Stem Cell Modeling to Understand Genetic Risk and Endocytic Network Dysfunction in Alzheimer's Disease

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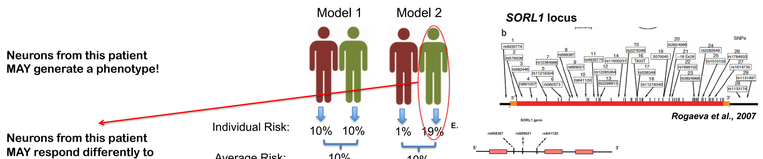
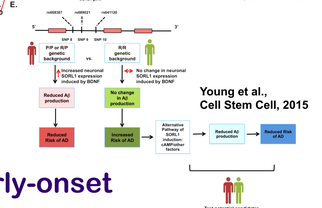
About our research:

1. Use stem cell models to understand human AD risk

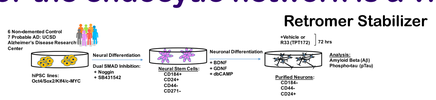


SORL1: **An AD risk gene that functions in APP trafficking and intracellular sorting.**

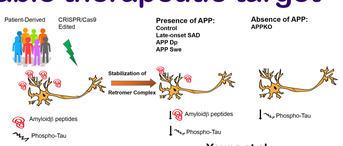
- ❖ Loss of SORL1 leads to early-onset AD in some families
- ❖ Identified via GWAS as a late-onset risk gene



2. Use stem cell models to test whether manipulation of the endocytic network is a viable therapeutic target

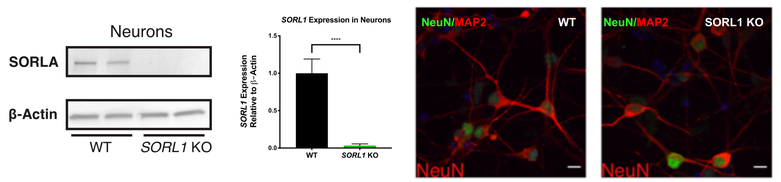


❖ Retromer is a multi-protein trafficking complex
❖ APP is retromer cargo via SORL1/SORLA

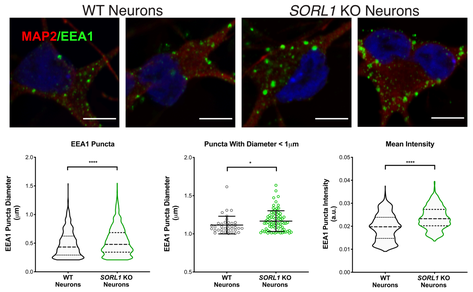


- ❖Stabilizing retromer reduces Aβ and pTau
- ❖De-stabilizing retromer increases Aβ and pTau
- ❖Changes in pTau due to retromer manipulation are APP independent
- ❖Endocytic Dysfunction may be an upstream driver of pathology that affects Aβ and pTau independently

Loss of *SORL1* does not affect neuronal differentiation



SORL1 null neurons have enlarged early endosomes

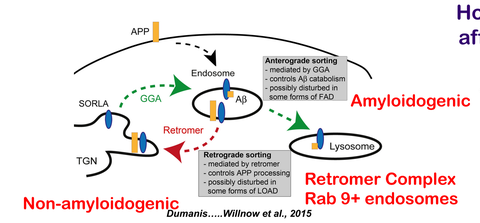


- ❖Loss of *SORL1* does not impair neuronal differentiation from hiPSCs.
- ❖Previous studies have implicated endosome enlargement, indicative of traffic jams, as an early phenotype in AD.
- ❖*SORL1* null cells have more intensely stained and larger early endosomes.
- ❖Loss of *SORL1* phenocopies an early AD cytopathology.

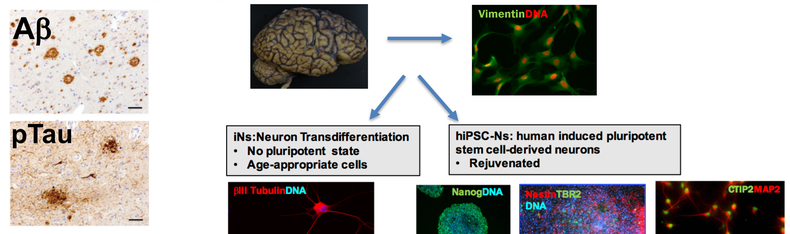
How do endosomal traffic jams affect cargo transport through the endocytic network?

- ❖Colocalization analysis of APP with markers of the endo-lysosomal network.

EEA-early endosomes
Rab7-late endosomes
Rab9-late endosome/Golgi Recycling
TGN-Golgi



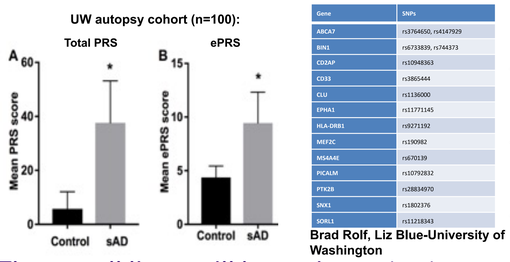
2. Generate hiPSC-derived and directly reprogrammed neurons from pathologically characterized tissue.



Rose et al., JNEN, 2018

- ❖ We generated matched cell lines from patients with a comprehensive post-mortem neuropathologic examination.
- ❖ We used leptomeningeal cells due to proximity to the brain and relative protection from environmental exposures.
- ❖ We demonstrated that we can both directly transdifferentiate neurons and reprogram hiPSCs from these samples. Direct transdifferentiation may preserve the aged transcriptome and epigenome.
- ❖ This gives us a source of "aged" and "rejuvenated" cells to interrogate the effects of brain aging on cellular phenotypes.

3. Generate hiPSC lines and neural cells from subjects with high and and low AD polygenic risk.

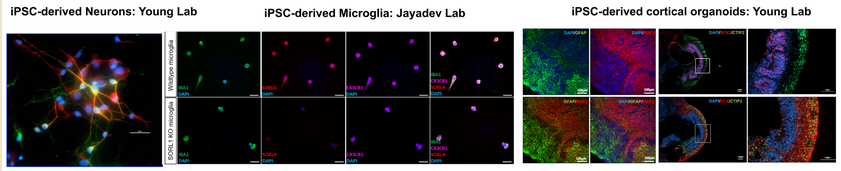


Gene	SNPs
ABCA7	rs3764650, rs4147929
BIN1	rs6733839, rs744373
CD2AP	rs10948363
CD33	rs3865444
CLU	rs1136000
EPHA1	rs11771145
HLA-DRB1	rs9271192
MEF2C	rs190982
MS4A6E	rs6670139
PICALM	rs10792832
PTK2B	rs28834970
SNX1	rs1802376
SORL1	rs11218343

Brad Rolf, Liz Blue-University of Washington

- ❖Genetic contribution to SAD is likely a cumulative effect of multiple variants.
- ❖We selected AD risk loci in endocytic network genes to determine an ePRS (endocytic polygenic risk score).
- ❖ We genotyped samples from the subjects we are generating leptomeningeal cell lines from.
- ❖ Neuropathologic diagnosis of SAD have increased PRS and ePRS.

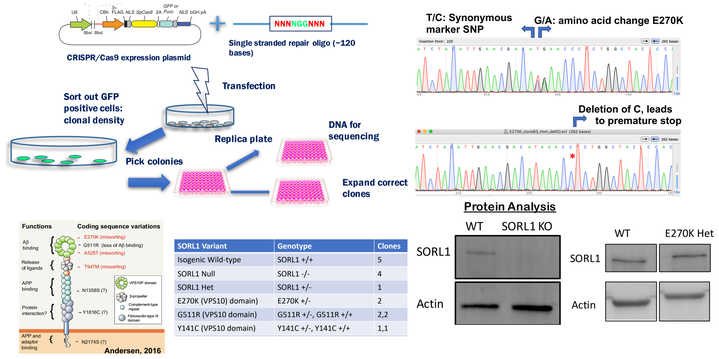
4. These cell lines will be an important resource for many collaborative projects



Hypothesis: Genetic Variation in Endosomal Network Genes is a Molecular Driver of AD Pathogenesis and Predicts Cellular and AD-Relevant Phenotypes in Human Neurons.

- ❖ Endocytic network pathology is apparent in AD
- ❖ Genes associated with endosomal trafficking are strongly associated with AD pathogenesis
- ❖ We can interrogate endocytic function and pathology in living human neurons (and other CNS cell types).

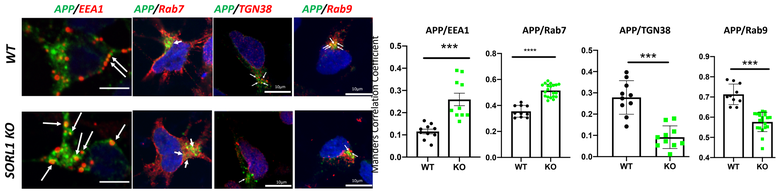
1. Use CRISPR/Cas9 genome engineering to generate null and AD-associated variants in *SORL1*.



SORL1 Variant	Genotype	Clones
Isogenic Wild-type	SORL1 +/+	5
SORL1 Null	SORL1 -/-	4
SORL1 Het	SORL1 +/-	1
E270K (VPS10 domain)	E270K +/-	2
G511R (VPS10 domain)	G511R +/-, G511R +/+	2,2
Y141C (VPS10 domain)	Y141C +/-, Y141C +/+	1,1

- ❖We employ a gene-editing pipeline to generate isogenic hiPSCs harboring one or two copies of pathogenic *SORL1* variants.
- ❖*SORL1* null lines are generated in the same editing experiments due to indels leading to frameshifts.
- ❖These cell lines are differentiated into neurons for functional experiments.

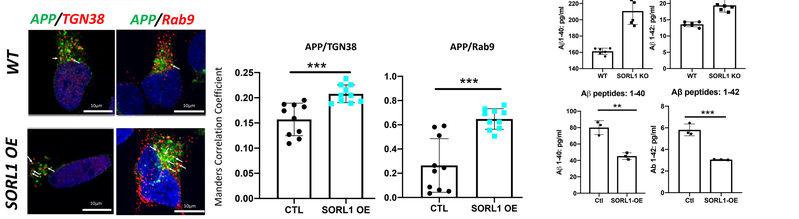
Modulating *SORL1* expression alters subcellular localization of APP and implicates various trafficking pathways



- ❖Loss of *SORL1* leads to increased APP in early/late endosomal compartments (EEA1 and Rab7+), likely leading to enhanced amyloidogenic processing.
- ❖Loss of *SORL1* leads to decreased APP the Golgi and in Rab9+ endosomes that recycle to the Golgi.

PiggyBac Expression system to overexpress *SORL1* cDNA in hiPSCs

- ❖ Differentiate to neurons
- ❖ Both CRISPR KO and PiggyBac OE yield clonal and isogenic cell lines



- ❖Enhancing *SORL1* expression does not change APP colocalization with early/late endosomes (not shown)
- ❖Enhancing *SORL1* expression leads to increase APP in the Golgi and in Rab9+ endosomes that recycle to the Golgi, likely leading to decreased amyloidogenic processing.

Conclusions and Future Directions

- ➢ *Loss of SORL1 phenocopies an early AD cytopathology*
- ➢ *Modulating SORL1 expression in human neurons demonstrates opposing effects on APP intracellular trafficking*
- ➢ *SAD patients have increased polygenic risk in endosome-associated genes*
- ➢ *Systematic analysis of pTau*
- ➢ *Can enlarged endosomes be adapted into a phenotypic screen?*
- ➢ *Analysis of how altered trafficking affects synaptic properties*
- ➢ *Test whether endocytic phenotypes are stronger with cellular age*
- ➢ *3D organoid models from cell lines with high AD risk:*
  - *Incorporation of multiple cell types*
  - *Improved neuronal maturation (?)*

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