

The Rare African GGA3 Ins545T Variant Drives Increasingly Alzheimer's Disease-Specific Pathway Dysfunction with Age in Humanized Mouse Models

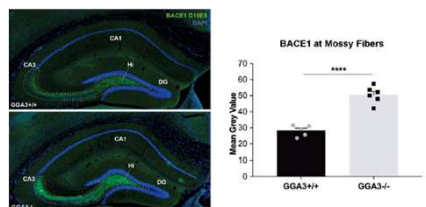
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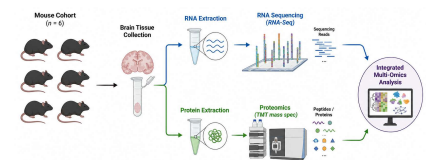
INTRODUCTION

Recently, we determined that genetic deletion of Gga3 triggers axonal trafficking disruption, resulting in BACE1 accumulation in axonal swellings in vitro and axonopathy in vivo. We recently found rs150787028, a GGA3 in-del, Ins545T, co-segregated with late onset AD (LOAD) in families with African ancestry. To investigate the potential of Ins545T in GGA3 to alter AD pathology and clinical outcomes, we generated humanized GGA3 wild-type (hGGA3^{WT/WT}) and humanized GGA3-Ins545T (hGGA3^{Ins545/Ins545}) knock-in mice. We compared this precision mouse model to the previous Gga3 knock-out mouse model to assess the AD-relevance of the human polymorphism.



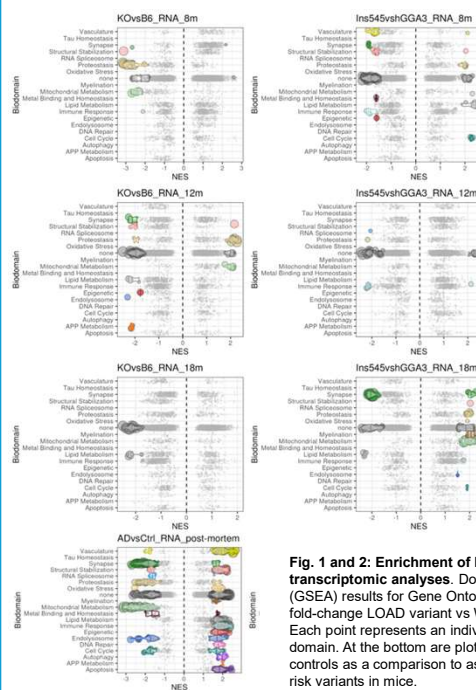
METHODS

We aged cohorts of Gga3 knock-out (Gga3^{-/-}) and humanized GGA3-Ins545T (hGGA3^{Ins545/Ins545}) knock-in mice along with their wildtype controls to assess changes in each model compared to the other and to human AD. We sampled both males and females at three timepoints: 8 months, 12 months, and 18 months. Hemi-brain samples from the same individual were sent for either RNA-Seq or TMT proteomics. Human data was accessed from the Accelerating Medicines Partnership for Alzheimer's Disease (Greenwood et al, 2020: doi.org/10.1002/cphg.105). We applied a system biology approach based on a phenotyping protocol developed by the Target Enablement to Accelerate Therapy Development for AD (TREAT-AD) (Cary et al, 2026: doi.org/10.1002/alz.71403), enabling us to identify transcriptional and proteomics disruptions relevant to human disease, even in models that do not fully exhibit LOAD pathology.



RESULTS

1. Gene expression changes in hGGA3^{Ins545/Ins545} become more AD-specific and stronger with age



2. Protein abundance changes in hGGA3^{Ins545/Ins545} correlate strongly with human AD at aged timepoint

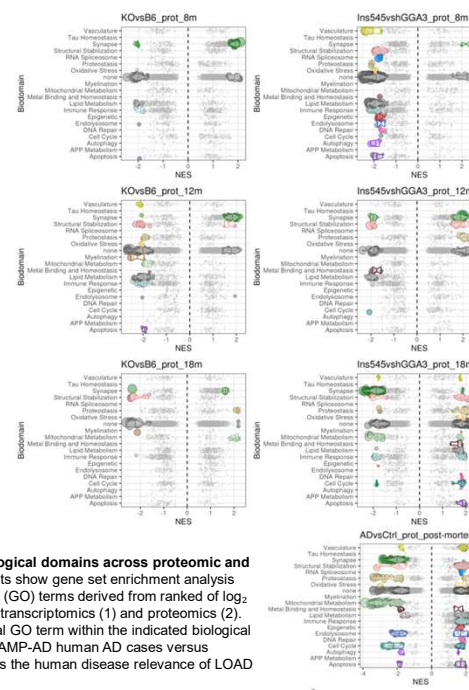


Fig. 1 and 2: Enrichment of biological domains across proteomic and transcriptomic analyses. Dot plots show gene set enrichment analysis (GSEA) results for Gene Ontology (GO) terms derived from ranked of log₂ fold-change LOAD variant vs WT transcriptomics (1) and proteomics (2). Each point represents an individual GO term within the indicated biological domain. At the bottom are plotted AMP-AD human AD cases versus controls as a comparison to assess the human disease relevance of LOAD risk variants in mice.

3. Correlations with human AD are strongest in 18-month hGGA3^{Ins545/Ins545} proteomics and are highly enriched for proteins involved in axonal and dendritic transport.

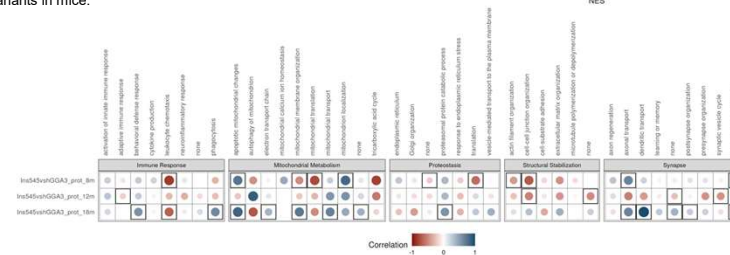


Fig. 3: Concordance of pathway-level transcriptional responses between human Alzheimer's disease and LOAD variant hGGA3^{Ins545/Ins545}. Dot plots showing Pearson correlations of NES for GO terms between human Alzheimer's disease (AD vs control) and hGGA3^{Ins545/Ins545} vs hGGA3^{WT/WT} protein abundance log₂ fold changes.

4. Changes in synapse transport proteins in hGGA3^{Ins545/Ins545} mirror changes seen in human AD

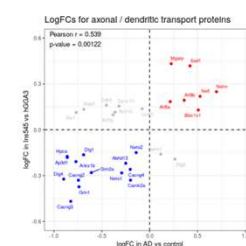


Fig. 4A: Strong correlation between protein expression changes in hGGA3^{Ins545/Ins545} and AD for axonal and dendritic transport genes. Scatter plot comparing log fold changes (logFC) in the Ins545 vs hGGA3 model (y-axis) and AD vs control (x-axis).

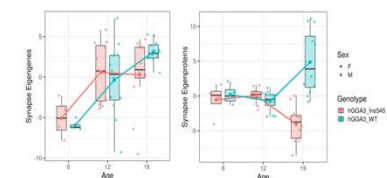


Fig. 4B: Age- and genotype-associated variation in synaptic gene expression and protein abundance. Boxplots summarize the PC1 values for gene expression (left) and protein abundance (right) within each genotype. PC1 summarizes the largest source of variation in synaptic gene expression among samples and shows distinct grouping by both age and genotype.

CONCLUSIONS

Multimodal analysis elucidates AD-relevant responses in both hGGA3^{Ins545/Ins545} and Gga3^{-/-} mouse models. As anticipated, tampering with GGA3 trafficking protein results in axonal and dendritic trafficking abnormalities, however hGGA3^{Ins545/Ins545} is a much more precise model of GGA3 impact in Alzheimer's disease than Gga3^{-/-} complete ablation. Finally, hGGA3^{Ins545/Ins545} may capture early AD pathology and could be a good experimental model for intervention.

ACKNOWLEDGMENTS

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