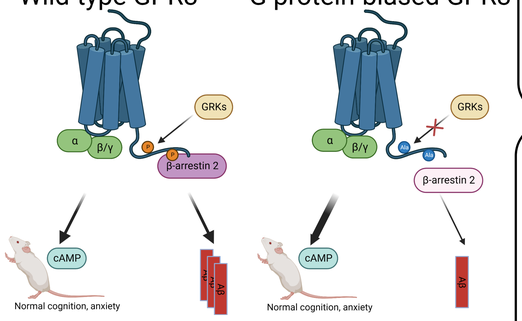
GRK2 activity promotes Aβ generation by altering GPR3 signaling and intracellular trafficking

William Lu[1], Nicholas Todd[1, 2], Yunhong Huang[1], Thais Rafael Guimaraes[1, 3], Amantha Thathiah[1, 4, 5]

[1]Department of Neurobiology, University of Pittsburgh School of Medicine, PA, USA; [2]Graduate Program in Molecular Pharmacology, University of Pittsburgh School of Medicine, PA, USA; [3]Center for Neuroscience, University of Pittsburgh School of Medicine, PA, USA; [4]University of Pittsburgh Brain Institute, University of Pittsburgh School of Medicine, PA, USA; [5]Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh School of Medicine, PA, USA

BACKGROUND

- Alzheimer's Disease (AD) is a neurodegenerative disorder pathologically characterized by the accumulation of intracellular neuronal tau tangles and extracellular amyloid-beta (Aβ) plaques[1].
- Biased G protein-coupled receptor (GPCR) signaling preferentially activates G protein- or β-arrestin-mediated signaling pathways and presents opportunities to develop more selective and safer therapeutics in the absence of side effects[2].
- Although GPCRs are implicated in the pathophysiology of AD[3], biased GPCR signaling is a largely unexplored area of investigation in AD.
- Recently, we developed a G protein-biased GPR3 AD mouse model, which does not recruit β-arrestin 2, that displays reduced Aβ pathology without adverse cognitive effects[4]. the



OBJECTIVE

To investigate the mechanism by which G protein-biased GPR3 signaling ameliorates Aβ pathology using *in vitro* cellular models

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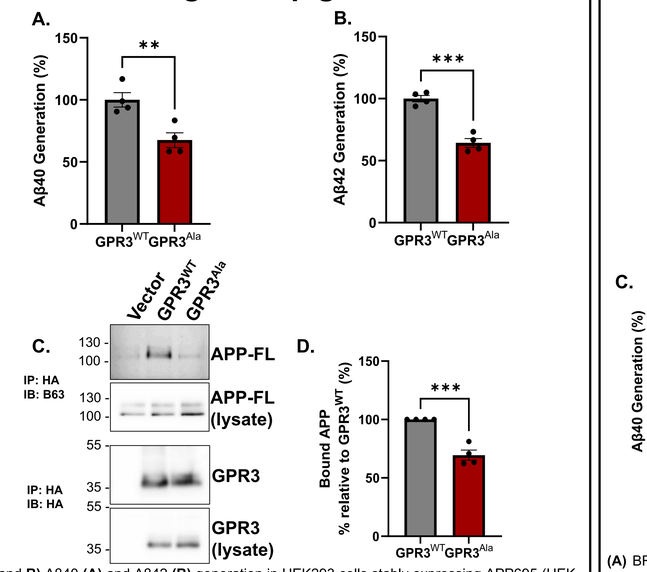
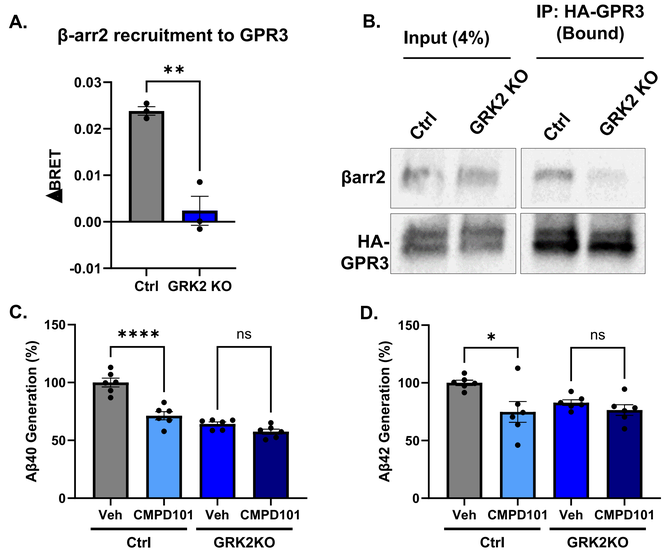
RESULTS

Figure 1. G protein-biased GPR3 displays reduced amyloid precursor protein (APP) binding and Aβ generation



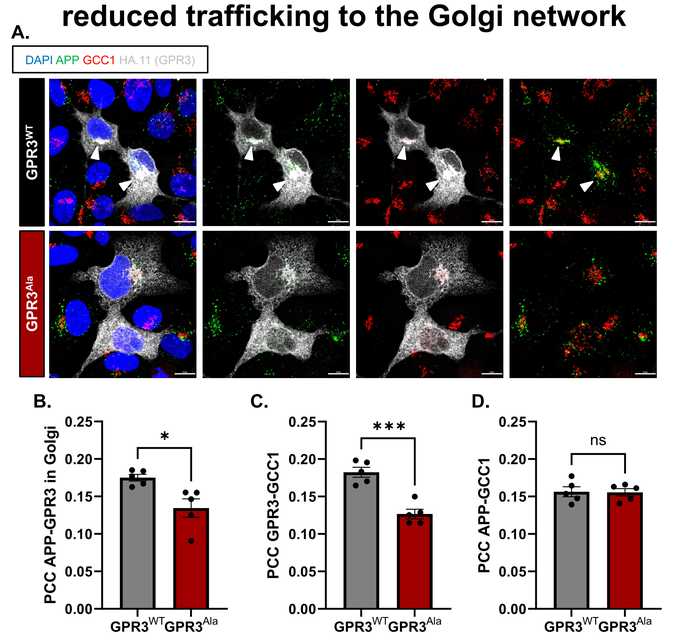
(A and **B)** Aβ40 **(A)** and Aβ42 **(B)** generation in HEK293 cells stably expressing APP695 (HEK-APP) and transfected with wild-type GPR3 ($GPR3^{WT}$) or G protein-biased GPR3 ($GPR3^{Ala}$). Data as mean±SEM. n=4 independent experiments. Unpaired two-tailed t-test. ** = p<0.01. *** = p<0.001. **(C** and **D)** Representative co-immunoprecipitation **(C)** and quantification **(D)** of full-length APP and $GPR3_{WT}$ or $GPR3^{Ala}$ from HEK-APP lysates. Data as mean±SEM. n=4 independent experiments. Unpaired two-tailed t-test. *** = p<0.001. Molecular weight in kDa.

Figure 2. GRK2 is involved in β-arrestin 2 recruitment to GPR3 and Aβ generation



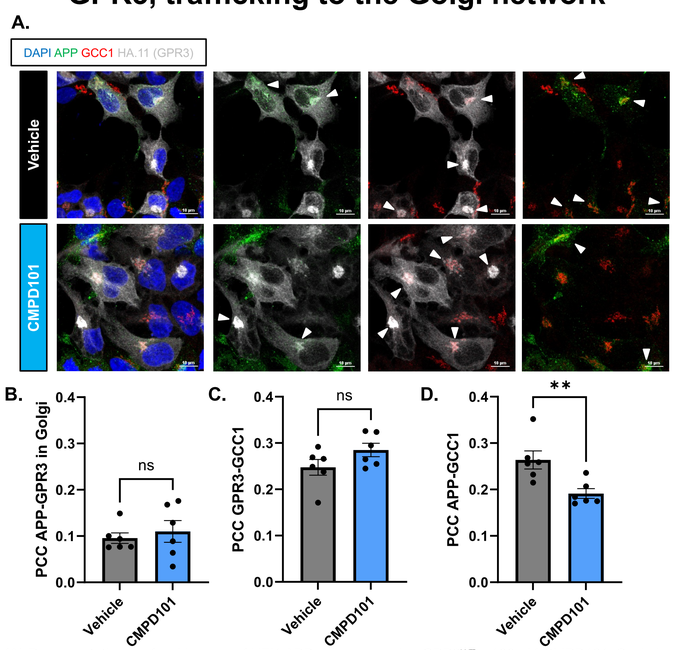
(A) BRET assay measuring βarr2 recruitment to $GPR3^{WT}$ in HEK293 control and GRK2 KO cells. Data as mean±SEM. n=3 independent experiments. Unpaired two-tailed t-test. ** = p<0.01 **(B)** Co-immunoprecipitation experiment in HEK293 control or GRK2 KO cells expressing $GPR3^{WT}$. **(C and D)** Aβ40 **(C)** and Aβ42 **(D)** generation in HEK293 CRISPR control and GRK2 KO cells expressing APP-C99 and $GPR3^{WT}$ ± 10 μM CMPD101. Data as mean±SEM. n=3 independent experiments run with 2 technical replicates each. Ordinary two-way ANOVA with Tukey's multiple comparisons test. * = p<0.05. **** = p<0.0001. ns = not significant.

Figure 3. G protein-biased GPR3 displays reduced trafficking to the Golgi network



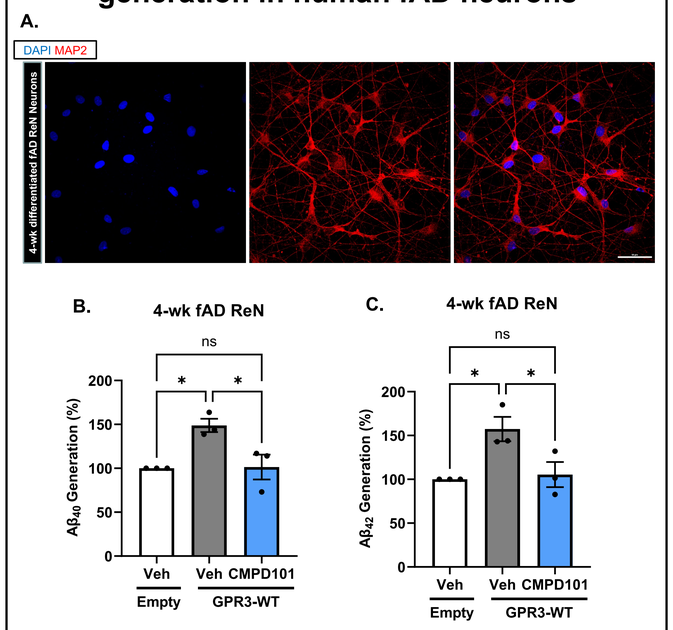
(A) Representative confocal images of HEK-APP cells expressing $GPR3^{WT}$ or $GPR3^{Ala}$. Scale bars are 10 μm. **(B-D)** Quantification of colocalization of APP and GPR3 in Golgi binary **(B)**, GPR3 and GCC1 **(C)**, and APP and GCC1 **(D)** with Pearson correlation coefficient. Data as mean±SEM. n=5 independent experiments, two technical replicates each. Unpaired two-tailed t-test. * = p<0.05. *** = p<0.001. ns = not significant.

Figure 4. GRK2 activity affects APP, but not GPR3, trafficking to the Golgi network



(A) Representative confocal images of HEK-APP cells expressing $GPR3^{WT}$ ± 100 μM CMPD101. Scale bars are 10 μm. **(B-D)** Quantification of colocalization of APP and GPR3 in Golgi **(B)**, GPR3 and GCC1 **(C)**, and APP and GCC1 **(D)** with Pearson correlation coefficient. Data as mean±SEM. n=6 independent experiments. Paired two-tailed t-test. ** = p<0.01. ns = not significant.

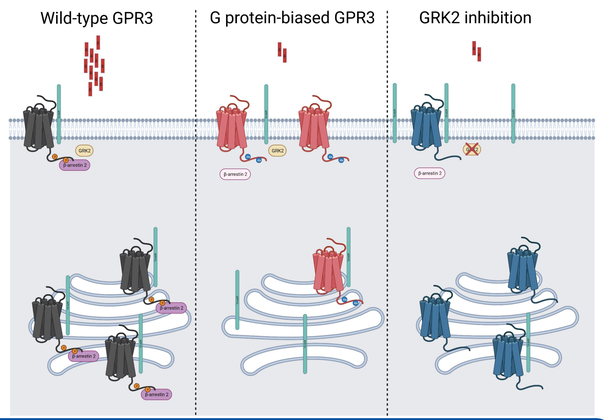
Figure 5. GRK2 inhibition reduces Aβ generation in human fAD neurons



(A) Representative confocal images of 4-week differentiated fAD neurons. Scale bars are 50 μm. **(B and C)** Aβ40 **(B)** and Aβ42 **(C)** generation in 4-week differentiated fAD neurons with and without $GPR3^{WT}$ expression and ± 10 μM CMPD101. Data as mean±SEM. n=3 independent experiments. Ordinary one-way ANOVA with Tukey's multiple comparisons test. * = p<0.05. ns = not significant.

SUMMARY

1. G protein-biased GPR3 shows reduced binding to APP and reduced localization to the Golgi network.
2. GRK2 is involved in β-arrestin 2 recruitment to GPR3 and Aβ generation.
3. Inhibition of GRK2 leads to a reduction in APP localization to the Golgi network.



NEXT STEPS

1. Identify putative GRK phosphorylation sites on GPR3 and APP
2. Determine the effect of GRK2 inhibition on GPR3-APP binding
3. Investigate the impact of biased GPR3 or GRK2 inhibition on GPR3 and APP trafficking to membrane/other subcellular compartments

*Schematics created with BioRender