

The microglial adaptor protein DAB2 links APOE signaling to dysregulated microglial states in Alzheimer's Disease (AD)

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DAB2 is a RELN- and APOE- endocytic adaptor protein that modulates microglial phagocytic, lipid-handling and complement pathways – its deficiency shifts microglia away from DAM/LDAM states and towards pro-inflammatory cytokine responsive states relevant to Alzheimer's Disease

INTRODUCTION

RELN-DAB1 modify the risk of familial¹ and sporadic² forms of Alzheimer's Disease (AD), through their interaction with APOE¹. While RELN and APOE4 have opposing effects on amyloid and Tau pathology in mice³, how risk is altered in the human brain is unclear. Schematic shows the hypothesised convergence of RELN and APOE signaling pathways in previously unassessed microglia, based on neuronal and brain endothelial cell⁴ studies.

METHODS

1) Analysis of human brain microglial transcriptomics
DeSeq2, fgsea, WGCNA

2) In-vivo Reln KO mouse
E14.5, P0, P22
Reln KO vs Reln WT

3) Functional DAB knock-down in human APOE iPSC microglia
APOE isogenic iPSC-microglia (BION37-A/A2/A4 & iCMG BtBio)
DAB1 siRNA, DAB2 siRNA
Opera Phenix, Harmony, Cell-paint algorithms, PCA
Bulk RNA Seq
DeSeq2, fgsea, WGCNA

RESULTS

1. RELN-APOE enrichment in human microglia with increasing amyloid pathology in AD
(Alzheimer's Disease Progression Atlas, Wachter et al., 2024)

Human brain microglia CERAD high vs CERAD low

Region	RELN core +DAB1 arm	RELN core +DAB2 arm
EC	+2.01	+1.98
ITG	+1.83	+1.85
PFC	+1.43	+1.35
V1	+1.52	+1.54

Human APOE-iPSC microglia APOE3/E3 APOE4/E4

Gene set enrichment of RELNcore+DAB1/+DAB2 gene pathways in human microglia in CERAD-high (moderate/frequent neuritic plaques, n=15 donors) vs CERAD-low (none/sparse, n=8 donors) brain regions: EC (high n=15, low n=8), ITG (18/13), PFC (18/10), and V1 (18/13), across pooled APOE genotypes. (APOE carriers: APOE3/E3 n=12, APOE3/E4 n=6, APOE4/E4 n=1). BH*padj<0.05**padj<0.01***padj<0.0001. Leading edge genes driving enrichment are shown. Color-bar: NES range of ± 2.0. Panels show APOE3/E3 (left) and APOE4/E4 (right) iPSC-microglia immunostained for DAB2 and actin-labelling phalloidin.

2. Microglial hypotrophied processes & upregulation of CD68 in Reln-deficient mice

P22 Caudal Cortex

3. DAB2 regulates gene networks associated with phagocytic/lysosomal DAM-states in the human AD brain, with deficiency shifting microglia towards pro-inflammatory cytokine-responsive states

Induced by DAB2 knockdown: brown TNFα-NFκB/metabolic/CRM (n=32, q=4e-6), green TNFα-NFκB/circadian (n=18, q=0.02).
Suppressed by DAB2 knockdown: purple Lysosomal/phagocytic/DAM/TREM2 (n=20, q=5e-3), salmon Innate Immune/Lysosomal/HM (n=31, q=5e-13), greenyellow Lysosomal/Lipid/DAM (n=16, q=0.03), blue Metabolic/lipid/mTORC1/signaling (n=42, q=4e-13), magenta Innate Immune/complement (n=42, q=2e-10).

Alzheimer's Disease

ARM (SalaFrigerio) $\rho=+0.80^{**}$ FDR=0.000 n=24

CRM-2 $\rho=-0.43^{*}$ FDR=0.044 n=24

Legend: ● AD Progression Atlas, ● APOE iPSC-MG (this study), ● APOE iPSC MG DAB KD (this study), ● Haney AD MG, ● Haney APOE iPSC MG, ▲ APOE4/E4 vs APOE3/E3

CONCLUSIONS

Cytokine-responsive iPSC-Microglia
↓ LDAM/DAM
↑ CRM

Activated iPSC-Microglia
↑ LDAM/DAM
↓ CRM

DAB2 silencing: Enhanced pro-inflammatory TNFα/NFκB, reduced phagocytosis, complement, Aβ clearance & lipid handling.

APOE4 microglia: Increased activation of DAM/LDAM states, extracellular matrix remodeling & reduced cholesterol biosynthesis.

Activated human microglia
↑ LDAM/DAM ↓ CRM
↑ APOE ↑ RELN/ITGB1

Cellular response to cytokine stimulus, Ubiquitin-dependent protein catabolism, PI3K-AKT signalling

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