Heterozygous *de novo* WHAMM mutation (c.1662C>T) alters cellular response to TNF in iPSC-derived MSCs from a patient with early onset axial spondyloarthritis

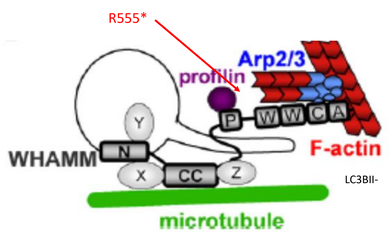
Yongquan Luo[1], Stephen Brooks[2], and Robert A. Colbert[1]

[1]Pediatric Translational Research Branch, NIAMS, [2]Biodata Mining and Discovery; Bethesda, MD 20892

Introduction

- WHAMM (Wiskott-Aldrich syndrome protein [WASP] Homolog-associated protein with Actin, Membranes and Microtubules) regulates ER to Golgi transport
  - Interacts with both actin and microtubule cytoskeletons to control membrane tubulation and dynamics at the Golgi apparatus
  - Participates in autophagosome formation and autolysosome reformation in autophagic processing, and apoptosis
  - Requires interaction between WCA domain and Arp2/3
- We discovered a de novo heterozygous mutation in WHAMM (c.1662C>T, p.R555Ter) in an individual with HLA-B27-positive Axial Spondyloarthritis (AxSpA)

WHAMM 15:83499372 CT
(c.1662C>T, p.R555Ter)



Arp2/3 mediated actin assembly

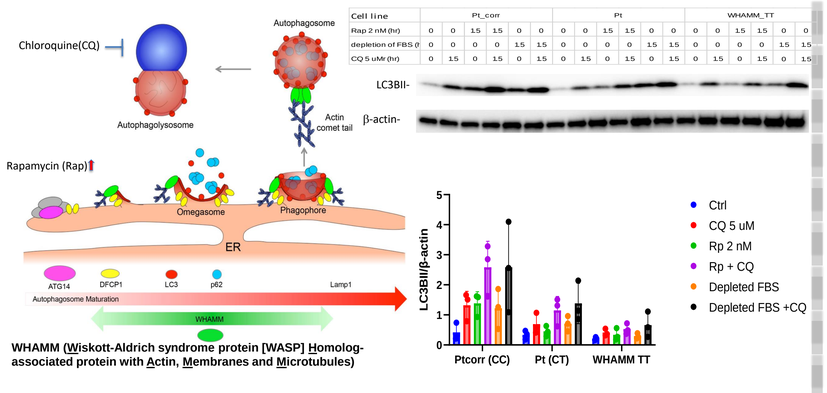
Goal: To investigate effects of the de novo WHAMM C>T mutation on autophagy and TNF response using RNASeq and iPSC-derived MSCs

Methods and Results

Generating isogenic WHAMM mesenchymal stem cells (MSCs)

WHAMM Patient (Pt)-derived fibroblast → (iPSC reprograming) → WHAMM Pt-derived iPSCs → (CRISPR/ Cas 9 editing) → Isogenic WHAMM iPSCs: Pt-Corr (CC), Pt (CT), Pt-homo mut (TT) → (Differentiation) → Isogenic WHAMM MSCs (CC, CT, TT)

Reduced autophagy by WHAMM C>T

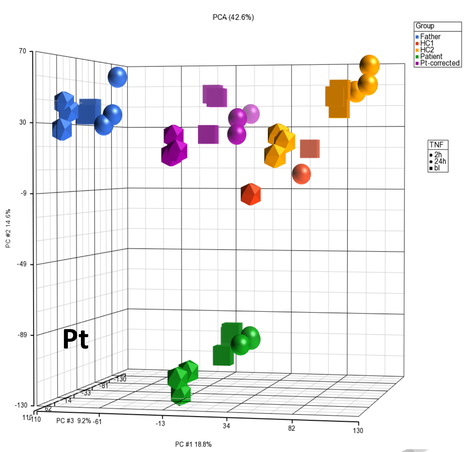


WHAMM (Wiskott-Aldrich syndrome protein [WASP] Homolog-associated protein with Actin, Membranes and Microtubules)

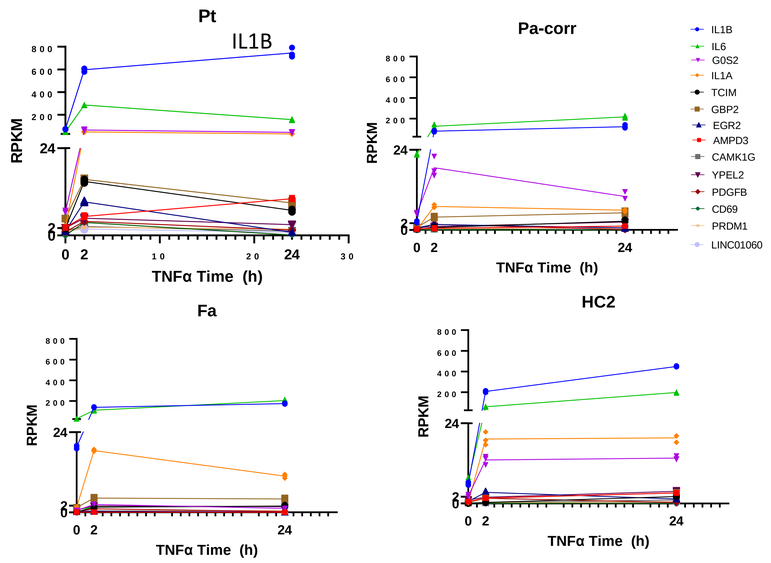
Distinguished Pt cell and its TNF response from controls in RNASeq

Control (0 h) — TNFα (30 ng/ml) 2 h — TNFα 24 h

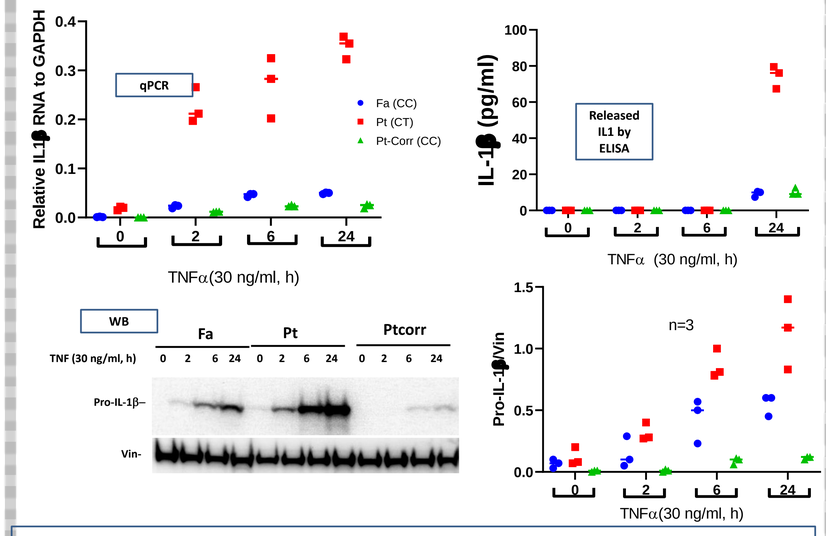
MSCs	WHAMM genotype	# replicates
HC1	CC	1
HC2	CC	3
Pt	CT	3
Fa	CC	3
Pt-corr	CC	3



Considerable higher TNF induced IL1 expression in Pt

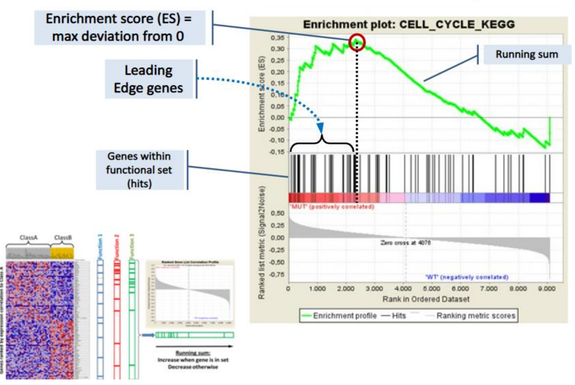


Confirmed studies

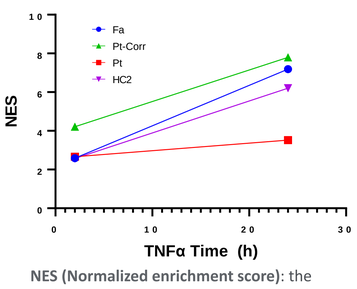


Reduced TNF induced late interferon stimulated genes (ISGs) expression in Pt

Gene Set Enrichment Analysis



IFNα response

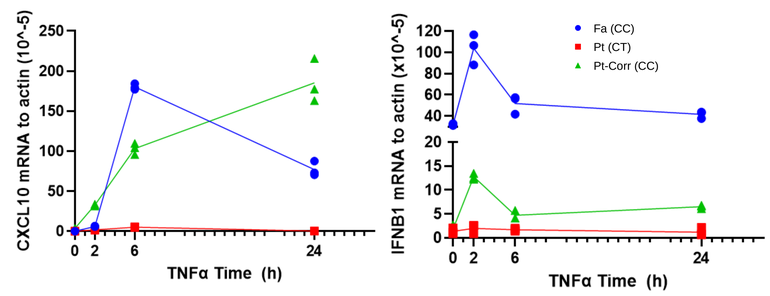


NES (Normalized enrichment score): the enrichment score for the gene set after it has been normalized across analyzed gene sets.

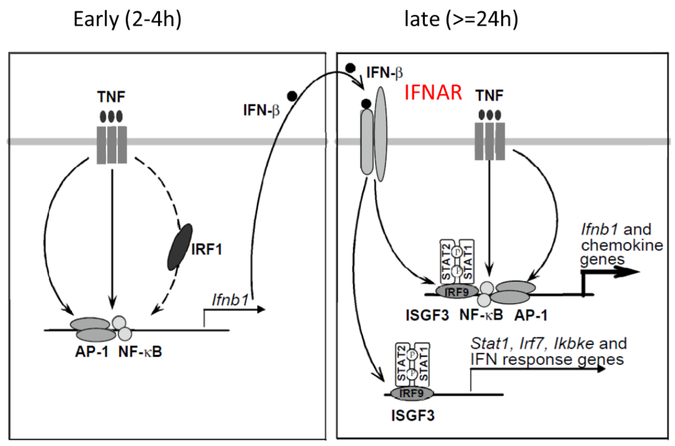
42 anti-viral related ISGs are not induced by TNFα in Pt at 24 h

- ADAR APOL6 C1R C1S CFB CMPK2 CXCL10 EIF2AK2 EPSTI1 HERC6 IFI44 IFI44L IFIT1 IFIT2 IFITM1 IL18BP IRF9 ISG15 ISG20 LGALS3BP MX1 MX2 MYD88 NMI OAS1 OAS2 OAS3 PARP9 PLSCR1 SAMD9 SAMHD1 SLC25A28 SP110 STAT1 TDRD7 TNFAIP6 TRIM14 TRIM5 UBA7 USP18 XAF1 ZNFX1

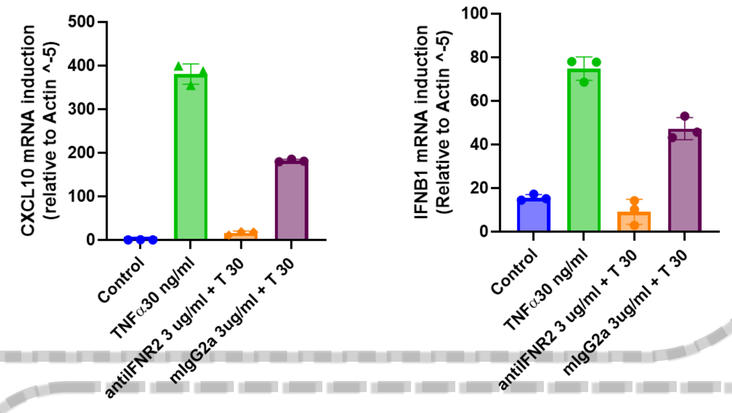
MSCs from Pt do not produce IFNB1 and CXCL10 by TNF



TNF induced late CXCL10 is mediated by IFN/IFNR pathway



2008: TNF/NFKB/IRF1/IRF1-depent autocrine loop of expressing chemokines and STAT1-dep ISGs



Summary and plan

Ptcorr, controls		Pt
↑	Autophagy	↓
↓	IL-1 inflammasome	↑
↑	TNF/IFN/ISGs	↓