A multi-discipline phenotyping platform for late-onset Alzheimer's disease employed on a novel, humanized *APOEε4.Trem2*R47H* mouse model

Kevin P. Kotredes[1], Adrian Oblak[2], Ravi S. Pandey[1], Peter Borchian[2], Dylan Garceau[1], Harriet Williams[1], Asli Uyar[1], Rita O'Rourke[1], Sarah O'Rourke[1], Cynthia Ingraham[2], Daria Bednarycek[1], Melisa Belanger[1], Zackary Cope[3], Kate E. Foley[1], Benjamin A Logsdon[4], Lara M. Mangravite[4], Stacey J. Sukoff Rizzo[3], Paul R. Territo[2], Gregory W. Carter[1], Michael Sasner[1], Bruce T. Lamb[2†] and Gareth R. Howell[1†].

[1] The Jackson Laboratory, Bar Harbor, ME, USA.

[2] Stark Neuroscience, Indianapolis, IN, USA.

[3] University of Pittsburgh, Pittsburgh, PA, USA.

[4] Sage Bionetworks, Seattle, WA, USA.

[†]Correspondence: gareth.howell@jax.org and btlamb@iu.edu

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ABSTRACT

Background: Late-onset Alzheimer's Disease (AD) (LOAD) is the most common neurodegenerative disease. Despite extensive efforts to understand disease progression there are currently no approved disease modifying interventions to delay or reverse neurodegeneration caused by AD. Repeated failures in human trials, despite promising preclinical results in amyloidogenic mouse models, highlight the need for animals that better model human AD. MODEL-AD (Model organism development and evaluation for late-onset AD) are identifying and integrating disease-relevant, humanized gene sequences identified from public AD data repositories to create more translatable mouse models relevant to AD.

Methods: Strong risk factors for LOAD, *APOEε4* and *Trem2*R47H*, were expressed alone or in combination on a congenic C57BL/6J (B6) background, in cohorts of mice established on multiple sites and aged to between 4-24 months. A deep phenotyping approach was employed to assess phenotypes relative to human AD.

Results: The LOAD1 mouse strain, expressing humanized *APOEε4* and *Trem2*R47H* alleles, was designed to elucidate the disease state of animals expressing the two strongest genetic risk factors of LOAD at endogenous levels. Robust analytical pipelines measured behavioral, transcriptomic, metabolic, and neuropathological phenotypes in cross-sectional cohorts for progression of disease hallmarks at all life stages. *In vivo* PET/MRI neuroimaging revealed regional alterations in glycolytic metabolism and vascular perfusion. Transcriptional profiling by RNA-Seq of brain hemispheres identified sex and age as the main sources of variation between genotypes including age-specific enrichment of AD-related processes. Similarly, age, but not genotype, was the strongest determinant of behavioral change. In the absence of mouse amyloid plaque formation, many of the hallmarks of AD were not observed in this strain. However, these two alleles together form a sensitized, background strain which will serve as a platform for the characterization of additional genetic and environmental LOAD risk factors.

Conclusions: Comprehensive phenotyping provided key insights into genetic and environmental effects aimed at modeling human disease, critical to understand the complex intergenic interactions and subsequent molecular signaling cascades. The data provided by these assays are important for understanding the relative contributions of subsequent risk factors amended to LOAD1.

BACKGROUND

Alzheimer's disease (AD) is the most common form of dementia, currently affecting 5.8 million patients in the United States (1), ~95% of which are late-onset (LOAD) (2). LOAD is a heterogeneous disease defined by a few widely accepted hallmarks: extracellular amyloid plaques, intracellular tau tangles, vascular disfunction, immune activation, synapse loss, neuron death, and cognitive decline (3). Dissecting the etiology of these properties in animal models has provided key insights into our understanding of the disease and propose strategies to treat it, however human clinical trials of resultant therapeutics have not yielded an approvable drug.

In 2015, the National Institutes on Aging (NIA) aimed to accelerate the AD drug testing pipeline by promoting the design of more predictive preclinical studies that better translate to human disease (4). Current popular animal models of AD rely on the expression of transgenic alleles to promote the aggregation of amyloid plaques or neurofibrillary tangles as drivers of subsequent disease processes, notably during adolescence and early adulthood (5). Therefore, these overexpression models do not fully capitulate the breadth of the disease observed in human patients, particularly elderly populations; many underlying biological pathways affected in human disease are not observed in these mice (6). From this need the MODEL-AD (Model Organism Development and Evaluation for Late-onset AD) consortium was established to provide the research community with the next generation of AD animal models (7). In an effort to design and validate novel mouse models of LOAD that better mimic human disease, we opted to create humanized coding and non-coding LOAD risk variants expressed at endogenous levels. To implement these considerations in the development of a new generation of preclinical animal models, candidate humanized gene variants or SNPs from a number of public data repositories (ADSP, AMP-AD, ADGC, ADNI, ROS/MAP, IGAP, M^2OVE-AD, Resilience-AD, ACT) are being identified and engineered into existing mouse genes under endogenous promoters (8). As a heterogenous disease, it is unlikely that a single genetic alteration will promote the complex set of endophenotypes observed in humans. Rather, a combination of genetic and/or environmental risk factors are likely needed to phenocopy human disease and a better understanding of the effects of the complex interrelationships between risk factors is required to identify prospective therapeutic avenues. To this end, new mouse strains and conditions better replicating human LOAD are needed to improve the clinical translation of mouse therapies in human patients.

To date, approximately 40 loci have been identified through genetic and genome wide association studies that increase risk for AD (9-11). The strongest of these risk factors are the ε4 allele of apolipoprotein E (*APOE*) and point mutations in triggering receptor expressed on myeloid cells 2 (*TREM2)* locus (2, 6, 10-13). The strongest genetic determinant of LOAD risk, *APOE*, has been the focus of extensive investigation for many years (14-16), including the development of multiple mouse models (17-20). *APOE* is important in lipoprotein metabolism and immunoregulation strongly associated with cardiovascular and Alzheimer's disease (2, 6, 8, 10-13, 21). Three isoforms of *APOE* are expressed in the human population: *APOEε2,* *APOEε3,* and *APOEε4* which confer increasing risk of LOAD, respectively. The ε4 isoform of *APOE* increases AD risk and decreases age of diagnoses, compared to *APOEε3* carriers (22, 23). The three isoforms differ by one amino acid each at positions 112 and 158 that has profound effects on their functions: *APOEε2* (Cys112, Cys158); *APOEε3* (Cys112, Arg158); and *APOEε4* (Arg112, Arg158). The APOE protein has been shown to stimulate binding, transport, and metabolism of lipoproteins, major cholesterol transporters in the central nervous system (CNS) resulting in hypocholesteremia, tight junction failure, and vascular dysregulation (24, 25). Additionally, reports of mice carrying human *APOEε4*-targeted replacement allele show evidence of blood-brain barrier (BBB) leakiness (26, 27), immune alterations (28-31), synaptic disfunction (19), and behavior deficits (32). Most importantly, APOE is also implicated in beta-amyloid and tau clearance (33, 34). However, many of the aspects of LOAD are not recapitulated upon expression of *APOEε4* alone*,* including formation of beta-amyloid plaques and neurofibrillary tangles (32).

TREM2 encodes a member of a receptor signaling complex with TYRO protein tyrosine kinase binding (TYROBP) protein, which activates microglia, macrophages, and dendritic cells during damage and immune responses (35-37) functioning in processes like debris clearance (38) and amyloid plaque response (39-41). Single nucleotide polymorphisms (SNP) found in *TREM2* have been shown to regulate microglial function (36, 42, 43), the most widely studied being the R47H missense mutation in exon 2. The *TREM2*R47H* mutation triples the carrier's likelihood of Alzheimer disease (2, 6, 10-13). The increased risk is suggested to be, in part, the result of decreases in the microglial receptor's interactions with ligands (phospholipids, APOE, and beta-amyloid) yielding chronic dysfunction in microglial phagocytosis and inflammatory pathways (8, 35, 42).

Here we describe the application of a deep phenotyping approach developed by MODEL-AD that included biometrics, behavioral assays, transcriptomics, neuroimaging, and immunohistochemistry to assess AD-relevant phenotypes in mice expressing combinations of humanized *APOEε4* and *Trem2*R47H* alleles by endogenous genetic elements.

RESULTS

To decipher how these two strong risk factors drive AD-relevant phenotypes, we created the LOAD1 mouse strain, a double homozygous B6.*APOE4.Trem2*R47H* model, accompanied by single genotype controls, on a C57BL/6J (B6) background ***(Supplemental Table 1)***. In appreciation of sexual dimorphism observed in human aging and disease, cohorts of males and females were established for phenotyping at 4-, 8-, 12- and 24-months using a cross-sectional design. To determine whether the LOAD risk variants *APOEε4, Trem2*R47H,* or the combination produced *in vivo* phenotypes independent from normal healthy aging, a comprehensive cross-sectional phenotyping battery was conducted (***Figure 1)*** and included *in vivo* frailty assessments, metabolic screening, microbiome sampling, biomarker evaluation, behavioral phenotyping, and *in vivo* imaging. Postmortem brain tissue was further examined for transcriptomic and neuropathological indications of disease. All accumulated data sets and observations are disseminated for public availability (44).

Biometric profiles of LOAD1 mice change with age

Comparison of biometric data from young (4 months) and aged mice (24 months), comprised of both sexes from four genotypes, revealed the expected age-related accumulation in physical frailty characteristics ***(Figure 2 A,B)*** with inverse correlations in body temperature ***(Supplemental Figure 1)*** and age-related increases in body weights ***(Figure 2 C,D)***. However, effects due to genotype were not observed overall (see also ***Supplemental Table 2***). Statistical analyses were constrained to contrasting only cohorts sharing littermates. To determine the effects of *APOEε4* and *Trem2*R47H* on mouse metabolome, terminal non-fasted blood plasma levels of metabolites were measured. Homozygous expression of humanized *APOEε4* resulted in a significant decrease of non-fasted serum low-density lipoproteins (LDL) in the absence of corresponding decreases in total cholesterol ***(Figure 2 E-H)***. Expected aging-specific-effects included

decreases in glucose and triglyceride levels in both sexes independent of genotypes ***(Figure 2 I-L)***. Other measurements and metabolic analytes included in our panel were unchanged between sex, age, and genotype cohorts ***(Supplemental Figure 1 C-F and Supplemental Table 3)***. Additional cohorts, investigating *APOEε4* allele alone compared with littermate B6 controls, were aged to 12 months. Consistent with initial aging related phenotypes in the 4- and 24-month cohorts, there was an expected increase in cumulative frailty scores, reductions in core body temperature and increase in body weight in both sexes with no genotype-driven differences observed ***(Supplemental Figure 1 G-I)***.

Age is the strongest determinant of performance by LOAD1 mice in behavioral assays

As part of the comprehensive phenotypic characterization, all mice were evaluated through a behavioral testing pipeline consisting of open field, spontaneous alternation (y-maze), rotarod motor coordination, and running wheel activity assessments. Age-dependent impairments were observed across all genotypes from 4 to 24 months in locomotor activity as measured by open field and entries in the y-maze, motor coordination as measured by rotarod, and wheel running activity ***(Figure 3; Supplemental Figure 2; Supplemental Figure 4)***. Spatial working memory was preserved up to 24 months of age with no deficits observed across genotype relative to 4 months of age. Interestingly, in the rotarod motor coordination assay, there was a greater impairment observed in B6.*Trem2*R47H* relative to B6.*APOE4.Trem2*R47H* or B6.*APOE4* alone at 24 months ***(Figure 3 A,B)***. Compared to 4-month-old animals, we observed a decrease in activity during the active period (dark phase) by 24 months ***(Supplemental Figure 4)***. At 24 months of age, home cage running wheel activity suggested a correlation between increased activity during the active period and expression of the *APOEε4* allele (both B6.*APOE4* and B6J.*APOE4/Trem2*R47H* mice) by way of total distance traveled and day-time activity in B6.*Trem2*R47H* mice ***(Supplemental Figure 4 E,F)***. In addition to monitoring physical wellness and behavior of otherwise healthy mice, we also wanted to track the effects of sex and genotype on animal longevity, in the absence of amyloid-associated AD. For this analysis, mortality was defined as subjects that were found dead with no obvious signs of infection, trauma, or intervention during daily monitoring. Mortality risk for each allele was determined by comparing survival scores of cohorts aged to 24 months. Overall, females had a greater risk than males, and survival

probabilities were lowest in both sexes for animals expressing both LOAD risk alleles ***(Supplemental Figure 5)***.

PET/MRI identified age- and genotype-dependent differences in glycolysis and tissue perfusion

In an effort to understand the role of risk alleles on regional glycolysis and tissue perfusion, translationally relevant regional measures were acquired via 18F-FDG and 64Cu-PTSM PET/MRI and autoradiography, respectively. By 12 months glycolysis was altered in key brain regions associated with sensory integration, cognition, vision and motor function in B6.*APOE4* and B6.*APOE4.Trem2*R47H* mice, when compared with controls ***(Figure 4)***, and were confirmed via post-mortem autoradiography, which has a 40 folder greater resolution than PET. As expected, these changes were greater in number of regions and magnitude of change in female mice when compared to males ***(Figure 4 B,C)***. These changes were similarly observed through time, where female mice showed significantly altered glycolysis at 4, 8 and 12 months, while male mice largely showed a hypoglycolytic phenotype at 8 months, that was virtually mitigated by 12 months ***(Supplemental Figure 6)***. Since these risk alleles can alter metabolic functionality and neuroinflammatory-driven tissue perfusion in an independent manner, we quantitatively measured changes in regional tissue perfusion via 64Cu-PTSM PET/MRI and confirmed this via autoradiography. Brain perfusion was significantly lower in regions associated with sensory integration, cognition, vision and motor function in both sexes by 12 months and confirmed via post-mortem autoradiography ***(Figure 5)***. Interestingly, these changes were manifested temporally, with the greatest reductions occurring across genotypes and regions in both sexes at 4 months ***(Supplemental Figure 7)***. Unlike glycolysis, these changes were largely resolved by 8 months in female mice, while males continued to show regional reduction in perfusion at this same age.

Biochemical and neuropathological effects of *APOEε4 and Trem2*R47H* alleles

Confirmation of protein expression levels in brain tissue were confirmed for alleles encoding human APOE4 and mouse TREM2 carrying the R47H mutation ***(Supplemental Figure 8)***. Similar to reports of R47H variant-mediated reduction in *Trem2* transcript levels (6), TREM2 protein levels in the brains of these animals were also decreased. However, instead of a near knock-out of all TREM2 that has been reported

previously (6), levels fell by approximately 50% in *Trem2*R47H* animals compared to C57BL/6J **(*Supplemental Figure 8A,B).*** We have previously shown APOE4 protein levels are similar to endogenous mouse APOE (14) and expression of APOE4 appeared similar between male and female LOAD1 mice ***(Supplemental Figure 8C)*** . Additional molecular characterization of these animals showed both age- and genotype-driven differences in levels of cytokines present in the brain and blood ***(Supplemental Figure 9)***. IL-6 and KC/GRO concentrations were highest in B6.*APOE4.Trem2*R47H* brain tissue at 8 months, while blood plasma concentrations continued to increase with age in those mice ***(Supplemental Figure 9 B,D,E)***. In multiple occasions a trend appeared to suggest increased cytokine concentrations in mice expressing mutated allele *Trem2*.

Neuropathological features of AD were then investigated by hematoxylin and eosin (H&E, structure) and luxol fast blue/cresyl violet (LFB/CV, myelin) staining but did not reveal any gross anatomical changes to tissue architecture or myelin ***(Figure 6A)***. Brain sections were also imaged via immunofluorescence and included neuritic plaque-reactive-microglia (X34/Lamp1/Iba1), vascular leakage (CD31/Iba1/Fibrin), and ThioflavinS (amyloid plaques and neurofibrillary tau tangles) ***(Figure 6B)***. No gross abnormalities in cell counts, nor additional neuropathological features were observed in 24 month B6.*APOE4.Trem2*R47H* mice. We focused particularly on the cortex and hippocampus where episodic memory (hippocampus) and memory behavior (cortex) are regulated. Amyloid plaques and hyperphosphorylated Tau were not observed in mice of any genotype at any age ***(Figure 6B)***.

Transcriptional profiling revealed Individual and synergistic effects of APOEε4, Trem2*R47H, and age

Brain hemispheres from 4 and 24 month male and female B6.*APOE4.Trem2*R47H* mice and single genotype and C57BL/6J controls were assessed using RNA-seq (see Methods). Transcriptomic analysis measured the expression levels (log-transformed TPM counts) of mouse *Apoe*, *Trem2*, and human *APOE* genes across all mouse models ***(Figure 7 A-C)***. We observed higher expression of human *APOE* gene in mice carrying humanized *APOEε4* (B6.*APOE4* and B6.*APOE4.Trem2*R47H* mice), whereas mouse *Apoe* gene was highly expressed in B6 and *Trem2*R47H* mice ***(Figure 7 A,B)***. As expected based on protein levels **(Supplemental Figure 8A,B)**, expression of *Trem2* was significantly reduced ($p < 0.05$) in

B6.*Trem2*R47H* and B6.*APOE4.Trem2*R47H* compared to age-matched B6 ***(Figure 7C),*** an effect likely caused by a novel effector splice site and truncation introduced by the R47H mutation. Furthermore, expression level of *Trem2* increased with age across all mouse models, but no such patterns were observed in the expression levels of mouse *Apoe* and human *APOE* genes ***(Figure 7 A,B)***. In addition, there was lower expression of *Trem2* in B6.*APOE4.Trem2*R47H* compared to B6.*Trem2*R47H* mice at advanced age (24 months), suggesting expression of *Trem2* might be suppressed by *APOEε4.* Next, principal component analysis (PCA) identified two distinct clusters corresponding to male and female samples separated along the first principal component (26% of total variance), suggesting sex-specific differences are profound in mice ***(Figure 7D)***. Analysis of samples from different age groups revealed a gradient of discrimination along the second principal component (14% of total variance) ***(Figure 7D)***, implying the presence of age-dependent molecular changes in the brain transcriptomes.

To identify molecular effects of the LOAD risk genes, we performed pairwise differential analysis between each genotype (B6.*APOE4,* B6.*Trem2*R47H* or B6.*APOE4*.*Trem2*R47H*) and age- and sex-matched B6 controls. At an early age (4 months), only a few genes were differentially expressed (DEG) ($p < 0.05$) for all genotypes for both sexes ***(Supplemental Figure 10A)*** and no KEGG pathways were enriched. At 8 months of age, there were 32 DEGs (3 upregulated, 29 downregulated) ($p < 0.05$) in male B6.*Trem2*R47H* mice, and 11 DEGs (2 upregulated, 9 downregulated) ($p < 0.05$) in female B6.*Trem2*R47H* mice ***(Supplemental Figure 10A)***. KEGG Pathway analysis identified enrichment of genes involved in immune related pathways such as 'complement and coagulation cascades' and 'staphylococcus aureus infection' in the downregulated DEGs in female B6.*Trem2*R47H* mice ***(Figure 8)***. In 8 months old B6.*APOE4* mice, a total of 145 genes were significantly differentially expressed (42 upregulated, 103 downregulated) ($p < 0.05$) in male mice, whereas a total of 25 genes were differentially expressed (11 upregulated, 14 downregulated) ($p < 0.05$) in female mice ***(Supplemental Figure 10A)***. Pathway enrichment analysis of upregulated genes in male B6.*APOE4* mice identified enrichment of 'platelet activation' pathway, whereas downregulated genes in female B6.*APOE4* mice were enriched for 'protein processing in endoplasmic reticulum' pathway ***(Figure 8)***. No KEGG pathways were enriched for downregulated DEGs in male B6.*APOE4* mice and upregulated DEGs in female B6.*APOE4* mice**.** DEGs in male B6.*APOE4.Trem2*R47H*

(7 upregulated, 32 downregulated), and female B6.*APOE4.Trem2*R47H* mice (2 upregulated, 1 downregulated) ($p < 0.05$) ***(Supplemental Figure 10; Figure 8)*** were not enriched in any KEGG pathway. At 12 months of age, there were a total of 206 DEGs (118 upregulated, 88 downregulated) in male B6.*Trem2*R47H* mice and 285 DEGs (113 upregulated, 172 downregulated) ($p < 0.05$) in female B6.*Trem2*R47H* mice. The upregulated DEGs in male *Trem2*R47H* mice were enriched in 'RNA transport' and 'spliceosome' pathways ***(Figure 8; Supplemental Figure 10)***, while genes in the 'oxidative phosphorylation' pathway were downregulated in male B6.*Trem2*R47H* mice ***(Figure 8; Supplemental Figure 10)***. Upregulated and downregulated DEGs in female B6.*Trem2*R47H* mice, were enriched for 'RNA transport' and 'lysosome' pathways respectively ***(Figure 8; Supplemental Figure 10)***. There were very few DEGs in B6.*APOE4* (1 in male, 3 in female) and B6.*APOE4.Trem2*R47H* mice (2 in male, 5 in female) ***(Supplemental Figure 10)*** at this age and therefore no enrichment of KEGG Pathways.

At 24 months old, there were a total of 144 DEGs (15 upregulated, 129 downregulated) in the male B6.*Trem2*R47H* mice and 748 DEGs (359 upregulated, 389 downregulated) ($p < 0.05$) in the female B6.*Trem2*R47H* mice. At this age, B6.*APOE4.Trem2*R47H* mice showed a greater number of DEGs ($p < 0.05$) in both male (24 upregulated, 197 downregulated) and female mice (83 upregulated, 400 downregulated) compared to at younger ages (4-12 months) ***(Supplemental Figure 10)***, suggesting that the most dramatic transcriptional changes arise between 12 and 24 months. We also found substantial overlap in downregulated DEGs between B6.*Trem2*R47H* and B6.*APOE4.Trem2*R47H* mice for both sexes ***(Supplemental Figure 10)***. This suggests that the *Trem2*R47H* allele is the major driving force in age-dependent transcriptional changes in B6.*APOE4.Trem2*R47H* mice. Downregulated DEGs were enriched in multiple AD-related pathways such as 'lysosome', 'osteoclast differentiation', 'phagosome", 'antigen processing and presentation', cytokine-cytokine receptor interaction', and 'complement and coagulation cascades' in both 24 months old B6.*Trem2*R47H* and B6.*APOE4.Trem2*R47H* mice ***(Figure 8; Supplemental Figure 10)***. In B6.*APOE4* mice, we observed only two DEGs (1 upregulated, 1 downregulated) in male and 24 DEGs (1 upregulated, 23 downregulated) in female mice. Downregulated genes in the B6.*APOE4* female mice were enriched in 'NOD-like receptor signaling' pathway ***(Figure 8; Supplemental Figure 10)***. We did not observe any enriched KEGG pathways in the upregulated list of genes across all genotypes at 24 months.

Home cage voluntary wheel running increased oxidative phosphorylation pathway in LOAD1 mouse brains

In the human population, sedentary lifestyle is correlated with an increased risk of LOAD (45-47). Therefore, to determine if physical activity influenced transcriptional changes in B6.*APOE4.Trem2*R47H* mice, a running wheel was provided in the home cage of 22 month old male mice for two months. Brain tissue from these animals was profiled by RNA-seq. A total of 292 DEGs (108 upregulated, 184 downregulated) were identified in the running B6.*APOE4.Trem2*R47H* mice compared to 24 months old B6 male mice. Enrichment analysis identified multiple enriched KEGG pathways such as "oxidative phosphorylation", "thermogenesis", and "retrograde endocannabinoid signaling" in the upregulated list of genes, whereas immune system associated pathways were enriched in the downregulated list of genes ***(Supplemental Figure 11A)***. To identify the effect of exercise, running B6.*APOE4.Trem2*R47H* male mice were compared with sedentary 24 month old B6.*APOE4.Trem2*R47H* male mice and there was a total of 600 DEGs (312 upregulated, and 288 downregulated). Upregulated DEGs were enriched in pathways such as "oxidative phosphorylation" and "Ribosome" ***(Supplemental Figure 11A)***. The expression of these upregulated DEGs enriched for oxidative phosphorylation showed reduced expression in age- and sex-matched B6.*APOE4* and B6.*Trem2*R47H* compared to running B6.*APOE4.Trem2*R47H* mice ***(Supplemental Figure 11B)***. Finally, the expression of the upregulated DEGs associated with oxidative phosphorylation pathway were assessed in transcriptional data from AMP-AD. Reduced expression of these running signature genes was observed in AD cases compared to controls across multiple brain regions such as parahippocampal gyrus (PHG) and frontal pole brain regions (FP) ***(Supplemental Figure 11C)***. This suggests that exercise induces beneficial effects on health by increasing the expression of oxidative phosphorylation pathway genes that are down regulated across multiple brain regions in AD patients.

DISCUSSION

MODEL-AD was established in response to the many shortcomings of existing mouse models of AD. Aspects of human pathology have been replicated in mouse strains, most prominently the formation of beta amyloid plaques via transgenic over-expression of brain-specific mutant human amyloid beta precursor

protein (*APP*), presenilin-1 (*PS1*), and/or microtubule associated protein tau (*MAPT*) bearing familial Alzheimer's disease (FAD) mutations (48). Legacy preclinical models rely heavily on alleles that overexpress transgenes, resulting in the removing or masking of important human-relevant biological interactions. These mouse strains have been invaluable for understanding the molecular and behavioral phenotypes of early-onset Alzheimer's disease (EOAD) driven by rapid and robust formation of plaques and tangles in the brain and correlating hyperactivity which is a confound of many cognitive behaviors. However, LOAD is ~20x more prevalent than EOAD and further implicates aging, inflammation, environmental, and many more genetic risk factors in disease development. Our LOAD1 mice were healthy late into life allowing a better understanding of the effect of AD risk factors in the context of aging ***(Supplemental Figure 5)***. As the heterogeneity of this disease becomes more appreciated, so is the importance of appropriate disease staging. Molecular targets of interest may only be available during particular evolving disease stages: debris (cell fragments, plaques, tangles, etc.) accumulates over time and inflammation, interruption/loss of neuronal function all also change with disease progression. In light of the repeated short-comings of "fit-for-all" therapies, efforts may be better directed at targeted therapies (19, 49, 50). Faithfully modeling a complex, polygenic disease will be aided by the creation of platform strains that carry multiple genetic risk factors to motivate with a scientific rationale rather than a grant-focused one. Of the candidate risk variants identified, expression of the ε4 allele of *APOE* and the R47H mutation in *TREM2* were identified as the strongest candidates for initial development of a novel LOAD mouse strain. Introduction of the R47H mutation into *Trem2* resulted in the creation of a novel murine splice site yielding a decrease in approximately 50% of TREM2 protein ***(Supplemental Figure 8 A,B)***, and 20% decrease in *Trem2* transcript ***(Figure 7C)***. Similar models have shown similar decreases in R47H-mediated *Trem2* expression and function (51-53). Efforts are ongoing to develop a *Trem2* allele expressing the full-length R47H risk factor at levels similar to wild-type *Trem2*. *APOEε4* is strongly associated with disease development and severity (15, 54) and at least one allele is present in approximately 65% of AD patients (55). Unfortunately, endogenous *Apoe* in mice does not express the isoform diversity seen from the *APOE* allele in humans. Insertion of humanized *APOE* alleles into mouse genomes has been a successful strategy to dissect the biology of APOE isoforms in mice (17-20, 56). The MODEL-AD *APOEε3* and *APOEε4* allelic series on a C57BL/6J background has subsequently be shown to consistently reproduce the biology

presented in other *APOE* mouse models and most importantly, human patients (14, 56). Therefore, *APOE4* formed the basis for multiple platform strains that include: B6.*APOE4.Trem2*R47H* (for which we have provided the identifier 'LOAD1').

APOE binds to high-density lipoproteins to facilitate cholesterol and phospholipid transport to LDL receptors. As expected, (16, 56), mice expressing the humanized *APOEε4* allele showed decreased plasma lipoprotein levels across all time points ***(Supplemental Table 3)***. We observed an age-dependent decrease in glucose levels across both sexes and all genotypes ***(Figure 2L)***, an indication of increased frailty and aging that is common in aging-related-disease studies (57). The contributions of metabolic and vascular factors are strongly implicated in disease progression, and how APOE or any other metabolic trait, via systemic pathways, can influence CNS function should be a continued focus for intervention (58). The brain has one of the richest networks of blood vessels and are especially vulnerable (block or reduce blood flow, oxygen and nutrients). For example, the respective influence of *APOEε4*, with and without *Trem2*R47H* expression, in regional changes in brain glycolytic metabolism (59) ***(Figure 4; Supplemental Figure 6)***, tissue perfusion (60, 61) ***(Figure 5; Supplemental Figure 7)*** observed in human AD remain under further study. Additional analyses of this *APOE4* allele have also revealed changes in cholesterol metabolism and transcriptional signatures in the brain compared to carriers of the *APOE3* allele (14).

Despite the heterogeneity of AD, age is the strongest risk factor in the human population. As an aging disease, monitoring and evaluating mouse models in relation to age is crucial for understanding onset and progression of disease over time. We selected timepoints that reflected different life stages of an adult mouse: Mature, by 3-6 months (beyond development but not yet affected by senescence); Middle-aged, 10-14 months (some senescent changes detected in some, not all, biomarkers of aging); and Aged, 18-24 months (senescent markers can be observed in all animals) (62). Non-invasive testing of motor activity, behavior, and cognition have been shown to be reliable phenotypes for staging disease onset and trajectory (63, 64). Aged mice equally displayed the expected trial dependent increases in consecutive rotarod trials and decreases in total distance traveled over time in the open field assay, regardless of genotype ***(Supplemental Figure 3)***. Animal activity, measured by total distance traveled during open field assays ***(Figure 3 and Supplemental Figure 2)***, also decreased equally with age. Similarly, home cage wheel running assays, which provide a more comprehensive activity phenotype than the 1-hour open field test,

also showed a decrease in activity levels during the active (dark phase) (63) ***(Supplemental Figure 4)***. Interestingly, at 24 months some indications of increased activity during the active phase in animals carrying the *APOEε4* allele (both B6.*APOE4* and B6J.*APOE4/Trem2*R47H*) and day-time activity in only B6.*Trem2*R47H* mice suggesting a dominant phenotype produced by expression of *APOEε4* ***(Supplemental Figure 4 C,D; Supplemental Figure 10)***.

In the absence of additional environmental or genetic risk factors, B6.*APOE4/Trem2*R47H* mice did not display penetrant behavioral phenotypes beyond the expected aging-related changes but did exhibit decreased survival probabilities by 24 months ***(Supplemental Figure 5)***. Very few C57BL/6J mice succumbed during the 24-month aging process (<5%), whereas mortality was higher in mice expressing both LOAD alleles in both males (~20%) and females (~35%). Male mice expressing either allele alone had survival probabilities similar to C57BL/6J, whereas females with *APOEε4* or *Trem2*R47H* showed a mortality rate of ~20%. Therefore, it would seem that these two LOAD risk alleles show an equal and additive risk when expressed together, but in females their interaction appears synergistic.

We investigated the molecular signatures in the brain transcriptomes of LOAD mouse models at different ages in both sexes. We identified age-dependent molecular changes associated with LOAD pathologies in mouse models. Introduction of the R47H mutation revealed a novel *Trem2* isoform identical to primary transcript, but truncated by 119 bp from its start position in exon 2 (See Materials and Methods) (44). Expression of this novel isoform resulted in a decrease in both transcript and protein compared to wild-type *Trem2* carriers ***(Figure 7C; Supplemental Figure 8 A,B)***. Despite the decrease in *Trem2* expression, mouse models carrying R47H mutation in *Trem2* gene did not exhibit any significant transcriptional changes at young age, in contrast APOE4 mice exhibited significant changes only at 8 months of age. We further identified significant downregulation of genes associated with oxidative phosphorylation pathway in the 12 months old B6.*Trem2*R47H* mice, suggesting that the oxidative phosphorylation could be prominent early feature for the onset of neurodegeneration/inflammation process. Subsequently, multiple immune related processes were disrupted in 24 months old B6.*Trem2*R47H* and B6.*APOE4.Trem2*R47H* mice, supporting the profound relationship between aging, *Trem2* and AD. Interestingly, at 12 months of age we did not observe any significant transcriptional changes in B6.*APOE4.Trem2*R47H* mice compared to control mice, suggesting that the effect of *Trem2* gene is suppressed due to the presence of *APOEε4*. Similarly, when

mouse models were compared with human co-expression modules, we observed strong negative correlation between the B6.*Trem2*R47H* mice and immune-related human co-expression modules from multiple brain regions, and this inflammatory response is dampened in the presence of *APOEε4* in the B6.*APOE4.Trem2*R47H* mice. Distinct mouse models showed concordance with distinct human co-expression modules reflecting a different transcriptional response driven by the human *APOEε4* and *Trem2*R47H* risk variants. We also observed age dependent shift in co-expression patterns associated with LOAD pathologies. A strong negative correlation between co-expression modules associated with cell cycle and DNA repair was observed in the early-aged mouse B6.*APOE4* model, whereas advanced-aged B6.*APOE4* female mice showed strong positive correlation with these co-expression modules. This overlap with human late-onset co-expression signatures early in life was observed for a number of different brain regions and was absent in Trem2*R47H knock-in mice. Furthermore, aged B6.*Trem2*R47H* mice showed a moderate overlap with several human neuronal co-expression modules enriched for genes that play an important role in synaptic signaling and myelination. At advanced age, a strong correlation between the mouse models and immune related human co-expression modules highlights the important role of the LOAD associated *APOEε4* and *TREM2* R47H variant in Alzheimer's related immune processes. Our experiments predict that *APOEε4* functions through the suppression of effects brought out by expression of the *Trem2*R47H* allele: 455 genes are upregulated by $TREM2^{R47H}$ but suppressed by *APOEε4* for ***(Supplemental Figure 10)***. Our results mirror some emerging evidence that *APOEε4* suppresses *Trem2*R47H* in AD risk, that there are some suggestions that *APOEε4* carriers don't have increased AD risk with *Trem2*R47H* and *Trem2*R47H* only increases risk on *APOEε3* carriers (65, 66). Additionally, we observe more differentially expressed genes at middle age than at a later age supporting evidence of an earlier aging phenotype than C57BL/6J mice, with a realignment of transcriptomes at later timepoints (58, 67). We employed a weighted gene co-expression network analysis (WGCNA) used to identify modules of correlated genes. Each module was tested for differential expression by strain, then compared with human postmortem brain modules from the Accelerating Medicine's Partnership for AD (AMP-AD) to determine the LOAD-related processes affected by each genetic risk factor (6, 68, 69). This will be a useful tool in identifying differentially expressed genes correlated with molecular pathways tied to inflammation and

identifying a mouse strain that exhibits a similar transcriptional signature to human patients with true neuroinflammation.

Amyloid plaque formation is a primary diagnostic measure of Alzheimer's disease with both APOE and TREM2 linked to amyloid deposition (2, 3, 9, 40, 48, 67, 70, 71). For example, TREM2 can bind amyloid, altering microglial function, linking the TREM2-APOE pathway directly to amyloid-driven disease progression (72, 73). Loss of functional Trem2 in mice resulted in plaques that contained reduced amounts of APOE and promoted amyloidogenesis in mice by reducing microglial function (71, 74) indicating that microglia, through TREM2 mediated signaling, can regulate APOE co-deposition around amyloid deposits. Further, TREM2 KO prevented infiltration of blood-derived myeloid cells and ameliorated plaque burden in *APP/PS1* mice (40) and disease-related mutations impair many of its functions (75). However, current amyloidogenic mouse models develop amyloid plaques at very young ages, within a few months ***(Supplemental Figure 7B)***, whereas in human patients the average age of AD onset is at older ages, ~80 years, potentially causing the disparity in therapeutic outcomes between mouse models and human patients. In the absence of amyloid deposition, many hallmarks of LOAD can be investigated for *APOEε4*- and *Trem2*-influenced effects that precede and may contribute to onset of AD. However, current work is evaluating the effects of APOE and TREM2 risk alleles in the context of humanized Abeta. For instance, we are currently evaluating a novel B6.*APOE4*.*Trem2*R47H*.*hAbeta* (LOAD2) strain and in the process of incorporating humanized Tau (MAPT) alleles into forthcoming strains. These novel platform or AD-sensitized strains (e.g., LOAD1, LOAD2 etc.) are being used to assess the contribution of additional genetic risk factors identified through genetic and genome-wide association approaches using LOAD1 as a platform strain. These include variations in genes commonly associated with AD including *ABCA7*, *PLCG2*, *CR1*, *BIN1* and *SORL1*. The new strains are prioritized for extensive phenotyping using a primary screening approach centered on transcriptional profiling of nearly 800 genes known to be differentially expressed in human AD brains compared to unaffected controls. (76). Platform stains are also ideal for studying age-dependent effects of environmental risk factors, such as diet, as well as genetic context. Amendments to the current phenotyping strategy are also in consideration to expand characterizations of the metabolome, proteome, and electrophysiology of LOAD animals. Ultimately, strains carrying combinations of risk factors

that more closely align with human disease will be incorporated into the pre-clinical testing core of MODEL-AD to assess the potential of prioritized compounds to treat AD.

CONCLUSIONS

The MODEL-AD consortium has established the LOAD1 model to study the effects of two strong risk factors of LOAD, *APOEε4 and Trem2*R47H*. In the absence of amyloid plaque formation, changes in the cellular dynamics observed in the diseased human brain were not recreated in the young or aged cohorts. However, metabolic traits thought to exacerbate disease severity were replicated. *APOEε4* expression altered cholesterol and lipid metabolism pathways. In addition to *APOEε4*, the R47H mutation of *Trem2* also caused changes in glycolysis and tissue perfusion in many separate brain regions, similar to clinical observations. Transcriptional analysis of brain hemispheres highlighted alterations to disease-related processes and, when compared to human data sets, an indication that expression of *APOEε4 and Trem2*R47H* yield an aged mouse model more representative of human patients than C57BL/6J control mice. Subsequent data sets with risk factor additions, or exclusions, will further improve our understanding of the relative contributions of individual risk factors and how best to engineer additional models. The LOAD1 strain (available from The Jackson Laboratory) and associated data (uploaded to the AD Knowledge Portal: https://adknowledgeportal.org; https://doi.org/10.7303/syn23631984) provide a valuable resource to study the combined effects of age, APOE, and TREM2 LOAD risk factors in the absence insult from pathogenic protein aggregates.

LIST OF ABBREVIATIONS

AD: Alzheimer's disease

ADGC: Alzheimer's Disease Genetics Consortium

ADNI: Alzheimer's Disease Neuroimaging Initiative

ADSP: Alzheimer's Disease Sequencing Project

AMP-AD: Accelerating Medicines Partnership - Alzheimer's Disease

B6: C57BL/6J

BBB: Blood-brain barrier

CNS: Central nervous system

DEG: Differentially expressed genes

FP: frontal pole brain regions

HDL: high-density lipoproteins

IGAP: International Genomics of Alzheimer's Project

LOAD: Late-onset Alzheimer's disease

LDL: low-density lipoproteins

M^{2}OVE-AD: Molecular Mechanisms of the Vascular Etiology of Alzheimer's Disease

MRI: Magnetic resonance imaging

PET: Positron emission tomography

PHG: parahippocampal gyrus

ROS/MAP: Religious Orders Study and Memory and Aging Project

METHODS

Model backgrounds

All animals were obtained from The Jackson Laboratory. Mouse models of Late-onset Alzheimer disease (LOAD) developed by MODEL-AD (Model Organism Development & Evaluation for Late-onset Alzheimer's Disease) are congenic to the C57BL/6J (JAX# 000664) (B6) strain. Genetic variants, identified from human data compiled by the AMP-AD (Accelerating Medicines Partnership – Alzheimer's Disease) project, expressed in MODEL-AD-generated strains are listed on the MODEL-AD strain table at https://www.model-ad.org/strain-table/, along with relevant links for allele descriptions, data, distribution, and legal disclaimers.

*LOAD1 (B6J.APOE4.Trem2*R47H) mice*

The LOAD1 double mutant strain created at The Jackson Laboratory in Bar Harbor, Maine carries two primary risk alleles found in Alzheimer's disease patients. The humanized ApoE knock-in allele, in which a portion of the mouse Apoe gene (exons 2, 3, a majority of exon 4, and some 3' UTR sequence) of the mouse Apoe gene was replaced by the corresponding sequence of the human APOE4 gene (available as B6(SJL)-Apoe$^{tm1.1(APOE*4)Adiuj}$/J ; https://www.jax.org/strain/027894) (14). The second allele, *Trem2*, contains

the R47H point mutation and two additional silent mutations (available as C57BL/6J-Trem2^{em1Adiuj}/J; https://www.jax.org/strain/027918). The human R47H variant, when expressed in mouse brains, also confers a novel splice variant due to a cryptic slice acceptor site in exon 2 (52). See additional information in the Jackson Laboratory APOE4.Trem2R47H mouse (JAX strain #028709) strain data sheet. APOE4.Trem2R47H mouse strain data sheet at https://www.jax.org/strain/028709.

Animal housing conditions

All experiments were approved by the Animal Care and Use Committee at The Jackson Laboratory and the Institutional Animal Care and Use Committee at Indiana University. To minimize gene expression variation between mice, animal housing conditions were replicated between both Bar Harbor and Indianapolis campuses. Mice were bred in the mouse facility at Indiana University or The Jackson Laboratory and maintained in a 12/12-hour light/dark cycle, consisting of 12 hours-ON 7am-7pm, followed by 12 hours-OFF. Room temperatures are maintained at 65-75°C with 40-60% humidity. All mice were housed in positive, individually ventilated cages (PIV). Standard autoclaved 6% fat diet, (Purina Lab Diet 5K52) was available to the mice *ad lib*, as was water with acidity regulated from pH 2.5-3.0. All breeder and experimental mice were housed in the same mouse room and were aged together. All behavioral characterization was conducted in the Mouse Neurobehavioral Core Facility (MNBF) at The Jackson Laboratory. Briefly, mice were relocated from the housing room in which they were reared to the MNBF in an adjacent building on the Bar Harbor campus. Mice were individually housed at minimum 5 days prior to behavioral testing. The dedicated MNBF housing room consists of PIV caging with temperature controlled at a setting of 72±2°F and humidity at 50±20%. The testing facility was on a 12:12 L:D schedule (lights on at 6:00am) with all testing performed during the light cycle (typically between 7:00am and 5:00pm, with the exception of wheel running which was continuous 24-hour testing for up to 5 days). All subjects were randomized and counterbalanced for testing order across multiples of instrumentation and time of day for each test day, with a simplified testing ID number (e.g., #1-100), with all technicians blinded to genotype (e.g., coded as A, B, C, etc.). The blind was maintained throughout testing and until after the data were analyzed with no subjects or data excluded based on any mathematical outliers.

Behavioral testing

Behavioral tests were conducted as previously reported (63) in the following order with at minimum a 1-2-day rest period between tests: Frailty assessment with core body temperature recording, open field test, spontaneous alternation, rotarod, and wheel running activity. On each test day, subjects were transported from the adjacent housing room into the procedure room, tails were labeled with a non-toxic permanent marker with the assigned subject ID number, and subjects were left to acclimate undisturbed to the testing environment for a minimum 60 minutes prior to testing. Between subjects, all testing arenas were sanitized with 70% ethanol solution and dried prior to introducing the next subject. Lighting in the testing rooms were consistent with the housing room (~500 lux) unless where specifically noted. At minimum 5 days post the conclusion of behavioral testing, mice were sent for tissue harvesting.

Frailty assessment

Similar to as previously described (53), subjects were individually evaluated for the absence or presence of 26 aging related characteristic traits and scored a 0, 0.5, or 1 (based on presence/absence, and severity) for each assessment by a trained observer, blind to genotype/age, and included the following assessments: alopecia; loss of fur color; dermatitis/skin lesions; loss of whiskers; coat condition; piloerection; cataracts; eye discharge/swelling; microphthalmia; nasal discharge; rectal prolapse; vaginal/uterine/penile; diarrhea; vestibular disturbance; vision loss assessed by visual placing upon subject being lowered to a grid; menace reflex; tail stiffening; impaired gait during free walking; tremor; tumors; distended abdomen; kyphosis; body condition; breathing rate/depth; malocclusions; righting reflex. The frailty index score was calculated as the cumulative score of all measures with a maximum score of 26.

Core body temperature

Core body temperature was recorded just prior to the conclusion of the frailty assessment via a glycerol lubricated thermistor rectal probe (Braintree Scientific product# RET 3; measuring 3/4" L .028 dia. .065 tip) inserted ~2cm into the rectum of a manually restrained mouse for approximately 10 seconds. Temperature was recorded to the nearest 0.1°C (Braintree Scientific product#TH5 Thermalert digital thermometer).

Open field activity

Versamax Open Field Arenas (40cm x 40cm x 40cm; Omnitech Electronics, OH USA) were used for this test. Arenas were housed within sound attenuated chambers with lighting in the testing room and arenas consistent with the housing room (~500 lux). Mice were placed individually into the center of the arena and infrared beams recorded distance traveled (cm), vertical activity, and perimeter/center time. Data were collected in 5-minute time-bins for duration of 60 minutes.

Spontaneous alternation

Mice were acclimated to the testing room under ambient lighting conditions (~ 50 lux). A clear polycarbonate y-maze (in-house fabricated; arm dimensions 33.65cm length, 6cm width, 15cm height) placed on top of an infrared reflecting background (Noldus, The Netherlands), surrounded by a black floor-to ceiling curtain to minimize extramaze visual cues was used for this test. Mice were placed midway of the start arm (A), facing the center of the y for an 8-minute test period and the sequence of entries into each arm are recorded via a ceiling-mounted infrared camera integrated with behavioral tracking software (Noldus Ethovision XT). Percent spontaneous alternation is calculated as the number of triads (entries into each of the three different arms of the maze in a sequence of three without returning to a previously visited arm) relative to the number of alteration opportunities.

Rotarod test for motor coordination

An accelerating Rotarod (Ugo-Basile; model 47600) is used for this test. Lighting in the testing room is consistent with the housing room (~ 500 lux). The trial began with mice being placed on the rotating rod (4 rpm), which accelerates up to 40 rpm over the course of 300 seconds. Each mouse is subjected to 3 consecutive trials with an ~1 min inter-trial interval to allow cleaning of the rod between trials. Latency to fall (sec) is measured. Subjects that fall upon initial placement on the rod, before acceleration begins, are scored as 0 sec for that trial.

Wheel running activity

Subjects were individually housed into a clean cage with a running wheel (Med-Associates, Vermont, USA) and with food and water ad libitum. The light cycle was identical to the housing room with 12:12 L:D (lights on at 6:00am). Running Wheels were equipped with a wireless transponder that recorded activity on the running wheels (revolutions) in sync with a computer that time stamps events. Mice were left undisturbed throughout the testing period with the exception of daily welfare checks. Data were evaluated for time spent running (min), total distance traveled (meters), and speed (revolutions per min) over the course of three 24-hour periods

Behavioral data analysis

Prior to data analysis and while still blinded, results were adjusted to exclude data only from mice which could not be tested or which data was not available inclusive of any equipment failures, escape episodes, etc. Subjects were not excluded by any mathematical determination. Data was analyzed under coded genotypes (A, B, C, etc.) within sex, as one-way or two-way ANOVA as appropriate versus sex- and age-matched WT control. The blind was revealed at the conclusion of the data analysis for interpretation.

Fasted blood glucose collection and measurement

Fasted mice were placed into a fresh cage, free of food but with fresh water, at 6am – the beginning of the light-ON cycle. Mice were fasted for 6 hours, until 12pm, at which time blood glucose levels were analyzed. Prior to mouse restraint, a Contour Next EZ blood glucose monitor (Ascensia, Parsippany, NJ) was calibrated with Contour glucose control solution and Contour Next test strips. While restraining the animal, with a 5.0mm lancet a stab incision was made into and perpendicular to the cheek, located dorsal to the to the cheek skin gland at a distance equal to the height of the eye and caudal distance equal to the length of the eye. One drop of blood, approximately 10μl, was applied to a blood glucose test strip and readings were recorded.

Fecal collection

Parallel with measurement of animal weight, animals were placed in a clean container on a scale. Mouse weight was recorded and upon production, fecal sample was collected with forceps to prevent

contamination. Sample was placed in a pre-marked 1.5mL tube and snap-frozen immediately on dry ice. Container and forceps were cleaned with 70% ethanol before collecting from subsequent mice. Fecal samples were stored long term at -80°C until analyzed.

Animal anesthesia

Upon arrival at the terminal endpoint for each aged mouse cohort, individual animals were weighed prior to intraperitoneal administration of either: (A) ketamine (100mg/kg) and xylazine (10mg/kg); or (B) tribromoethanol (1mg/kg). Routine confirmation of deep anesthesia was performed every 5 minutes by toe pinch. First confirming deep anesthetization via toe pinch, an incision along the ventral midline to expose the thorax and abdomen, followed by removal of the lateral borders of the diaphragm and ribcage revealed the heart. If desired, prior to perfusion blood and CSF samples must be collected. To perfuse the animal, a small cut was placed in the right atrium to relieve pressure from the vascular system before perfusing the animal transcardially with 1XPBS via injection into the left ventricle. Completion of perfusion and clearance of the vascular system was indicated by a blanching of the liver.

Whole Animal Perfusion

First confirming deep anesthetization via toe pinch, animals are secured to a surgical board or tray using needles or pins and abdomen wetted with 70% ethanol followed by an incision along the ventral midline along the entire ventral surface, exposing the underlying muscle of the thorax and abdomen. An additional incision is made into this underlying muscle and cut to puncture the diaphragm, taking care not to cut any major blood vessels or the lungs. To expose the heart the ribcage can be cut along the lateral borders and removed. A small incision is made in the right atrium of the heart to relieve diastolic pressure and begin removal of blood from the vascular system. To clear the vascular system of all blood a butterfly catheter needle is inserted into the left ventricle attached to a perfusion pump. Approximately 10mL of 1xPBS solution will clear the system of a 20g animal. Once the system has been cleared of blood the liver will appear very pale and PBS will be noticed exiting the right atrium. At this time organs of interest were collected as indicated.

Non-fasted blood collection and analysis

Blood was collected by cardiac puncture from non-fasted, anesthetized animals (see Perfusion method) at harvest prior to incision of the right atrium and subsequent perfusion. A 25-gauge EDTA-coated needle, attached to a 1mL syringe, is inserted into the right atrium of the exposed heart and the plunger gently pulled to slowly aspirate approximately 500mL of blood, avoiding entrapping air in the syringe to prevent hemolysis. After removal of the needle from the syringe, the blood was slowly injected into a 1.5mL EDTA coated MAP-K2 blood microtainer (363706, BD, San Jose, CA) on ice. Blood tubes were spun at 4°C and 4,388xg for 15 minutes. Blood serum is then removed and aliquoted equally into three replicate 1.5mL tubes on ice. Tubes were then snap frozen on dry ice and stored long-term at -80°C. Thawed blood plasma collected from non-fasted mice was then analyzed by Beckman Coulter AU680 chemistry analyzer (Beckman Coulter, Brea, CA) and Siemens Advia 120 (Germany) for levels of non-fasted glucose, total cholesterol, LDL (low-density lipoproteins), HDL (high-density lipoproteins), triglycerides, and NEFA (non-essential fatty acids).

Brain harvest

Anesthetized and subsequently perfused animals were decapitated, and heads submerged quickly in cold 1xPBS. The skin was cut from the base of the neck, over the top of the skull, between the ears, stopping between the eyes, and separated to either side to expose the skull. The skull was cut dorsally until the cerebellum. Two opposite, horizontal cuts were made in the skull under, but without cutting, the cerebellum to ease separation of the skull at the midline. On the top of the skull, scissor blades were inserted superficially at the bregma and slowly expanded to separate the skull down the midline. With a pair of blunt-end forceps, the two skull plates were removed to expose the brain. The brain was carefully removed from the skull, weighed, and divided midsagitally, into left and right hemispheres, using a brain matrix. The right hemisphere was quickly homogenized on ice and equally aliquoted into three cryotubes for metabolomic, proteomic, and transcriptomic analysis. Cryotubes were immediately snap frozen on dry ice, and stored long-term at -80°C. The left hemisphere was immediately placed in 5mL 4% PFA at 4°C for no less than 24 hours, but no longer than 30 hours. The left hemisphere was then moved from PFA solution to 10mL 15% sucrose at 4°C for 24 hours, or until it sinks in the sucrose, when it was then transferred to a 30% sucrose

for 24 hours at 4°C, or until it sinks in the solution. The left hemisphere was then removed from 30% sucrose solution, snap frozen on a flat mold, cut-side down, floating in 2-methylbutane solution cooled by dry ice. Once frozen the left hemisphere is then placed into a cryotube and stored at -80°C until used for microtome sectioning and immunohistochemistry analysis.

Immunohistochemistry and microscopy imaging

During harvest, whole mouse brains were removed and weighed. Using a brain matrix, left and right hemispheres were separated along the midsagittal plane. The left hemisphere was placed in 5mL of 4% PFA at 4°C overnight, then moved to 10mL of 15% sucrose at 4°C overnight, before finally being incubated in 10mL of 30% sucrose at 4°C overnight or until brain sinks to bottom of the tube. The left hemisphere was then snap frozen and stored at -80°C until sectioned. Left hemispheres (see preparation in Brain harvest method) were cut via Thermo Scientific HM430 sliding microtome at 25μm thickness. Coronal brain tissue sections were oriented to capture the cortex and hippocampus at approximately Bregma: -2.75mm and Interaural 1.05mm. Each section was placed into cryoprotectant buffer (37.5% 1xPBS, 31.25% glycerol, 31.25% ethylene glycol) for immediate use or long-term storage at -20°C. Each section was tracked so as to store 100 sections equally distributed over 10 groups, so each of the 10 groups had equal representation of the 10 sections from forebrain to hindbrain and hippocampus. Of these 10 groups of 10 25-micron sections, 7 will be used for standardized staining combinations highlighting cell types and markers of interest: (1) Vascular [CD31/Iba1/Fibrin/DAPI]; (2) Neuritic plaques [Lamp1/Iba1/X34]; (3) Astrocytes and microglia [GFAP/Iba1/S100b/DAPI]; (4) Neurons [NeuN/Ctip2/DAPI]; (5) Plaques [ThioS]; (6) Luxol Fast Blue/Cresyl Violet; (7) Haematoxylin and eosin; and (8) Prussian blue (Iron stain). Floating sections were then blocked prior to immunohistochemical staining and mounting. After blocking slides with 10% normal donkey serum or normal goat serum diluted in 1xPBS+0.5%Triton wash buffer all antibodies were washed floating in 1xPBT (1x PBS with 0.5% Triton) wash buffer after blocking for 1 hour at room temperature on shaker in 10% NGS (normal goat serum) or 10% NDS (normal donkey serum) in 1xPBT. Secondary antibodies were incubated in 10% NGS or NDS in 1xPBT for 1 hour at room temperature, followed by washes in 1xPBT before mounting on to slides. Each staining combination of 10 sections were placed onto one slide. DAPI, X34, and ThioS stains were performed on the slide following immunostaining. Slides were

then imaged on a Leica Versa slide scanner, automated fluorescent microscope system (Leica, Allendale, NJ). For further analysis, regions of the cortex and hippocampus were processed using CellProfiler (Cambridge, MA) or Imaris (Bitplane, Concord, MA) software to quantify cell counts, fluorescence intensity, and surface area ratios. [CD31 (R&D Systems, MAB3628, 1:500), Iba1 (Wako, 019-19741, 1:300), Fibrin (abcam, ab118533, 1:500), DAPI (1:1000), Lamp1 (abcam, ab25245, 1:500), X34 (0.04% in 40% ethanol), GFAP (Origene, AP31806PU-N, 1:1000), S100b (Thermo Fisher, PA175395, 1:1000), NeuN (abcam, ab104225, 1:500), Ctip2 (abcam, ab18465, 1:1000), and ThioS (1% in 50% ethanol)]

Radiopharmaceuticals

Regional brain glycolytic metabolism was monitored using 2-[18F]-fluoro-2-deoxy-D-glucose (18F-FDG) and was synthesized, purified, and prepared according to established methods (77), where clinical unit doses ranging from 185 to 370 MBq (5 to 10 mCi) were purchased from PETNet Indiana (PETNET Solutions Inc). To evaluate region brain perfusion, Copper(II) pyruvaldehyde bis(N4-methylthiosemicarbazone) labeled with 64Cu (64Cu-PTSM) was synthesized, purified, and unit doses (i.e., 370 to 740 MBq (10 to 25 mCi)) dispensed by the PET Radiochemistry Core Facility at Indiana University according methods described previously (78, 79).

Magnetic Resonance Imaging (MRI)

To provide high contrast grey matter images, at least two days prior to PET imaging, mice were induced with 5% isoflurane (balance medical oxygen), placed on the head coil, and anesthesia maintained with 1-3% isoflurane for scan duration. High resolution T2-weighted (T2W) MRI images were acquired using a 3T Siemens Prisma clinical MRI scanner outfitted with a dedicated 4 channel mouse head coil and bed system (RapidMR, Columbus OH). Images were acquired using a SPACE3D sequence (80) using the following acquisition parameters: TA: 5.5min; TR: 2080ms; TE: 162ms; ETL: 57; FS: On; Ave: 2; Excitation Flip Angle: 150; Norm Filter: On; Restore Magnetization: On; Slice Thickness 0.2mm: Matrix: 171x192; FOV: 35x35mm, yielding 0.18 x 0.18 x 0.2mm resolution images. At the completion of the imaging period, mice we returned to their warmed home cages, and allowed to recover.

Positron Emission Tomography (PET) Imaging

To evaluate changes in cerebral glycolysis (18F-FDG) and cerebral perfusion (64Cu-PTSM) mice were placed in a restrainer and consciously injected into the peritoneal or tail vein, respectively, with 3.7-11.1 MBq (0.1-0.3 mCi) of purified, sterile radiotracer, where the final volume did not exceed 10% of the animal's body weight. Each animal was returned to their warmed home cage and allowed 30 min (18F-FDG) or 5 min (64Cu-PTSM) to allow for uptake and cellular trapping (81, 82). Post-uptake, mice were induced with 5% isoflurane gas, placed on the scanner imaging bed, and anesthesia maintained at 1-3% isoflurane (balance medical oxygen) during acquisition. In all cases, calibrated PET acquisition was performed in list mode for 15 (18F-FDG) or 30 (64Cu-PTSM) min on an IndyPET3 scanner (83), where random prompts did not exceed 10% of the total prompt rate. Post-acquisition, the images were reconstructed into a single-static image with a minimum field of view of 60 mm using filtered-back-projection (FBP), and were corrected for decay, random coincidence events, and dead-time loss (84).

Autoradiography

To provide secondary confirmation of the in vivo PET images, and to quantify tracer uptake regionally, brains were extracted post rapid decapitation, gross sectioned along the midline, slowly frozen on dry ice, then embedded in cryomolds with Optimal Cutting Temperature (OCT) compound (Tissue-Tek). Thin frozen sections (20 um) were obtained via cryotomy at prescribed bregma targets (n=6 bregma/mouse, 6 replicates/bregma) according to stereotactic mouse brain coordinates (85). Sections were mounted on glass slides, air dried, and exposed on BAS Storage Phosphor Screens (SR 2040 E, Cytiva Inc.) for up to 12 hrs. Post-exposure, screen were imaged via Typhoon FL 7000IP (GE Medical Systems) phosphor-imager at 25 um resolution along with custom 18F or 64Cu standards described previously (86).

Image Analysis

All PET and MRI images were co-registered using a ridged-body mutual information-based normalized entropy algorithm (87) with 9 degrees of freedom, and mapped to stereotactic mouse brain coordinates (85) using Analyze 12 (AnalyzeDirect, Stilwell KS). Post-registration, 56 regions bilateral regions were extracted via brain atlas, and averaged to yield 27 unique volumes of interest that map to key cognitive and

motor centers that includes: Agranular Insular Cortex; Auditory Cortex; Caudate Putamen, Cerebellum; Cingulate Cortex; Corpus Callosum; Dorsolateral Orbital Cortex; Dorsintermed Entorhinal Cortex; Dysgranular Insular Cortex; Ectorhinal Cortex; Fornix; Frontal Association Cortex; Hippocampus; Lateral Orbital Cortex; Medial Orbital Cortex; Parietal Cortex; Parietal Association Cortex; Perirhinal Cortex; Prelimbic Cortex; Primary Motor Cortex; Primary Somatosensory Cortex; Retrosplenial Dysgranular Cortex; Secondary Motor Cortex; Secondary Somatosensory Cortex; Temporal Association Cortex, Thalamus; Ventral Orbital Cortex; Visual Cortex. For autoradiographic analysis, tracer uptake was quantified on hemi-coronal sections by manually drawing regions of interest for 17 regions of interest (i.e. Auditory Cortex, Caudate Putamen, Cerebellum, Cingulate Cortex, Corpus Callosum, Dorso-intermed Entorhinal Cortex, Dysgranular Insular Cortex, Ectorhinal Cortex, Hippocampus, Hypothalamus, Medial Septum, Primary Motor Cortex, Primary Somatosensory Cortex, Retrosplenial Dysgranular Cortex, Temporal Association Cortex, Thalamus, Visual Cortex) on calibrated phosphor screen at bregma 0.38, -1.94, and -3.8 mm using MCID (InterFocus Ltd). To permit dose and brain uptake normalization, Standardized Uptake Value Ratios (SUVR) relative to the cerebellum were computed for PET and autoradiograms for each subject, genotype, and age as follows:

$$SUVR(s,R,g,a) = \frac{R(s,g,a)}{C(s,g,a)} \quad (1)$$

where, s, g, a, R, and C are the subject, genotype, age, region/volume of interest, cerebellum region/volume of interest. In all cases, region/volumes of interest were analyzed for differences with time and genotype using a Two-Way ANOVA (Prism, GraphPad Inc.), where significance was taken at $p<0.05$.

Immunoprecipitation

Tissue samples were homogenized in tissue protein extraction reagent (T-PER ThermoScientific) supplemented with protease and phosphatase inhibitors cocktail (Sigma-Aldrich). Protein concentration was measured using bicinchoninic acid (BCA) (Pierce). Immunoprecipitation was performed by incubating a total of 1500ug of brain protein extract with 1ug of biotinylated sheep anti-Trem2 antibody (RnD systems BAF1729) overnight at 4C, followed by incubation with streptavidin sepharose beads (CST 3419) for 6 hours at 4C, washed 3 times with ice cold PBS with 0.1% Tween 20. Protein was eluted in sample loading buffer with 1mM DTT followed by separation via Western blot (see below).

Western immunoblot

Snap-frozen right hemispheres were homogenized by hard tissue homogenizer (USA Scientific, Ocala, FL) and lysed in 1mL RIPA buffer (R0278, Sigma, St. Louis, MO) supplemented with protease and phosphatase inhibitor reagents (1861281, Thermo Fisher Scientific, Waltham, MA). Lysates were incubated for 1 hour at 4°C before pelleting insoluble proteins by spinning at 4°C, 11,000xg for 15 minutes. Protein concentration was determined by Bradford protein assay (Biorad, Hercules, CA), according to manufacturer's instructions. Samples were mixed with 10x Laemlli buffer (42556.01, Amsbio, Cambridge, MA), boiled for 10 minutes, and run on 12% SDS PAGE gels (456-1044, BioRad) with colorimetric ladder (RPN800E, GE, Boston, MA). Gels were transferred to PVDF membranes for immunoblotting and imaging using an iBlot2 dry blotting system (Thermo Fisher). Membranes were blocked in 5% non-fat dry milk in 1xPBS+0.1% Tween20 for 1 hour prior to incubating with primary antibodies diluted in 5% non-fat dry milk in 1xPBS+0.1% Tween20 for 1 hour at room temperature. Membranes were washed in 1xPBS+0.1% Tween20 before incubating with secondary antibodies diluted in 5% non-fat dry milk in 1xPBS+0.1% Tween20. HRP-conjugated secondary antibodies targeting primary antibody host IgG were incubated at 1 hour at room temperature. Membranes were washed in 1xPBS+0.1% Tween20 before digital imaging with SuperSignal West Pico PLUS chemiluminescent substrate (34579, Thermo Fisher). Images for immunoblot were quantified using ImageJ 1.8.0 version. Proteins of interest were visualized with the following primary antibodies against: ACTIN (Abcam, ab179467), GAPDH (abcam, ab9483), APOE4 (Novus, NBP1-49529), TREM2 (R&D Systems, MAB1729), and Alpha-tubulin (Sigma-Aldrich, T9026-100UL).

Cytokine Panel Assay

Hemibrains were homogenized in tissue homogenization buffer containing fresh protease inhibitor cocktail and aliquoted. Supernatant was utilized for the cytokine analysis. Mouse hemibrain samples were assayed in duplicate using the MSD mouse proinflammatory Panel I, a highly sensitive multiplex enzyme-linked immunosorbent assay (ELISA). The panel quantifies 10 cytokines: interferon gamma (IFN-γ), interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and tumor necrosis factor α (TNFα) from a single small sample volume (25µL) using an electrochemiluminescent detection method (MesoScale Discovery,

Gaithersburg, MD, USA). The mean intra-assay coefficient for each cytokine was <8.5%, based on cytokine standards. Any value that was below the lowest limit of detection (LLOD) for the cytokine assay was replaced with ½ LLOD of the assay for statistical analysis.

RNA-sequencing experimental design

RNA-Seq data were obtained from whole left hemisphere brain samples from APOE4 KI mouse, carrying a humanized version of the prominent *APOEε4* genetic risk factor for LOAD, and the Trem2*R47H mouse, carrying a rare deleterious variant R47H allele of *Trem2* gene. In addition, a mouse model expressing both human *APOEε4* and the *Trem2*R47H* mutation was used to compare the transcriptional changes in mice carrying both variants to mice carrying only a single risk allele and B6 controls. Whole-brain left hemispheres were collected at 4, 8, 12, and 24 months of age from both sexes.

Study population:

Mouse Models	**4M**		**8M**		**12M**		**24M**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
C57BL/6J	12	12	6	6	6	6	7	6
APOE4 KI	13	12	6	6	4	5	5	6
TREM2*R47H	12	12	6	6	6	6	6	3
APOE4.TREM2*R47H	10	12	5	6	8	5	7	6

RNA sample extraction

Total RNA was extracted from snap frozen right brain hemispheres using Trizol (Invitrogen, Carlsbad, CA). mRNA was purified from total RNA using biotin-tagged poly dT oligonucleotides and streptavidin-coated magnetic beads and quality was assessed using an Agilent Technologies 2100 Bioanalyzer (Agilent, Santa Clara, CA).

RNA-Sequencing assay library preparation

Sequencing libraries were constructed using TruSeq DNA V2 (Illumina, San Diego, CA) sample prep kits and quantified using qPCR (Kapa Biosystems, Wilmington, MA). The mRNA was fragmented, and double-stranded cDNA was generated by random priming. The ends of the fragmented DNA were converted into phosphorylated blunt ends. An 'A' base was added to the 3' ends. Illumina®-specific adaptors were ligated to the DNA fragments. Using magnetic bead technology, the ligated fragments were size-selected and then a final PCR was performed to enrich the adapter-modified DNA fragments, since only the DNA fragments with adaptors at both ends will amplify.

RNA-Sequencing

Libraries were pooled and sequenced by the Genome Technologies core facility at The Jackson Laboratory. All samples were sequenced on Illumina HiSeq 4000 using HiSeq 3000/4000 SBS Kit reagents (Illumina), targeting 30 million read pairs per sample. Samples were split across multiple lanes when being run on the Illumina HiSeq, once the data was received the samples were concatenated to have a single file for paired-end analysis.

RNA-Sequencing data processing

Sequence quality of reads was assessed using FastQC (v0.11.3, Babraham). Low-quality bases were trimmed from sequencing reads using Trimmomatic (v0.33) (88). After trimming, reads of length longer than 36 bases were retained. The average quality score was greater than 30 at each base position and sequencing depth were in range of 60 - 80 million reads. RNA-Seq sequencing reads from all samples were mapped to the mouse genome (version GRCm38.p6) using ultrafast RNA-Seq aligner STAR (v2.5.3) (89). To measure human *APOE* gene expression, we created a chimeric mouse genome by concatenating human *APOE* gene sequence (human chromosome 19:44905754-44909393) into mouse genome (GRCm38.p6) as a separate chromosome (referred as chromosome 21 in chimeric mouse genome). Subsequently, we added gene annotation of human *APOE* gene into mouse gene annotation file. Additionally, we have also introduced annotation for novel *Trem2* isoform in mouse gene annotation file

(GTF file), that is identical to primary transcript, but truncated exon2 by 119 bp from its start position. Afterwards, a STAR index was built for this chimeric mouse genome sequence for alignment, then STAR aligner output coordinate-sorted BAM files for each sample mapped to chimeric mouse genome using this index. Gene expression was quantified in two ways, to enable multiple analytical methods: transcripts per million (TPM) using RSEM (v1.2.31) (90), and raw read counts using HTSeq-count (v0.8.0) (4).

Differential expression analysis

Differential expression in mouse models was assessed using the R Bioconductor package DESeq2 (v1.16.1) (91). DESeq2 takes raw read counts obtained from HTSeq-count as input. Genes with the Benjamini-Hochberg corrected p-values < 0.05 were considered as significantly differentially expressed genes.

Principal component analysis

We analyzed a total of 234 RNA-Seq samples originating from different mouse models at different ages and sex. First, dispersion parameter for each gene was estimated using DESeq2 R package (91). Afterwards, we applied the varianceStabilizingTransformation (vst) function of DESeq2 (91) to the read count data in order to produce a data matrix in which expression levels are homoscedastic. Finally, we extracted the principal components using the plotPCA function of DESeq2 in R.

Functional enrichment analysis

Functional annotations and enrichment analyses were performed using the R Bioconductor package clusterProfiler (92), with Gene Ontology terms and KEGG pathways enrichment analyses performed using functions enrichGO and enrichKEGG, respectively. The function compareCluster was used to compare enriched functional categories of each gene module. The significance threshold for all enrichment analyses was set to 0.05 using Benjamini-Hochberg adjusted p-values.

Human post-mortem brain cohorts and co-expression module identification

Whole-transcriptome data for human post-mortem brain tissue was obtained from the Accelerating Medicines Partnership for Alzheimer Disease-(AMP-AD) consortium, which is a multi-cohort effort to harmonize genomics data from human LOAD patients. Harmonized co-expression modules from the AMP-AD data sets were obtained from the AD Knowledge Portal (DOI: 10.7303/syn11932957.1). The human co-expression modules derive from three independent LOAD cohorts, including 700 samples from the ROS/MAP cohort, 300 samples from the Mount Sinai Brain bank and 270 samples from the Mayo cohort. A detailed description on post-mortem brain sample collection, tissue and RNA preparation, sequencing, and sample QC has been provided elsewhere (12, 13, 93). As part of a transcriptome-wide meta-analysis to decipher the molecular architecture of LOAD, 30 co-expression modules from seven different brain regions across the three cohorts have been recently identified (6). Briefly, Logsdon et al. (6) identified 2,978 co-expression modules using multiple techniques across the different regions after adjusting for co-variables and accounting for batch effects (10.7303/syn10309369.1). A total of 660 co-expression modules were selected based on a specific enrichment in LOAD cases when compared to controls (10.7303/syn11914606). Finally, multiple co-expression module algorithms were used to identify a set of 30 aggregate modules that were replicated by the independent methods (6).

Mouse-Human correlation analysis

First, we performed differential gene expression analysis for each mouse model compared to age and sex-matched B6 control mice using the limma (94) package in R. Afterwards, we computed correlation between changes in expression (log fold change) for all DE genes in a given module with the fold changes for each mouse model (specified by genotype, sex, diet, and age). Correlation coefficients were computed using cor.test function in R as:

$$\text{cor.test}(\ \text{LogFC(h)}, \text{LogFC(m)}\ ) \qquad (1)$$

where LogFC(h) is the log fold change in transcript expression of human AD patients compared to control patients and LogFC(m) is the log fold change in expression of mouse transcripts compared to control mouse models. LogFC values for human transcripts were obtained via the AD Knowledge Portal (https://www.synapse.org/#!Synapse:syn11180450).

Statistical analysis

Statistical analyses was constrained to comparisons between littermate-controlled subjects only. Student's t-test was employed on data sets differing by a single variable (e.g., age, sex, genotype). ANOVA was used in data sets where multiple factors are considered and combined for possible synergistic effects.

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FIGURE LEGENDS

Figure 1. Phenotyping strategy for novel mouse strains carrying late-onset Alzheimer's disease (LOAD) risk factors. Cohorts aged 4, 8, 12, and 24 months first undergo physical wellness and behavioral evaluation to identify age-related deficits in locomotor activity and cognition. Live assessment also includes in vivo imaging by PET/MRI to measure changes in brain glucose metabolism and perfusion. Post-mortem analysis of brain tissue and non-fasted blood serum aimed to identify biomarkers of inflammation, metabolic disease, gene expression, and neurodegeneration.

Figure 2. Age is primary factor driving increases in measures of frailty. Cross-sectional cohorts of young and old mice inspected for measures of physical well-being, or frailty (A,B), including body weight (C,D). Post-mortem, non-fasted, blood biochemistry analysis provided serum levels of cholesterol,

lipoproteins, lipids, and glucose (E-L). Age-dependent differences within genotype determined by ANOVA: *p<0.05; **p<0.01; ***p<0.001. All alleles expressed were homozygous.

Figure 3. Behavioral testing of LOAD1 mice to identify functional neurophenotype. Performance of young and aged mice carrying combinations of *APOEε4* and *Trem2*R47H* alleles were evaluated for measures of coordination (rotarod; A,B), locomotor activity (open field; C-F), exploratory drive (open field; G,H), and spatial working memory (spontaneous alternation in Y-maze; I-L). Age-dependent differences within genotype determined by ANOVA: *p<0.05; **p<0.01; ***p<0.001. All alleles expressed were homozygous.

Figure 4. Variations in regional glucose metabolism due to expression of *APOEε4* and *Trem2*R47H*. Positron emission tomography (PET; red scale) of radioactive 18F-FDG marker was used to measure tissue glucose uptake, guided by magnetic resonance imaging (MRI) (black and white) mapping to brain regions of interest, indicated by bregma coordinates (far left) (A). Intensity of PET signal in brains regions, normalized to cerebellum, are quantified in B,C. Post-mortem autoradiography of coronal brain tissue is represented in A (rainbow; far right). Genotype-dependent differences determined by ANOVA: *p<0.05; **p<0.01; ***p<0.001. All alleles expressed were homozygous.

Figure 5. *In vivo* neuroimaging reveals differences in regional perfusion driven by expression of risk alleles. Positron emission tomography (PET; red scale) of radioactive 64Cu-PTSM marker was used to measure tissue perfusion, guided by magnetic resonance imaging (MRI) (black and white) mapping to brain regions of interest, indicated by bregma coordinates (far left) (A). Intensity of PET signal in brains regions, normalized to cerebellum, are quantified in B,C. Post-mortem autoradiography of coronal brain tissue is represented in A (rainbow; far right). Genotype-dependent differences determined by ANOVA: *p<0.05; **p<0.01; ***p<0.001. All alleles expressed were homozygous.

Figure 6. Neuropathological analysis of cortex and hippocampus. Hematoxylin and eosin (H&E), luxol fast blue/cresyl violet (LFB/CV), and Prussian blue staining tissue staining protocols (A) to identify

anatomical changes in the cortex and hippocampus of young and aged male mice. Thioflavin S (Thios) and four combinations of known neuropathological markers of LOAD in aged male mice (B); 5xFAD included as amyloidogenic positive control. All alleles expressed were homozygous.

Figure 7. Overview of Brain Transcriptome. Expression levels of mouse *Apoe* (A), human *APOE* (B), and mouse *Trem2* (C) genes in the B6.*APOE4,* B6.*Trem2*R47H,* B6.*APOE4.Trem2*R47H* and C57BL/6J mice at 4, 8, 12 and 24 months in both sexes. Principal component analysis (PCA) of RNA-Seq transcriptomics data from all 234 samples (D). The percent of variation explained by each principal component is displayed on the corresponding axis. Female and male samples are represented as circles and triangles, respectively. Genotypes are shown by different colors and increasing size of points correspond to increasing age of mice (4, 8, 12, and 24 months respectively). All alleles expressed were homozygous.

Figure 8. KEGG pathways enrichment analysis. Significantly enriched KEGG pathways ($p < 0.05$) in the downregulated and upregulated list of genes across mouse models at different ages for both sexes. All alleles expressed were homozygous.

TABLES

Table 1

Common name	JAX stock #	Strain	Background	Gene location	Allele name	Allele type	Additional considerations
B6	000664	C57BL/6J	-	-	-	-	-
Trem2*R47H	027918	C57BL/6J-Trem2^{em1Adiuj}/J	C57BL/6J	Chr17:48346401-48352276	Trem2 R47H KI	Cas9 endonuclease-mediated (humanized sequence)	- Two silent mutations (lysine AAG>AAA and alanine GCC>GCA) into *Trem2* - R47H mutation also introduces a cryptic splice acceptor site in exon 2, creating a novel splice variant with a deletion of 119bp at the 5' end of exon 2.
APOE4	027894	B6(SJL)-Apoe$^{tm1.1(APOE*4)Adiuj}$/J	C57BL/6J	Chr7:19696109-19699188	APOE4 KI	FRT site flanked PGK- neo cassette targeted mutation (gene replacement)	- Exons 2, 3 and a majority of exon 4 of the mouse *Apoe* gene were replaced by exons 2, 3, and 4 of the human *APOE* gene sequence (including a portion of the 3' UTR sequence) - Expression of FLP recombinase was used to remove the FRT site flanked PGK-neo cassette and subsequently backcrossed to remove FLP recombinase
APOE4. **Trem2*R47H**	028709	B6(SJL)-Apoe$^{tm1.1(APOE*4)Adiuj}$ Trem2^{em1Adiuj}/J	C57BL/6J	(see above)	(see above)	(see above)	(see above)

Table 1. Description of novel mouse strains expressing human LOAD risk alleles. Mouse strains expressing *Trem2*R47H* and *APOEε4* human late-onset Alzheimer Disease risk factors, alone or in combination, developed on the C57BL/6J background and distributed by The Jackson Laboratory [https://www.jax.org/mouse-search].

Table 2

		Cumulative Frailty Index Score								Core Body Temperature (°C)								Animal Weight							
		Female				Male				Female				Male				Female				Male			
	Genotype	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM
4 month	C57BL/6J	16	1.375	55.55%	0.1909	16	1.938	57.99%	0.2809	16	36.36	1.34%	0.1221	16	36.39	1.98%	0.1802	16	21.76	8.61%	0.4684	16	28.68	4.89%	0.3507
	Trem2*R47H	11	1.682	33.30%	0.1689	12	2.542	37.94%	0.2784	11	36.05	0.53%	0.05778	12	36.36	1.23%	0.1294	11	19.21	5.84%	0.3383	12	26.5	5.23%	0.4002
	APOE4	12	1.625	26.65%	0.125	12	2.875	40.70%	0.3378	12	36.45	1.48%	0.1555	12	36.25	1.16%	0.1215	12	22.25	7.71%	0.4949	12	28.32	10.49%	0.8576
	APOE4. Trem2*R47H	10	1.5	60.86%	0.2887	11	2.636	31.84%	0.2531	12	36.45	1.48%	0.1555	12	36.25	1.16%	0.1215	12	22.25	7.71%	0.4949	12	28.32	10.49%	0.8576
8 month	C57BL/6J	18	2.111	38.58%	0.192	18	3.167	29.66%	0.2214	18	36.48	1.07%	0.09202	18	36.29	2.03%	0.1737	18	25.09	9.16%	0.5418	18	34.09	5.58%	0.4487
	Trem2*R47H	12	1.75	31.06%	0.1569	12	2.458	25.22%	0.179	12	36.21	1.13%	0.1184	12	36.06	1.38%	0.1433	12	22.25	3.90%	0.2503	12	26.97	9.27%	0.722
	APOE4	12	2.208	26.37%	0.1681	12	3.583	18.66%	0.193	12	36.5	0.90%	0.09535	12	36.05	1.95%	0.2024	12	22.66	13.79%	0.9023	12	31.32	11.53%	1.042
	APOE4. Trem2*R47H	11	2.364	30.05%	0.2142	12	3.083	28.37%	0.2525	12	36.5	0.90%	0.09535	12	36.05	1.95%	0.2024	12	22.66	13.79%	0.9023	12	31.32	11.53%	1.042
12 month	C57BL/6J	21	5.048	16.55%	0.1823	23	5.043	12.65%	0.133	21	35.97	0.93%	0.07314	23	35.5	1.16%	0.08562	21	31.93	16.48%	1.148	23	41.31	8.02%	0.6906
	Trem2*R47H	11	3.136	31.96%	0.3022	13	5.038	11.07%	0.1546	11	36.24	1.44%	0.1568	13	35.36	1.17%	0.1147	11	25.47	16.86%	1.295	13	36.53	11.14%	1.128
	APOE4	10	2.75	33.47%	0.2911	11	5.273	18.17%	0.2889	10	36.13	1.05%	0.1202	11	35.62	1.43%	0.153	10	23.6	8.96%	0.6687	11	37.2	16.59%	1.861
	APOE4. Trem2*R47H	10	2.71	24.50%	0.21	12	5.042	24.46%	0.356	10	36.13	1.05%	0.1202	11	35.62	1.43%	0.153	10	23.6	8.96%	0.6687	11	37.2	16.59%	1.861
24 month	C57BL/6J	9	5	43.59%	0.7265	12	6.125	22.59%	0.3995	9	36.48	1.44%	0.1746	12	35.85	2.71%	0.28	9	29.91	12.29%	1.225	12	34.9	19.76%	1.99
	Trem2*R47H	14	3.714	42.15%	0.4184	13	5.731	22.13%	0.3518	14	36.51	1.00%	0.09713	13	36.48	1.26%	0.1272	14	30.34	14.08%	1.142	13	40.68	15.90%	1.794
	APOE4	11	4.045	28.96%	0.3533	11	6.091	11.50%	0.2113	11	36.2	1.19%	0.13	11	36.04	2.44%	0.2647	11	32.14	14.00%	1.356	11	37.29	10.50%	1.18
	APOE4. Trem2*R47H	5	4.5	33.33%	0.6708	8	6.313	13.35%	0.2979	5	36.5	1.71%	0.2793	8	35.89	2.53%	0.3215	5	32.4	13.44%	1.948	8	36.24	13.32%	1.706

Table 2. Complete data set of frailty assay measures of LOAD strains by age. Individual animals from cross-sectional cohorts housed to the ages indicated were assessed by 27 physical measures to provide a Cumulative Frailty Index Score. Animals were also measured for core body temperature and weight. All alleles expressed were homozygous.

Table 3

		Total Cholesterol (mg/dL)								LDL (mg/dL)								HDL (mg/dL)							
		Female				Male				Female				Male				Female				Male			
	Genotype	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM
4 month	C57BL/6J	13	66.23	17.70%	3.251	17	82.94	19.68%	3.96	13	4.784	29.30%	0.3887	17	3.368	51.05%	0.417	13	49.04	17.85%	2.427	17	62.94	18.62%	2.841
	Trem2*R47H	11	53.45	11.08%	1.786	12	82.58	22.02%	5.249	11	4.555	12.96%	0.178	12	2.041	22.38%	0.1319	11	40.9	9.43%	1.163	12	65.09	22.11%	4.154
	APOE4	10	55.4	14.52%	2.544	11	57.45	15.10%	2.616	10	1.244	34.83%	0.137	11	0.6791	52.92%	0.1084	10	39.07	16.36%	2.021	11	46.29	15.39%	2.148
	APOE4. Trem2*R47H	10	52.5	19.18%	3.184	10	58.6	27.75%	5.143	10	1.353	35.95%	0.1538	10	0.567	39.15%	0.07019	10	36.54	19.90%	2.3	10	48.61	27.79%	4.272
8 month	C57BL/6J	6	80.17	19.75%	6.462	12	81.92	16.12%	3.813	6	5.435	27.64%	0.6134	12	3.712	54.96%	0.5889	6	56	24.70%	5.647	12	60.25	16.47%	2.865
	Trem2*R47H	11	58.64	12.11%	2.142	11	72.27	18.52%	4.036	11	4.421	15.95%	0.2125	11	1.905	23.06%	0.1324	11	41.14	17.10%	2.122	11	56.25	17.65%	2.993
	APOE4	12	49.5	15.80%	2.258	11	64	17.73%	3.422	12	1.375	34.94%	0.1387	11	0.6582	44.12%	0.08755	12	34.63	22.43%	2.242	11	50.42	20.76%	3.155
	APOE4. Trem2*R47H	11	55.55	25.30%	4.237	11	71.55	23.44%	5.057	11	1.179	41.38%	0.1471	11	0.5991	26.80%	0.0484	10	40.15	30.99%	3.935	10	56.81	26.57%	4.774
12 month	C57BL/6J	11	75.27	16.37%	3.715	10	87.9	15.04%	4.181	12	3.858	31.94%	0.3558	11	2.55	15.34%	0.118	12	56.44	16.36%	2.665	11	70.79	20.51%	4.377
	Trem2*R47H	10	67.6	22.28%	4.764	12	93.92	17.46%	4.733	11	5.204	28.39%	0.4454	13	2.761	49.52%	0.3792	11	51.31	17.45%	2.7	13	73.06	17.20%	3.485
	APOE4	14	51.29	11.35%	1.556	13	56.31	18.43%	2.879	13	0.7246	44.08%	0.0886	14	0.3929	75.55%	0.07932	14	37.7	16.57%	1.67	14	45.96	19.08%	2.344
	APOE4. Trem2*R47H	3	53.67	5.99%	1.856	8	61.13	14.00%	3.026	4	1.013	57.61%	0.2916	8	0.5313	59.98%	0.1127	4	39.98	3.60%	0.7204	9	50.72	16.56%	2.8
24 month	C57BL/6J	13	71	28.76%	5.664	15	88.47	25.69%	5.869	13	5.576	36.69%	0.5675	14	4.811	44.53%	0.5725	13	49.53	24.92%	3.423	17	63.38	39.15%	6.018
	Trem2*R47H	13	64.69	31.49%	5.649	13	89.08	35.66%	8.809	13	3.378	83.16%	0.7791	12	4.32	58.79%	0.7331	13	44.88	29.78%	3.708	13	62.94	43.50%	7.593
	APOE4	10	60	32.06%	6.083	10	74	18.65%	4.364	8	1.293	59.70%	0.2728	10	1.024	73.53%	0.2381	10	40.35	25.04%	3.195	10	56.59	22.69%	4.06
	APOE4. Trem2*R47H	5	73	28.29%	9.236	8	90.88	33.19%	10.66	4	0.8675	38.43%	0.1667	7	0.3329	133.09%	0.1674	5	47.64	29.38%	6.26	8	66.89	10.55%	2.495
		Glucose (mg/dL)								NEFA (mg/dL)								Triglycerides (mg/dL)							
		Female				Male				Female				Male				Female				Male			
	Genotype	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM
4 month	C57BL/6J	13	491.6	16.08%	21.93	17	544.3	18.24%	24.07	13	0.3846	38.98%	0.04158	17	0.3271	22.54%	0.01788	13	114.3	31.45%	9.971	17	95.41	47.51%	10.99
	Trem2*R47H	11	446.5	20.24%	27.25	12	558.3	15.78%	25.44	11	0.3509	20.96%	0.02218	12	0.3533	19.09%	0.01948	11	76.45	20.55%	4.737	12	90.33	42.04%	10.96
	APOE4	10	469.6	20.96%	31.12	11	549.9	18.18%	30.14	10	0.383	17.97%	0.02176	11	0.3045	16.04%	0.01473	10	109.5	29.05%	10.06	11	94.64	48.15%	13.74
	APOE4. Trem2*R47H	10	456	20.26%	29.22	10	556.5	27.00%	47.51	10	0.367	22.73%	0.02638	10	0.317	17.02%	0.01707	10	104	38.64%	12.71	10	86.4	32.77%	8.953
8 month	C57BL/6J	6	539.5	25.07%	55.21	12	548.2	17.88%	28.3	6	0.3267	40.32%	0.05377	12	0.2892	23.15%	0.01932	6	107.3	25.66%	11.24	12	82.67	36.13%	8.622
	Trem2*R47H	11	444.9	15.03%	20.17	11	543.3	18.33%	30.03	11	0.2364	26.91%	0.01918	11	0.1836	20.56%	0.01138	11	81.73	35.40%	8.724	11	97.45	31.00%	9.108
	APOE4	11	438.7	19.20%	25.4	11	621.6	23.97%	44.93	12	0.275	22.50%	0.01786	11	0.2536	25.57%	0.01955	12	103.4	22.94%	6.848	11	112.4	17.64%	5.976
	APOE4. Trem2*R47H	11	487.1	16.85%	24.75	12	548.2	17.88%	28.3	11	0.2836	24.99%	0.02137	11	0.2455	21.89%	0.0162	11	109.4	24.24%	7.992	11	118.3	31.06%	11.08
12 month	C57BL/6J	12	535.1	16.00%	24.72	11	548.5	26.20%	43.33	12	0.2225	60.00%	0.03854	11	0.2836	21.63%	0.0185	12	95.17	27.64%	7.595	11	91.82	46.99%	13.01
	Trem2*R47H	11	472	19.56%	27.84	13	572.6	27.03%	42.93	11	0.1845	44.31%	0.02466	13	0.1954	32.61%	0.01767	11	87.09	29.82%	7.829	13	105.6	36.34%	10.64
	APOE4	15	426.1	19.48%	21.43	14	528.4	17.79%	25.13	15	0.2733	42.05%	0.02968	14	0.2814	39.44%	0.02967	15	86.93	21.51%	4.829	14	77.21	34.56%	9.208
	APOE4. Trem2*R47H	4	431	10.96%	23.63	9	599.9	26.28%	52.56	4	0.175	9.90%	0.00866	9	0.2367	24.46%	0.01929	4	111.5	2.97%	1.658	9	139.7	60.38%	28.11
24 month	C57BL/6J	13	347.1	18.85%	18.14	17	294.1	28.96%	20.66	13	0.2762	35.59%	0.02726	16	0.2363	33.75%	0.01993	13	59.62	35.97%	5.947	17	54.24	51.90%	6.827
	Trem2*R47H	13	381.8	15.87%	16.8	13	300.7	43.59%	36.36	13	0.4123	27.26%	0.03118	13	0.2692	33.92%	0.02533	12	68.33	27.51%	5.426	13	52.08	28.81%	4.161
	APOE4	10	303.5	18.33%	17.59	10	336.6	22.70%	24.16	10	0.313	27.65%	0.02737	10	0.259	20.14%	0.0165	10	77.4	42.84%	10.49	10	63.3	36.30%	7.266
	APOE4. Trem2*R47H	5	342.2	13.09%	20.04	8	360.4	16.26%	20.72	5	0.43	22.31%	0.0429	8	0.2475	22.42%	0.01962	4	73	17.44%	6.364	8	52.13	34.66%	6.388

Table 3. Complete data set of non-fasted blood serum biochemistry analysis from novel LOAD strains by age. Blood serum of individual animals from cross-sectional cohorts housed to the ages indicated were measured for a panel of analytes. All alleles expressed were homozygous.

Table 4

		Latency to Fall (sec)							
		Female				Male			
	Genotype	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM
4 month	C57BL/6J	16	183.8	26.34%	12.1	16	151.5	28.86%	10.93
	Trem2*R47H	11	154.8	24.06%	11.23	12	142.3	24.86%	10.21
	APOE4	11	139	29.94%	12.54	12	121.9	54.33%	19.11
	APOE4.Trem2*R47H	10	156.4	33.74%	16.69	11	141.5	33.27%	14.19
8 month	C57BL/6J	18	168.8	26.13%	10.4	18	88.89	51.70%	10.83
	Trem2*R47H	12	122	30.94%	10.89	12	123.9	50.16%	17.94
	APOE4	11	143.4	55.85%	24.15	12	84.47	68.54%	16.71
	APOE4.Trem2*R47H	11	128.7	60.97%	23.66	12	88.64	59.80%	15.3
12 month	C57BL/6J	22	118.5	55.26%	13.96	22	56.8	54.53%	6.603
	Trem2*R47H	10	102.7	47.03%	15.28	13	50.1	83.97%	11.67
	APOE4	10	83.3	78.62%	20.71	11	33.12	151.29%	15.11
	APOE4.Trem2*R47H	10	85.63	63.15%	17.1	12	48.11	84.89%	11.79
24 month	C57BL/6J	14	114.9	52.62%	16.16	16	62.69	74.02%	11.6
	Trem2*R47H	13	72.77	87.45%	17.65	13	19.21	107.48%	5.725
	APOE4	10	52.7	76.40%	12.73	11	63.21	86.31%	16.45
	APOE4.Trem2*R47H	7	88.33	45.15%	15.07	8	54.67	95.66%	18.49

Table 4. Complete data set of rotarod assay measures of LOAD strains. Individual animals from cross-sectional cohorts housed to the ages indicated were assessed by rotarod assay. Three trials were performed and the average latency to fall is provided. All alleles expressed were homozygous.

Table 5

		Percent Alternation (%)								Total Arm Entries							
		Female				Male				Female				Male			
	Genotype	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM
4 month	C57BL/6J	16	47.34	13.60%	1.085	16	56.18	15.06%	1.592	16	46.5	18.46%	2.26	16	43	20.12%	2.055
	Trem2*R47H	11	49.79	13.32%	1.946	11	53.72	18.66%	2.863	11	45.45	20.10%	2.912	12	38.42	16.76%	1.877
	APOE4	11	48.83	16.15%	2.879	12	52.16	20.94%	2.612	11	42.91	27.44%	3.672	12	42.33	24.13%	2.709
	APOE4.Trem2*R47H	10	45.1	29.90%	3.054	11	54.46	18.01%	2.065	10	47.1	23.25%	2.759	11	41.64	18.61%	2.383
8 month	C57BL/6J	18	51.6	16.97%	2.165	18	56.88	17.81%	1.799	18	40.06	17.53%	1.626	18	42.44	19.35%	2.182
	Trem2*R47H	12	56.18	20.21%	2.543	12	54.88	22.55%	2.714	12	41.67	13.82%	1.437	12	40	18.89%	2.25
	APOE4	12	52.05	17.05%	2.813	12	57.45	10.90%	1.759	12	35.83	29.90%	3.035	12	33.75	25.65%	2.437
	APOE4.Trem2*R47H	12	53.15	16.66%	2.539	12	59.13	25.16%	3.457	12	35.42	18.66%	1.921	12	36.25	18.82%	1.919
12 month	C57BL/6J	21	50.18	18.60%	2.103	22	53.91	20.90%	1.634	21	35.33	28.24%	1.934	22	36.64	24.46%	1.825
	Trem2*R47H	11	58.27	13.32%	3.355	13	58.94	20.18%	2.444	11	38.82	14.00%	1.911	13	32.31	23.09%	2.173
	APOE4	10	47.5	19.07%	2.422	11	50.04	17.65%	3.332	10	35.2	18.39%	1.971	11	36.82	17.96%	2.161
	APOE4.Trem2*R47H	10	53.77	20.99%	2.841	12	55.58	22.55%	3.726	10	40.5	14.80%	1.635	12	33.58	25.89%	2.294
24 month	C57BL/6J	13	58.13	17.74%	2.227	16	56.64	23.28%	2.831	13	33.38	37.52%	3.607	16	31.31	26.83%	1.974
	Trem2*R47H	12	55.59	18.90%	3.042	13	60.72	22.94%	3.271	12	39.25	30.03%	2.66	13	29.92	19.88%	1.666
	APOE4	11	54.53	15.20%	1.788	11	58.09	11.86%	2.35	11	39.91	16.09%	2.095	11	33.36	20.97%	1.918
	APOE4.Trem2*R47H	6	54.7	17.54%	2.927	8	58.1	12.51%	2.777	6	39.17	20.44%	3.06	8	31.38	37.64%	3.751

Table 5. Complete data set of spontaneous alternation assay measures of LOAD strains. Individual animals from cross-sectional cohorts housed to the ages indicated were assessed by spontaneous alternation assay in Y-maze. Percentage of successive entries into all three arms in series compared to all arm entries is provided. All alleles expressed were homozygous.

Table 6

		Sum Distance Traveled (cm; 0-60 min)								Total Sum Vertical Activity (0-60min)								Sum Margin Time (sec)							
		Female				Male				Female				Male				Female				Male			
	Genotype	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM	n=	Mean	Coeff. Variation	SEM
4	C57BL/6J	16	10706	18.93%	506.7	16	10184	24.07%	612.8	16	730.9	27.51%	50.27	16	857.1	30.29%	64.91	16	3128	6.17%	48.27	16	3174	7.34%	58.27

	Trem2*R47H	11	8783	20.58%	545	12	8174	18.83%	444.3	11	537.1	29.73%	48.15	12	719.4	30.75%	63.85	11	3251	4.57%	44.77	12	3128	4.44%	40.12
	APOE4	11	7998	23.29%	561.5	12	8900	14.02%	360.3	11	485.4	37.51%	54.89	12	779.8	20.91%	47.06	11	3155	6.60%	62.81	12	3060	8.80%	77.78
	APOE4. Trem2*R47H	10	8919	11.95%	336.9	11	8562	20.11%	519.1	10	474.2	27.62%	41.42	11	670.3	44.54%	90.02	10	3181	4.20%	42.21	11	3077	5.89%	54.67
8 month	C57BL/6J	18	7822	28.80%	531	18	8971	16.67%	352.6	18	448.8	48.00%	50.78	18	963.9	28.22%	64.11	18	3324	4.34%	33.99	18	3202	4.10%	30.93
	Trem2*R47H	12	8488	28.60%	700.7	12	7756	26.28%	588.4	12	411.2	50.29%	59.69	12	709.8	30.83%	63.18	12	3159	7.55%	68.83	12	3056	6.19%	54.64
	APOE4	12	8286	23.34%	558.2	12	7248	23.19%	485.1	12	458.3	37.34%	49.41	12	710.8	34.75%	71.3	12	3079	6.66%	59.23	12	3080	8.18%	72.76
	APOE4. Trem2*R47H	11	7564	21.71%	495.2	12	7656	20.54%	453.9	11	400.6	46.73%	56.45	12	759.8	23.06%	50.58	11	3218	4.01%	38.9	12	3089	6.81%	60.76
12 month	C57BL/6J	22	7217	35.62%	548.1	21	7391	26.08%	420.7	22	379	62.50%	50.5	21	908	32.23%	63.86	22	3360	5.20%	37.25	21	3101	7.58%	51.29
	Trem2*R47H	11	6971	18.38%	386.3	13	5839	18.11%	293.3	11	372.5	39.26%	44.09	13	553.2	33.82%	51.89	11	3166	7.43%	70.9	13	3027	10.29%	86.34
	APOE4	10	6446	14.89%	303.4	11	6665	33.08%	664.7	10	359.4	38.73%	44.02	11	648	38.23%	74.7	10	3149	6.63%	65.99	11	3113	8.64%	81.13
	APOE4. Trem2*R47H	10	7161	29.92%	677.5	12	6841	37.98%	749.9	10	508.3	42.44%	68.22	12	781.1	50.02%	112.8	10	3271	5.76%	59.6	12	3141	7.78%	70.53
24 month	C57BL/6J	15	6916	32.47%	579.8	16	6616	25.76%	426.1	15	538.1	50.26%	69.84	17	767	51.80%	96.36	15	3143	6.29%	51.06	17	2834	10.52%	72.33
	Trem2*R47H	13	7118	21.23%	419.2	13	5238	23.10%	335.6	6	343.3	31.99%	44.85	13	675.6	33.93%	63.59	13	2945	10.77%	87.96	13	2912	9.70%	78.34
	APOE4	11	7996	32.55%	784.6	11	6614	36.78%	733.5	11	333	37.44%	37.59	11	737.8	37.12%	82.59	11	3007	8.01%	72.65	11	2818	10.19%	86.57
	APOE4. Trem2*R47H	6	7409	32.62%	986.5	8	5846	26.45%	546.6	6	343.5	32.01%	44.88	8	708.9	40.04%	100.3	6	3033	6.32%	78.28	8	2798	18.23%	180.4

Table 6. Complete data set of open field assay measures of LOAD strains over time. Individual animals from cross-sectional cohorts housed to the ages indicated were assessed by open field assay. All alleles expressed were homozygous.

DECLARATIONS

Ethics approval

No human subjects or data was used in this study. All experiments involving mice were approved by the Animal Care and Use Committee at The Jackson Laboratory in accordance with guidelines set out in The Eighth Edition of the Guide for the Care and Use of Laboratory Animals. All euthanasia used methods were approved by the American Veterinary Medical Association.

Consent for publication

Not applicable

Availability of data and materials

The LOAD1 data sets are available via the AD Knowledge Portal (https://adknowledgeportal.org). The AD Knowledge Portal is a platform for accessing data, analyses, and tools generated by the Accelerating Medicines Partnership (AMP-AD) Target Discovery Program and other National Institute on Aging (NIA)-supported programs to enable open-science practices and accelerate translational learning. The data, analyses and tools are shared early in the research cycle without a publication embargo on secondary use. Data is available for general research use according to the following requirements for data access and data attribution (https://adknowledgeportal.org/DataAccess/Instructions).

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Competing interests statement

The authors declare that they have no competing interests

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Author contributions

K.P.K: Data curation, Formal analysis, Investigation, Methodology, Writing; A.O.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing; R.P.: Data curation, Formal analysis, Investigation, Methodology, Writing; P.B.: Formal analysis, Investigation; D.G.: Investigation, Methodology; H.W.: Data curation, Formal analysis, Investigation, Methodology; A.U.: Data curation, Formal analysis, Investigation; R.O.: Investigation; S.O.: Investigation; C.I.: Investigation; D.B.: Investigation; M.B.: Investigation; Z.C.: Investigation; K.E.F.: Investigation; B.A.L.: Methodology, Investigation; L.M.M.: Methodology, Investigation; S.J.S.R.: Conceptualization, Data curation, Investigation, Methodology, Writing; P.R.T.: Conceptualization, Data curation, Investigation, Methodology, Writing; G.W.C.: Conceptualization, Data curation, Methodology, Writing; M.S.: Conceptualization, Methodology, Writing; B.T.L.: Conceptualization, Methodology, Writing; G.R.H.: Conceptualization, Investigation, Methodology, Writing.

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