Protective mechanisms of SNPs in SH3RF3/POSH2 for Alzheimer's disease

COLUMBIA
TAUB INSTITUTE FOR RESEARCH ON ALZHEIMER'S DISEASE AND THE AGING BRAIN

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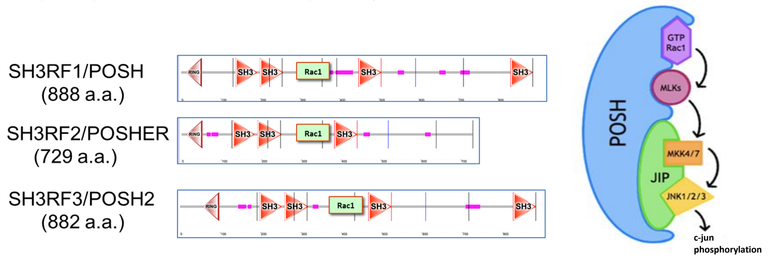
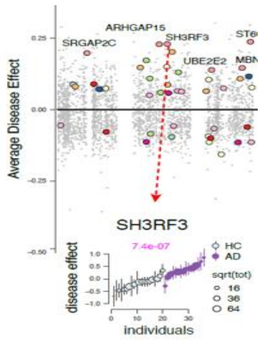
Background

- Alzheimer's disease (AD) is the most common form of dementia worldwide. Accumulation of extracellular amyloid-beta plaques and intracellular tangles comprised of hyperphosphorylated tau are clinical hallmarks of AD, although therapies targeting these neuropathological changes have limited efficacy so far in the clinic. Understanding how some high-risk individuals are protected from developing AD may elucidate novel therapeutic targets.
- Dr. Lee and colleagues have identified protective genetic variants in a Caribbean Hispanic cohort carrying the PSEN1 G206A mutation sufficient to cause early-onset AD. The initial discovery SNP rs6542814 in SH3RF3/POSH2 was associated with delayed age at onset of AD for 9.2 years. (Lee et. al, JAMA Neurol., 2015).



(Xu et al., *EMBO* 2003)

- SH3RF family proteins promote JNK and NFκB signaling
- Protective SNPs are thus predicted to be associated with reduced SH3RF3 expression, which our preliminary *in silico* analysis supports
- Published scRNAseq data shows higher expression of SH3RF3 is associated with higher disease effect in AD microglia compare to healthy controls.
- We used hPSC-derived neurons and microglia (iMGLs) combined with siRNA-mediated SH3RF3 knockdown to assess AD-related neuronal or microglia phenotypes.



(Park et. al., MedRxiv, 2022)

SNPs in SH3F3 affect age of FAD onset in PSEN1 G206A carriers

				WGS All: 329 Subjects				WGS G206A Only: 199 Subjects			
				AGE -Follow Up				AGE-Follow Up			
SNP	Location	A1	A2	MAF	Z	Beta	PVAL	MAF	Z	Beta	PVAL
rs116472630	intronic	A	G	0.009	2.59	9.24	0.00965	0.010	3.365	12.64	0.00077
rs113229964	intronic	T	A	0.038	2.38	4.23	0.01733	0.040	3.282	6.23	0.00103
rs17035072	intronic	C	A	0.009	2.11	7.53	0.03483	0.010	2.815	10.77	0.00488
rs79006626	intronic	T	C	0.009	2.11	7.53	0.03483	0.010	2.815	10.77	0.00488
rs34792138	intronic	C	CTT	0.009	-3.60	-15.88	0.00032	0.013	-3.203	-13.81	0.00136
rs1270158111	intronic	*	TGAGA	0.009	-2.48	-9.00	0.01317	0.013	-3.074	-10.45	0.00211
rs113788343	intronic	A	G	0.038	2.38	4.23	0.01733	0.040	3.282	6.23	0.00103
rs111377907	intronic	C	T	0.038	2.38	4.23	0.01733	0.040	3.282	6.23	0.00103
rs113489262	intronic	G	A	0.038	2.38	4.23	0.01733	0.040	3.282	6.23	0.00103
rs12616600	intronic	G	A	0.026	1.88	3.88	0.05988	0.025	2.149	4.79	0.03162
rs117050389	intronic	T	C	0.026	1.88	3.88	0.05988	0.025	2.149	4.79	0.03162
rs76814213	intronic	A	G	0.026	1.88	3.88	0.05988	0.025	2.149	4.79	0.03162
rs114238146	intronic	A	G	0.011	1.06	4.25	0.28863	0.003	3.944	31.55	0.00008
rs192679474	exonic; S700F	T	C	0.002	2.56	21.47	0.01039	0.003	3.035	22.24	0.00240

Table 1: SNPs in SH3RF3 affect age of onset of FAD in PSEN1 G206A carriers. Highlight of some newly identified SNPs in SH3RF3 from whole genome sequencing of PSEN1 G206A and control subjects. Beta refers to the effect on age of onset of AD in years. SNPs in red are in relative close proximity to the discovery SNP rs6542814 (Lee et. al, *JAMA Neurology* 2015).

Enhanced Aβ42:40 ratio in PSEN1 G206A neurons is not affected by SH3RF3 KD

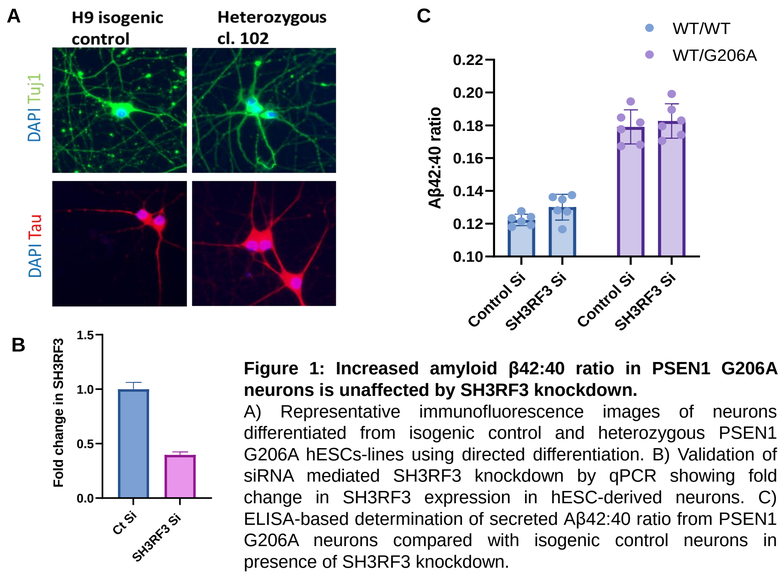


Figure 1: Increased amyloid β42:40 ratio in PSEN1 G206A neurons is unaffected by SH3RF3 knockdown. A) Representative immunofluorescence images of neurons differentiated from isogenic control and heterozygous PSEN1 G206A hESCs-lines using directed differentiation. B) Validation of siRNA mediated SH3RF3 knockdown by qPCR showing fold change in SH3RF3 expression in hESC-derived neurons. C) ELISA-based determination of secreted Aβ42:40 ratio from PSEN1 G206A neurons compared with isogenic control neurons in presence of SH3RF3 knockdown.

SH3RF3 KD minimally affects tau phosphorylation in human neurons under basal and stressed conditions

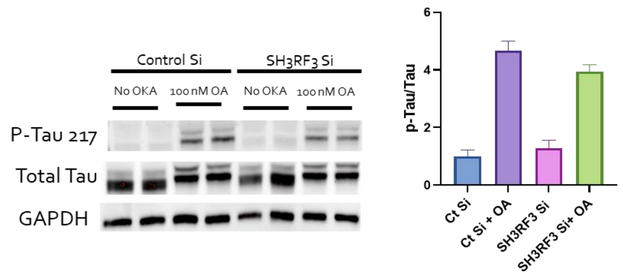


Figure 2: Okadaic acid induced tau phosphorylation shows mild reduction with SH3RF3 knockdown. Western blot analysis of phospho-Tau 217 and total tau with and without okadaic acid treatment +/- SH3RF3 knockdown in NGN2-mediated transdifferentiated neurons. OKA stimulation was for 2hr. p217tau:total tau ratio determined by densitometry, relative quantification is shown. GAPDH is used as loading control.

SH3RF3 KD reduces inflammatory phenotypes in iMGLs treated with a viral mimic via JNK and NFkB pathways

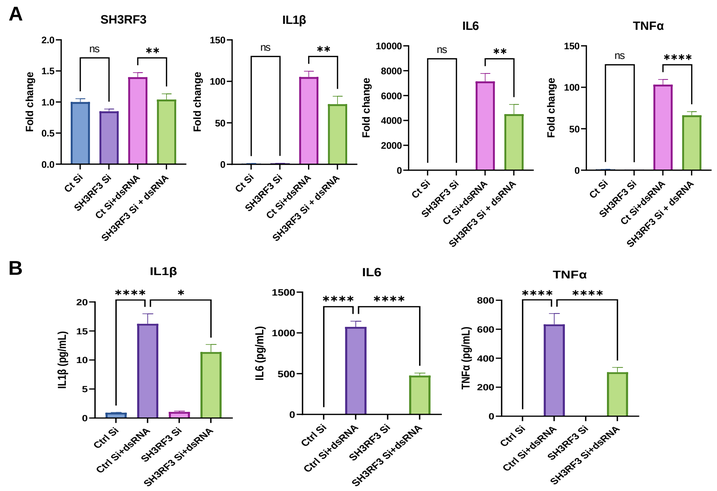


Figure 3: SH3RF3 knockdown reduces inflammatory cytokines induced by Poly(I:C)- a viral mimic(dsRNA). A) qPCR analysis of SH3RF3, IL1β, IL6 and TNFα in iMG upon SH3RF3 knockdown, samples labelled dsRNA are poly(I:C) stimulated for 2 hr. B) ELISA-based quantification of released inflammatory cytokines from iMG with poly(I:C) stimulation for 24hr.

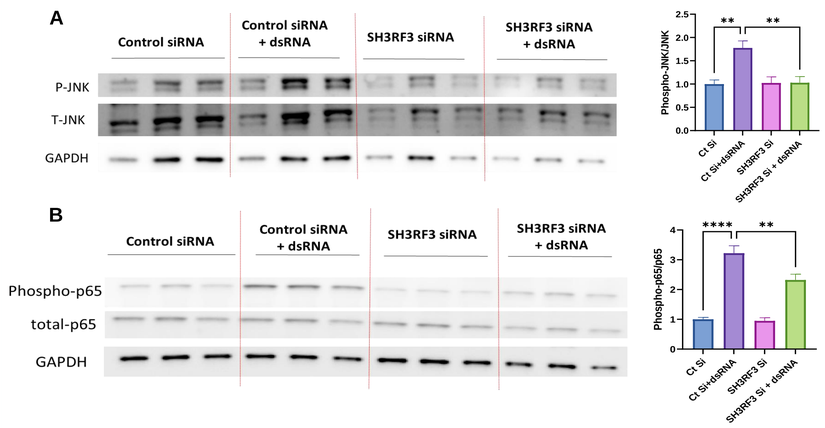


Figure 4: JNK and NFkB pathway activation induced by poly(I:C) are inhibited by SH3RF3 knockdown A) & B) Western blot analysis of JNK and NFkB pathway components with and without poly(I:C) stimulation with SH3RF3 knockdown. Representative blots phosphorylated JNK, total JNK, phosphorylated p65 and total p65 with densitometry quantification is shown. GAPDH is used as loading control. poly(I:C) stimulated for 6 hr.

SH3RF3 KD reduces inflammatory phenotypes in iMGLs treated with oligomeric Aβ42

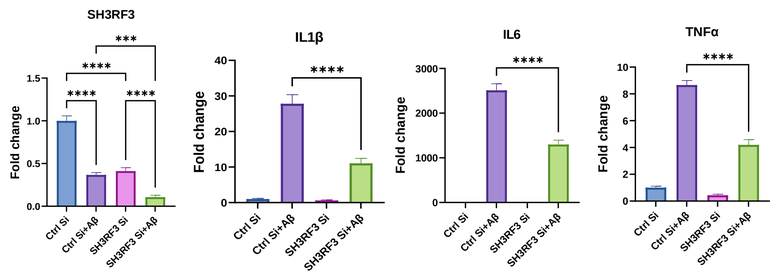


Figure 5: Oligomeric amyloid-β (oAβ) induces inflammatory phenotypes similar to poly(I:C) stress and can be mitigated by SH3RF3 knockdown. qPCR analysis of inflammatory cytokines and SH3RF3 upon 5 μM oAβ stress (24 hr).

SH3RF3 KD reduces activation of JNK and NFkB pathways in iMGLs treated with oligomeric Aβ42

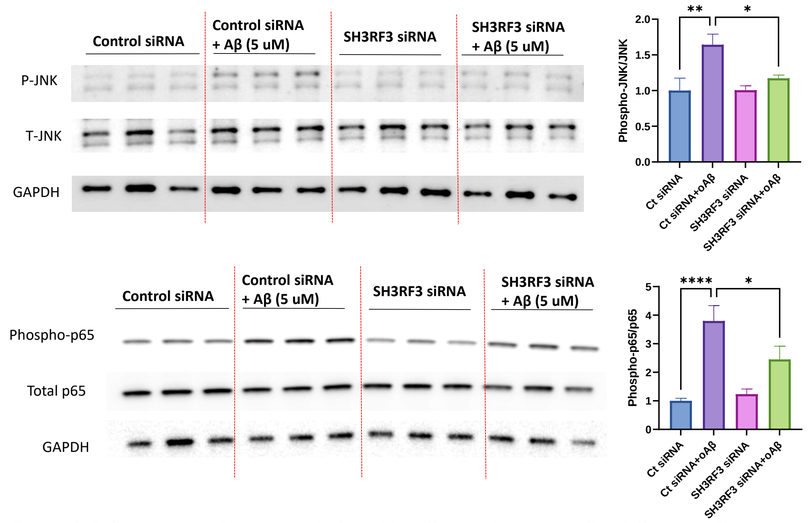


Figure 6: Inflammatory phenotypes induced by oligomeric amyloid-β (oAβ) are mediated by activation of JNK and NFkB signaling similar to poly(I:C) stress and can be mitigated by SH3RF3 knockdown. Representative immunoblots and densitometry analysis of JNK and NFkB pathway components treated with oAβ stress for 3h.

Pharmacological inhibition of JNK and NFkB pathways phenocopies SH3RF3 knockdown.

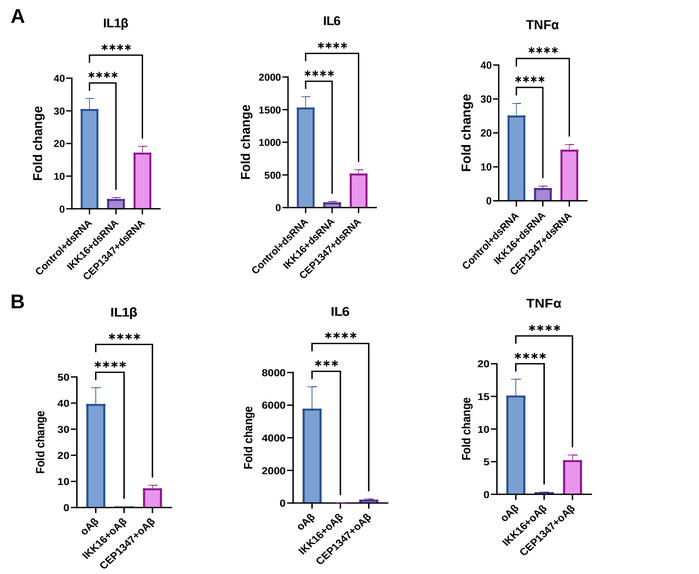


Figure 7: Inhibition of JNK and NFkB pathways by small molecule inhibitors in iMGLs treated with with poly(I:C) or oAβ. A) qPCR analysis of IL1β, IL6 and TNFα in iMGLs treated with poly(I:C) for 2 hr, with the presence or absence of pathway inhibitors. CEP1347 is a JNK pathway (MAP3K) inhibitor and IKK16 is a NFkB pathway inhibitor. B) qPCR for inflammatory cytokines after 12 hr oAβ42 stimulation in presence or absence of vehicle, CEP-1347, or IKK16

Conclusions & Future Directions

- Dr. Lee and his team have identified additional variants in SH3RF3 that affect age of onset of FAD from analysis of WGS data from larger cohort of PSEN1 G206A subjects and controls. Some new variants are shown in Table 1, eQTL analysis is ongoing
- SH3RF3 KD inhibits inflammatory cytokine transcription and release induced by the viral mimic poly(I:C) and oligomeric Aβ42 in iMGLs
- Treatment with Poly(I:C) or oligomeric Aβ42 increases JNK and NFkB pathway activity which is mitigated by SH3RF3 KD
- Pharmacological inhibition of JNK and NFkB pathways shows similar (albeit stronger) phenotypes as SH3RF3 KD upon poly(I:C) and oAβ treatment, consistent with SH3RF3 modulating these pathway to affect inflammatory cytokine production and release
- We are also currently investigating PSEN1 G206A iMGLs respond to inflammatory stimuli relative to isogenic controls, +/- SH3RF3 KD

Acknowledgements

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