



Variant-to-gene-mapping implicates Alzheimer's disease risk variant rs74504435 as a cell type specific regulator of the Epidermal Growth Factor Receptor (EGFR)

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Introduction

❖ Alzheimer's disease (AD) is the most common neurodegenerative disease affecting millions of aging adults worldwide, characterized by the presence of pathologic protein aggregates that promote neuronal death¹.

❖ Effective disease-modifying therapies for AD remain limited. Treatment is challenging because the etiology is not fully understood, though genetics plays an important role.

❖ Genome-wide association studies (GWAS) identify loci in the genome harboring single-nucleotide polymorphisms (SNPs) associated with AD risk. However, there is a lag in functional validation studies, which impedes the translation of GWAS into effective therapies².

❖ Leveraging published AD GWAS data in combination with Capture C-based variant-to-gene (V2G) mapping and single-cell CRISPR interference (sc-CRISPRi) screens performed in our lab³, we have identified the AD SNP **rs74504435 (A/G)**, residing within an intergenic region on chromosome 7 and regulating *Epidermal Growth Factor Receptor (EGFR)*.

❖ EGFR is a well-known cell-surface signaling receptor involved in tissue homeostasis and tumorigenesis. Although EGFR is important for brain development, its role in AD is less well understood⁴.

❖ Understanding the mechanisms of the genetic contributions to AD is fundamental to developing novel diagnostics and treatments. **Our work can support the repurposing of EGFR pathway inhibitors as potential AD therapeutics.**

Variant-to-gene mapping and Perturb-seq screen identify cis regulatory element (CRE)-gene pairs

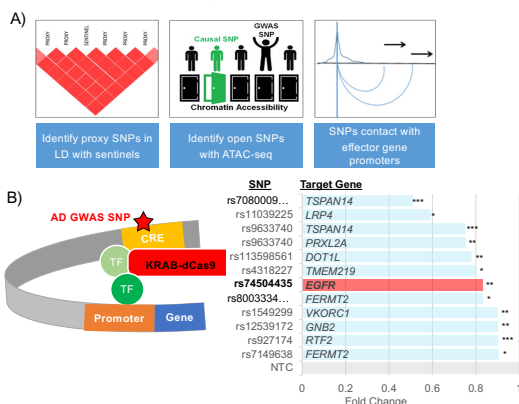


Fig 1. rs74504435 and EGFR were identified as a CRE-gene pair via V2G mapping and a Perturb-seq screen in microglia. A) Our V2G mapping approach overlays GWAS data with ATAC-seq and promoter-focused Capture C to nominate candidate causal variants and effector genes. Since 90% of GWAS SNPs reside in predicted transcription regulatory regions (CREs)², we anticipated that targeting them with the transcriptional repressor KRAB-dCas9 would knockdown effector genes. B) Pooled single-cell CRISPR interference (sc-CRISPRi) targeting putative AD-associated CREs in HMC3 microglial cells was coupled to single-cell RNA-seq and differential expression analysis using SCEPTRE. Bars show the effect of CRE perturbation on nearby genes. ***P<0.005; **P<0.05; *P<0.1.

Validation of rs74504435 (A/G) and EGFR as CRE-gene pair using bulk CRISPRi

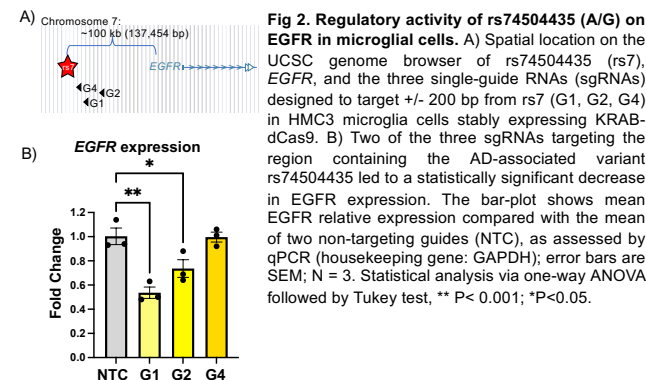


Fig 2. Regulatory activity of rs74504435 (A/G) on EGFR in microglial cells. A) Spatial location on the UCSC genome browser of rs74504435 (rs7), EGFR, and the three single-guide RNAs (sgRNAs) designed to target +/- 200 bp from rs7 (G1, G2, G4) in HMC3 microglia cells stably expressing KRAB-dCas9. B) Two of the three sgRNAs targeting the region containing the AD-associated variant rs74504435 led to a statistically significant decrease in EGFR expression. The bar-plot shows mean EGFR relative expression compared with the mean of two non-targeting guides (NTC), as assessed by qPCR (housekeeping gene: GAPDH); error bars are SEM; N = 3. Statistical analysis via one-way ANOVA followed by Tukey test, ** P< 0.001; *P<0.05.

Allele- and cell type- specific enhancer activity of rs74504435 (A/G)

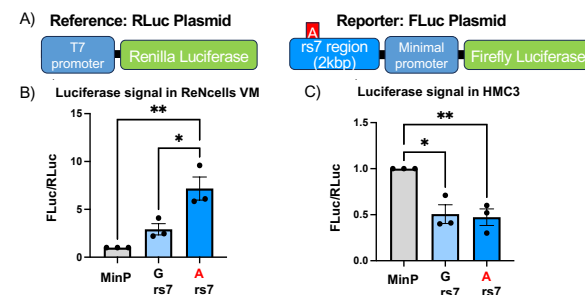


Fig 3. rs74504435 exhibits allele- and cell type- specific regulatory activity in neuronal and microglial models. A) The dual-luciferase reporter assay measures FLuc signal in relation to RLuc as a metric for enhancer activity. B) ReNcell VM (neuronal model) and C) HMC3 (microglial model) were co-transfected with Renilla luciferase (RLuc) and Firefly luciferase (FLuc) reporter plasmids containing either a minimal promoter alone (MinP) or a 2 kb region harboring the non-risk (G) or risk (A) allele of rs74504435 upstream of MinP. Bars show mean FLuc/RLuc activity normalized to MinP (± SEM; n = 3). In ReNcell VM, the rs74504435 risk allele (A) significantly increased reporter activity, consistent with enhancer function, whereas in HMC3, both alleles reduced activity relative to MinP, suggesting repressive activity in microglia. Statistical significance was assessed by one-way ANOVA with Tukey's post hoc test (**p < 0.01, *p < 0.05).

EGFR is increased in human brains with AD pathology

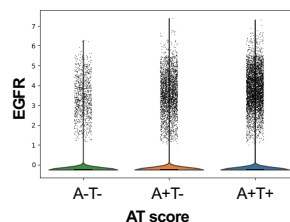


Fig 4. EGFR expression increases with Amyloid and Tau neuropathology score (AT score). Data from Dr. Edward B. Lee's group: a snRNA-seq study on postmortem human brain samples with varied neuropathology (AT score – a metric that reflects the presence or absence of Amyloid-β plaques and Neurofibrillary tangles)⁵. There is a positive correlation between EGFR expression and a higher AT score.

Future directions: dissecting the role of EGFR signaling in AD

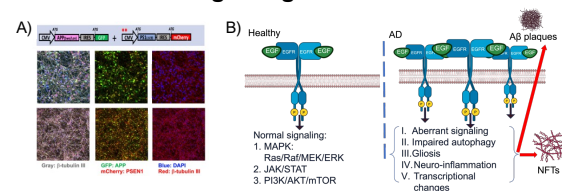


Fig 5. ReNcell VM AD cell model to study EGFR dysregulation in AD. A) Schematic of the construct expressed in the ReNcells VM AD cell line and 4-channel confocal images of 1-week-old cultures at 10x and 20x objectives. B) Model of EGFR dysregulation promoting AD. I hypothesize that in AD, EGFR expression/activation becomes dysregulated, resulting in elevated levels of total or phosphorylated EGFR relative to healthy. This leads to aberrant downstream signaling in pathways already compromised in AD, thereby promoting the formation of pathological protein aggregates (amyloid-β plaques and neurofibrillary tangles) and ultimately contributing to neurodegeneration.

Discussion/Conclusions

❖ Variant-to-gene mapping allows for tailored and biology driven functional validation of disease-associated SNPs from GWAS.

❖ The AD SNP rs74504435 resides in a functional CRE whose direction and magnitude of activity depend on both allele and cell type, consistent with complex cell type-specific regulation of EGFR.

❖ EGFR is overexpressed in AD brains, with levels of EGFR correlating with Amyloid-β and Tau pathology.

❖ EGFR is a receptor tyrosine kinase responsible for activating downstream signaling pathways such as the Ras/Raf/Mek, the PI3k/Akt/mTOR, and the Jak/Stat pathway.

❖ In the future, we will explore how dysregulation of EGFR levels and signaling activity contribute to AD in relevant AD cell types, such as a 3D AD model.

Acknowledgements

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