The Trem2*R47H variant alters expression and function in mouse models of Alzheimer's Disease

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ABSTRACT

Background: Alzheimer's disease (AD) is a debilitating neurodegenerative disorder with no effective treatment available. Research into understanding AD has been fueled by animal models with familial mutations. While these models have proved fruitful to understanding some baseline phenotypes, they have not been adequate to develop therapeutics to understand processes leading to AD. The brain's immune system has long been implicated as a key factor in the development of Alzheimer's disease, particularly microglia. Variants in the gene encoding TREM2 have been found to increase the risk for developing AD and TREM2 is predominately expressed on microglia.

Methods: We have created a mouse model carrying the Trem2*R47H KI allele using CRISP/Cas9 containing a nucleotide G>A point mutation for amino acid sequence change at R47H into the gene. Mice were aged to 2, 8 and 12 months. In addition, the 5xFAD model was crossed with mice carrying the Trem2*R47H variant. Tissue was analyzed using RNA-sequencing, IP Western Blotting and immunohistochemistry.

Results: In mice carrying the R47H variant, there is an alternate splicing event, resulting in a 119bp deletion inside exon 2 and decreased mRNA levels. This segment includes the R47H mutation. We have observed a similar alternate splicing event in some of non-mutant mice as well, but it is more prominent in Trem2*R47H sample as we have identified this alternate splice junction in 30 out of 34 Trem2*R47H mice. This finding suggested an alternate splicing effect on *Trem2* due to R47H mutation. Using IP Western Blots, we found the protein level of Trem2 to be reduced by approximately 60% in brain homogenates. This correlates with RNA-sequencing results.

CRISPR Strategies

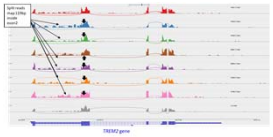
Human	TGG GGG AGG CGC AAG GCC TGG
Mouse	TGG GGG AGA CGC AAG GCC TGG
R47H	TGG GGG AGA CAC AAG GCC TGG

Mouse models created:

R47H (JAX)	TGG GGG AGA CAC AAG GCC TGG
R47H (IU)	TGG GGG AGA CAC AAG GCT TGG
R47H (Haass)	TGG GGT CGA CAC AAA GCC TGG

Human	VSCPYDSMKHWGRRKAWCRQLGEK
Mouse	VSCTYDAL KHWGRRKAWCRQLGEE

RNA-seq Data



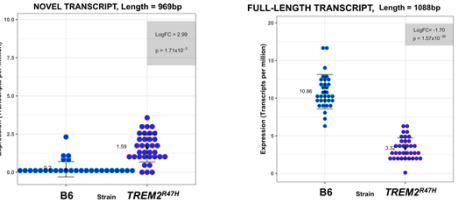
TREM2^{R47H} mice have a novel alternate splice junction at 119bp of Exon 2 in whole-brain.

Differential Expression of Trem2 isoforms

Transcript ID	Ensembl *TREM2*^{R47H}			Ensembl + Novel *TREM2*^{R47H}		
	Base Mean	Log2FC	padj	Base mean	Log2FC	padj
ENSMUST00000024791	104.2	-1.25	2.36×10^{-44}	94.2	-1.70	1.57×10^{-58}
ENSMUST00000113237	21.5	-0.62	0.83	21.8	-0.60	0.87
ENSMUST00000132340	53.7	0.19	0.99	53.7	0.19	0.99
ENSMUST00000148545	1.6	-1.48	0.99	2.1	-0.62	0.99
NOVEL ISOFORM	-	-	-	10.6	2.99	1.71×10^{-3}

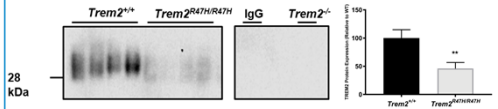
Consistent 30-50% reduction in *TREM2* in IU, JAX, nanostring, RNA-seq data.

Reduced Trem2 Expression in Homozygous R47H Knock-In Mice



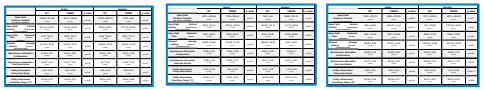
Alternate splicing effect. Sashimi plot of TREM2^{R47H} mice from IGV showing (A) all splice junction, (B) reads map 119 bp inside exon #2 of Trem2 primary isoform and map to novel exon 2 (selected in white bar) in custom isoform.

Reduced Trem2 Protein in Homozygous R47H Knock-In Mice



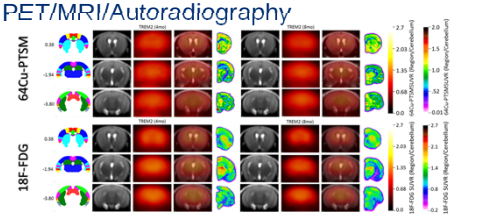
Immunoprecipitation was performed by incubating a total of 1500ug of brain protein extract with 1ug of biotinylated sheep anti-Trem2 antibody (RnD systems BAF1729) followed by incubation with streptavidin sepharose beads (CST 3419). Protein was reduced by 53% compared to the wildtype mice.

BEHAVIORAL STUDIES

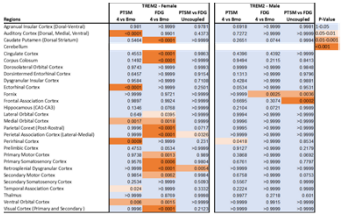


Trem2R47H mice differ from wild-type control on the following tests: Open field distance traveled and Open Field Vertical Activity (females only) at 4 months. Trem2R47H mice differ from wild-type control on the following tests: Open field perimeter time, Rotarod average latency (females only), frailty assessment (males only) at 6 months of age. Trem2R47H mice differ from wild-type control on the following tests: Open field distance traveled (males), open field vertical activity (males), open field perimeter time (females), spontaneous alternation (females) and frailty assessment (females) at 12 months of age.

PET/MRI/Autoradiography



Average MRI (left), PET (center-left), Fused (center-right), and Autoradiography (right) images at three bregma targets (0.38, -1.94, -3.80) as a function of time (top to bottom). 64Cu-PTSM PET/MRI labelling blood vessel perfusion. Images represent an average of 5 randomly selected male and females, while Autoradiography images are representative males or females.



Regional Analysis of 28 brain regions. Trem2R47H females demonstrate an reduction in blood flow as well as a reduction in glucose uptake between 4 to 8 months. Trem2R47H males have no change in blood flow, but do show a reduction in glucose uptake between 4 and 8 months.

CONCLUSIONS

- Despite the reduction in messenger RNA, there is significant full-length expression of mutant Trem2 mRNA in mice carrying the Trem2*R47H variant. This is consistent with the reduction in Trem2 protein is observed from brains.
- Furthermore, this model does not phenocopy a Trem2KO since significant expression of Trem2 protein in brain tissue is observed and transcriptome alterations are different and less pronounced.
- Further studies with this model will enable us to gain a deeper understanding of how Trem2 plays a role in the progression of AD.

For further information, please see

- MODEL AD: www.modelad.org
- AMP-AD Knowledge portal: www.ampadportal.org
- Jax AD models: https://www.jax.org/alzheimers
- AlzForum research models: http://www.alzforum.org/research-models

Acknowledgements

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