ASSESSING THE ROLE OF *PLCG2*M28L* RISK IN A NOVEL MOUSE MODEL LATE-ONSET ALZHEIMER'S DISEASE

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

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia, and 95% of patients have sporadic Late-Onset AD (LOAD). MODEL-AD has recently characterized mice LOAD1 mice which contain apolipoprotein E4 (*APOE4*), the greatest genetic risk factor of LOAD, and the R47H variant on the triggering receptor expressed on myeloid cell 2 (*TREM2*^{R47H}). In addition, GWAS studies have also identified the M28L variant of *PLCg2* (*PLCg2*^{M28L}), which plays a crucial role in signal transduction during phagocytosis, and introduced this variant on to LOAD1 (LOAD1.*PLCg2*^{M28L}). Clinically, environmental risk factors such as diet and exercise can significantly impact overall health including cognition and brain connectivity. Therefore, ***we hypothesize that combining LOAD risk alleles will result in AD-related cerebral metabolic and blood flow patterns, and that addition of a high fat diet will accelerate AD development***. To test this, we measured neurovascular uncoupling and brain connectomics in novel LOAD1 and LOAD1.*PLCg2*^{M28L} mouse models to assess their alignment human to genetic and environmental risk factors with and without a high fat diet.

GRAPHICAL ABSTRACT

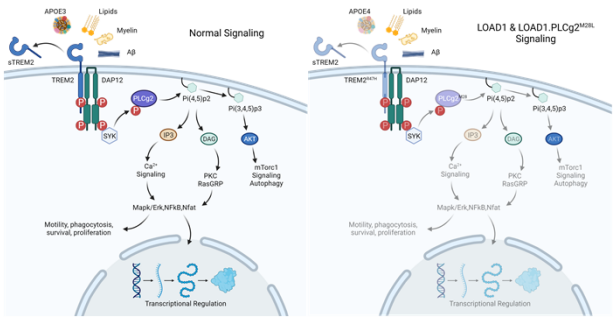

Figure 1. (A) Schematic diagram of TREM2 signaling via PLCg2 to regulate cellular function. (B) Diagram of LOAD1 (B6.APOE4, TREM2^{R47H}) and LOAD1.PLCg2^{M28L} signaling via TREM2, which is hypothesized to reduce microglial function and phenocopy AD.

METHODS AND MATERIALS

All studies were carried out in accordance with, and approval of, the IACUC of Indiana University Medical School and National Research Guide of the Care and Use of Laboratory animals, and were conducted according to the ARRIVE guidelines[1], where mice were randomized by sex and genotypes.

APOE4, TREM2^{R47H}, and *PLCg2*^{M28L}) allele were incorporated into C57BL/6J (B6) mice to produce LOAD1 (N=41) and LOAD1.*PLCg2*^{M28L} (N=71) mice . Mice of both sexes were fed control (male n=33, female n=25) or high fat diet (HFD) (male n=27, female n=27). Mice were administered 3.7-7.4 MBq of 18F-FDG (IP) and were allow 30 min conscious uptake[2], post-isoflurane (2-3%) anesthesia, mice were scanned in listmode for 15min. Blood flow in the same cohort was administered 3.7-7.4 MBq of ^{64}Cu-PTSM via tail vein (IV), allowed 5 min conscious uptake[2], and mice were scanned for 20min in listmode per ^{18}F-FDG.

Images were reconstructed via OSEM into a static volume and were co-registered to the Paxinos-Franklin[3] atlas, where regions were extracted using MIM (MIM Software). 28 (56 bilateral) PET SUVR (reference to cerebellum) brain regions were analyzed in this study to measure brain metabolism and blood flow. Neurovascular uncoupling was measured as bi-directional Z-Score changes for each tracer from normal diet group. Whole brain connectomic analysis was performed[4,5,7] on atlas segmented brain regions using a multi-resolution consensus clustering (MRCC)[6,7] approach to partition whole brain (28 regions) into consensus clusters. Using control diet mice as the reference population, consensus clusters were subjected to multi-p-value thresholding, and estimates of cluster-coefficients, degree, density, and nodal strength. All data were compared across sex and diet using CovNet developed in our lab[7].

RESULTS

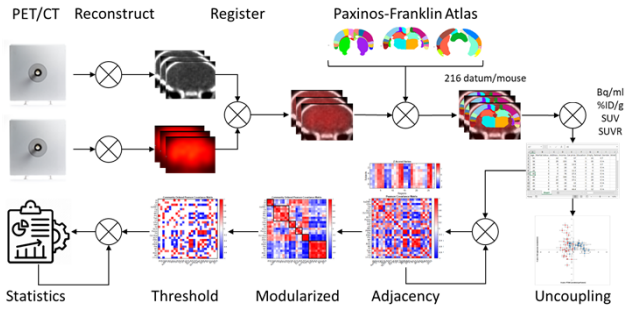

Figure 2. Imaging and data processing workflow for 18F-FDG and 64Cu-PTSM PET data derived from male and female mice fed control or HFD. Post segmentation, subject by region data are subjected to bi-directional uncoupling analysis by computing z-scores relative to control diet subjects. In a parallel analysis, these same regions are subjected to connectomics graph theory analysis, where adjacency matrices are modularized by multi-resolution consensus cluster analysis, subjected to multi-p-value thresholding, and estimates of cluster-coefficients, degree, density, and nodal strength measured. Post-thresholding, statistical analysis of MRCC modules aligned to the male control diet group, and univariate ANOVA conducted with sex and diet as factors for the main effect.

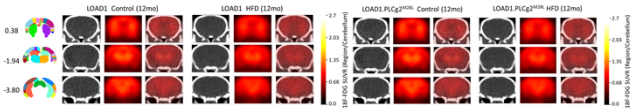

Figure 2. Representative ^{18}F-FDG PET/CT of LOAD1 and LOAD1.*PLCg2*^{M28L} mice at 12mo on control diet (left) compared to HFD (right) at 3 bregma targets

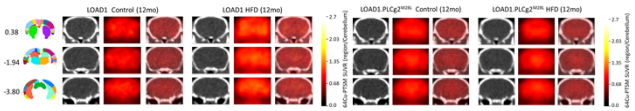

Figure 3. Representative ^{64}Cu-PTSM PET/CT of LOAD1 and LOAD1.*PLCg2*^{M28L} mice at 12mo on control diet (left) compared to HFD (right) at 3 bregma targets

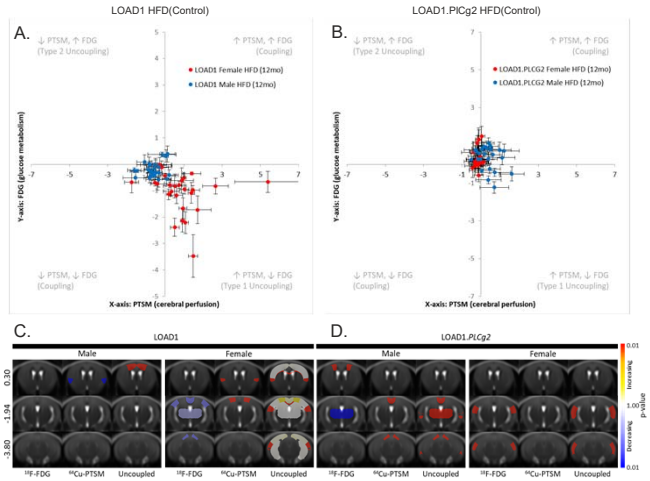

Figure 4. Uncoupling analysis (bi-directional z-score plots) of 28 brain (56 bilateral) regions in male (blue) and females (red) at 12mo on control diet (reference) compared to high fat diet for (**A**) LOAD1 and (**B**) LOAD1.*PLCg2*. Univariate statistical p-value maps for males (left) and females (right) at bregma 0.38, -1.94, and -3.80 mm for ^{18}F-FDG (left column), ^{64}Cu-PTSM (middle column), and uncoupling (right column). For uncoupling plots, data are presented as means±StDev, and for p-value maps color indicates the direction of the change (i.e. white-to-red indicates increasing and white-to-blue indicates decreasing z-scores) for ^{64}Cu-PTSM, ^{18}F-FDG, and Uncoupling.

RESULTS

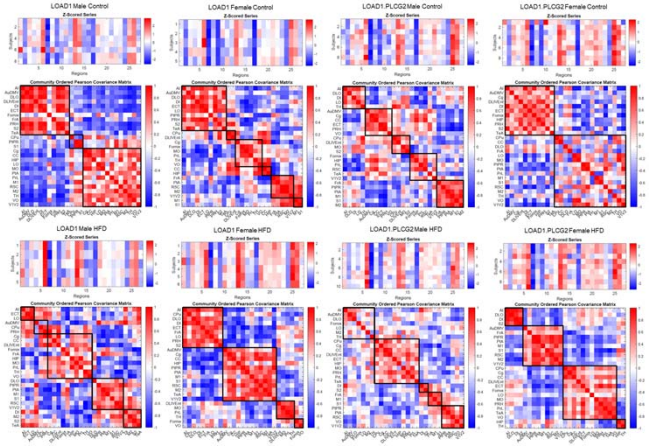

Figure 5. Z-Score series for the subject by regions which show consistency across subjects (top/each). Community ordered adjacency matrices of ^{18}F-FDG PET for 12mo LOAD1 and LOAD1.*PLCg2* mice on control diet (top row) compared to high fat diet (bottom row).

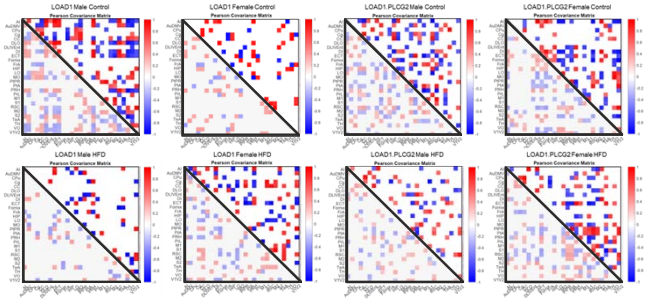

Figure 6. Community ordered adjacency matrices of ^{18}F-FDG PET for 12mo LOAD1 and LOAD1.*PLCg2* mice on control diet (top row) compared to high fat diet (bottom row) thresholded at the p<0.05. Shaded triangle represents the structures analyzed in Figure 7.

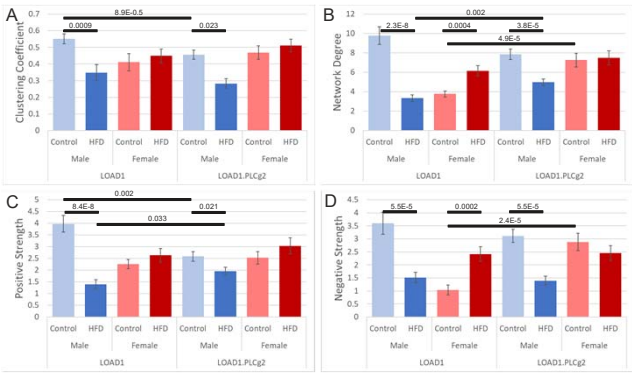

Figure 7. Statistical group analysis compared to a null distribution for 12mo male and female LOAD1 and LOAD1.*PLCg2* mice on control and high fat diet (HFD). (**A**) Clustering coefficient (sub-networks), (**B**) network degree, (**C**) positive and (**D**) negative nodal strength.

CONCLUSION

The combination of *APOE4*, *TREM2*^{R47H} genes in LOAD1 mice with HFD induced diet-dependent LOAD-relevant neurovascular uncoupling and network changes consistent with LOAD; however, the addition of *PLCg2*^{M28L} on LOAD1 did not enhance the disease phenotype, suggesting that the TREM2 dependent signaling was impaired when mice were fed a HFD.

ACKNOWLEDGEMENTS

MODEL-AD was funded from NIH/NIA Grants: U54 AG054345-01, U54 AG054349-01

- MODEL-AD: modelad.org
- AD Knowledge Portal: ampadportal.org
- Contact: pterrito@iu.edu

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