians were blind to genotype. An n=4 per sex per genotype per condition (control diet v high fat diet) were evaluated.
- There was a significant diet x genotype effect. LOAD2 mice demonstrated impaired acquisition of the task relative to WT controls. HFD mice (both WT and LOAD2) failed to meet advancement criteria for task acquisition to advance to pattern separation assessment.

Brain proteomic analysis alignment to human co-expression modules



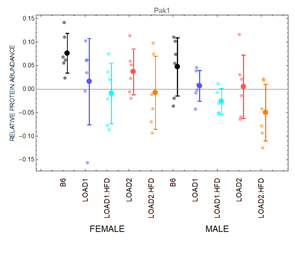
- Tandem Mass Tag (TMT) proteomics analysis of LOAD1 (lacking hAβ) and LOAD2 on control and HFD in comparison to C57BL6/J mice at 18 months of age.
- Blue dots indicate differential expression in the same direction as postmortem brain results using identical assays.
- Red dots indicate anti-correlated expression.
- Boxes indicate statistical significance at P<0.05.
- Human modules from Johnson ECB, et al, *Nat Neuro*, 2022.

Precision Medicine Approach: Mapping Mechanism to LOAD Models



PAK1 data sourced from: *ADKnowledgeportal.org*

- p21-activated kinase 1 (PAK1) is an AMP-AD nominated target
- PAK1 is down-regulated in AD patients relative to case controls at both the transcriptomic and proteomic level
- The Emory/Sage/SGC TREAT-AD Center is interested in developing tool compounds that modulate PAK1.
- In collaboration with TREAT-AD we evaluated the proteomics signatures of MODEL-AD mice to identify a mouse model that best recapitulates the omics signature of AD patients as an ideal model system for in vivo target engagement studies of PAK1 modulators



SUMMARY & CONCLUSIONS

- The biological construct of aging is an essential component for the study of Alzheimer's disease. Our MODEL-AD approach is to incorporate genetic risk for LOAD, aging, and environmental risk to improve translational relevance of mouse models for the study of AD.
- LOAD2 mice exposed to HFD from adolescence (LOAD2+HFD) demonstrated aging changes relative to LOAD2 mice in the absence of HFD, including presentation of insoluble Aβ42 in brain and plasma, and increased inflammatory cytokines. 12-month aged LOAD2+HFD mice also demonstrated increased NfL in CSF, as well as vascular and perfusion changes as measured by MRI. By 18 months, LOAD2+HFD mice demonstrated reductions in hippocampal neurons as well as cognitive impairment relative to LOAD2 mice in the absence of HFD on a translational touchscreen task. Intriguingly, gene expression profiles and proteomic signatures of aged LOAD2+HFD mice aligned with 'omics signatures of AD patients in the absence of core neuritic plaques, which were not detected up to 24 months of age.
- Mice with genetic risk for LOAD coupled with environmental risk factors demonstrate aging-dependent changes in line with a spectrum and trajectory of features of clinical LOAD. From a precision medicine approach, our MODEL-AD Preclinical Testing Core and our TREAT-AD colleagues are prioritizing LOAD2+HFD mice as an important model system for evaluating the therapeutic potential of non-amyloid targeting therapeutics as well as for prophylactic interventions initiated prior to significant amyloid accumulation.

FURTHER INFORMATION

- MODEL AD: model-ad.org
- AD Knowledge portal: adknowledgeportal.org
- AD Data Explorer: modeladexplorer.org
- TREAT-AD: treatad.org

ACKNOWLEDGEMENTS

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