The gain-of-function *TREM2*^{T96K} mutation increases Alzheimer's disease risk *via* impaired microglia function

#2300

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The AD-associated gain-of-function *TREM2*^{T96K} mutation impairs microglial clustering around Aβ plaques, lowers soluble Trem2, impairs Aβ uptake, and suppresses the DAM response in favor of a homeostatic-like state, specifically in female 5xFAD mice.

INTRODUCTION

Microglia are central regulators of Alzheimer's disease progression, and TREM2 is a key receptor controlling their activation and response to amyloid-β plaques. While most Alzheimer's-associated TREM2 mutations impair receptor function, the *T96K* enhances ligand-dependent signaling yet is associated with increased disease risk. We investigated how this apparent gain-of-function mutation influences microglial biology and Alzheimer's disease pathology.

METHODS

- Human whole-genome sequencing analyses to evaluate the association of **Trem2**^{T96K} with Alzheimer's disease risk.
- CRISPR/Cas9-generated **Trem2**^{T96K} knock-in mice crossed with **5xFAD** mice.
- Immunofluorescence/confocal imaging of Aβ pathology and microglial phenotypes.
- Flow cytometry, western blotting, ELISA, and Aβ uptake assays in primary microglia and engineered HMC3/BV2 cell lines.
- FACS-isolated microglia analyzed by bulk RNA-seq, single-cell RNA-seq, and qPCR.

RESULTS

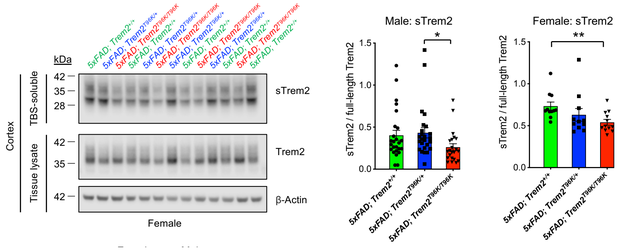
T96K increases AD risk

Meta-analysis of NIMH and ADSP cohorts showed TREM2-T96K associates with increased AD risk ($p = 0.000168$), driven by AA and HISP sub-cohorts, with earlier onset in T/T homozygotes

Rs2234253 (T96K)	NHW	AA	HISP	NIMH
Minor allele frequency (T)	0.0014	0.1390	0.0302	0.0050
Z-Score	1.4069	2.8135	2.7816	1.9220
p-value	0.1594	0.0049	0.0054	0.0546
3 subpopulations and NIMH families		Fisher score		30.56436
		Fisher *p*-value		0.000168

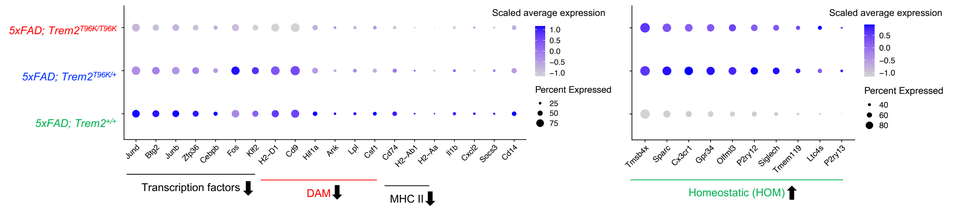
T96K lowers soluble TREM2

Reduced sTrem2 in mouse cortex and HMC3 cells, with reduced cell-surface TREM2



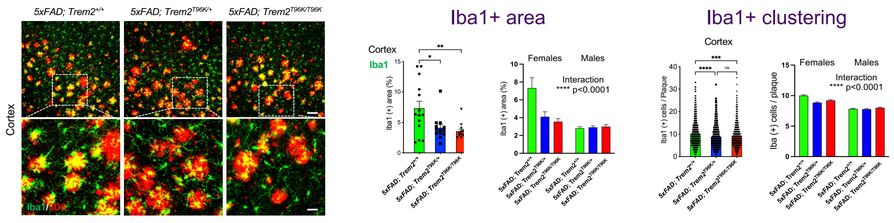
T96K suppresses DAM signature

scRNA-seq of 90,595 microglia showed reduced DAM population and downregulated DAM/inflammatory genes, with expanded homeostatic gene expression



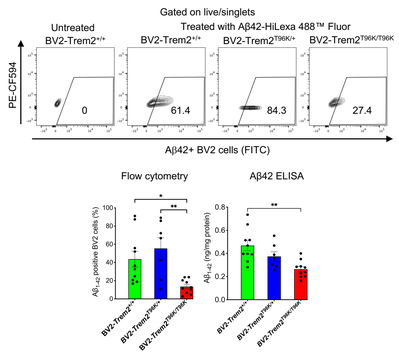
T96K reduces microglial clustering

Trem2^{T96K} reduced Iba1+ area and clustering around Aβ plaques in cortex and hippocampus of female, not male, 5xFAD mice



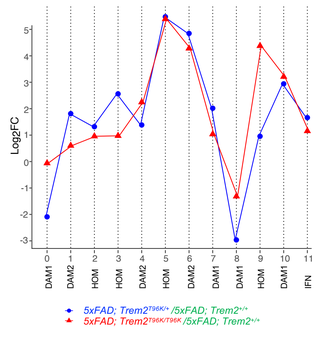
T96K impairs Aβ uptake

in BV2 knock-in microglia

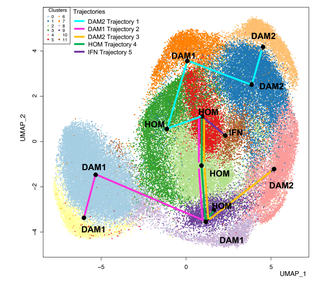


T96K blocks DAM differentiation trajectories

Log2 FC of cluster sizes (cell fractions) relative to control 5xFAD;Trem2$^{+/+}$



UMAP plot with the five inferred phenotypic cell trajectories



CONCLUSIONS

Although *TREM2*^{T96K} enhances receptor signaling *in vitro*, it paradoxically impairs microglial responses to amyloid pathology by reducing soluble/cell-surface TREM2, limiting plaque-associated microglial and suppressing DAM differentiation, thereby increasing Alzheimer's disease risk.

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