# Modeling Trem2 Variants in Mice

MODEL-AD
Model Organism Development & Evaluation for Late-Onset Alzheimer's Disease

473.06

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## ABSTRACT

Alzheimer's disease (AD) is the leading cause of dementia. The majority of AD cases are late-onset, but a causal mechanism remains unknown. However, there are likely multifactorial contributors like age, environment and genetics that increase the risk for developing the disease. Genetic factors such as rare variants of TREM2 (triggering receptor expressed on myeloid cells-2) strongly increase the risk of developing AD, confirming the role of microglia in AD pathogenesis. Several studies have examined the mechanisms by which TREM2 and its rare variants affect amyloid and tau pathologies and their consequences in both animal models and in human studies. The Model Organism Development and Evaluation Center for Late-Onset Alzheimer's Disease (MODEL-AD) has created a mouse models 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. In mice carrying the R47H variant, there is an alternate splicing event, resulting in a 119bp deletion inside exon 2 and decreased mRNA levels. The deleted segment includes the R47H mutation. This alternate splicing event occurs in some non-mutant mice, but is more prominent in Trem2*R47H samples. This finding suggests 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. PET imaging using 64Cu-PTSM and 19F-FDG shows a reduction in glucose metabolism of mice at older ages.

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 Trem2 knockout since significant expression of Trem2 protein in brain tissue is observed and transcriptome alterations are different and less pronounced. In addition, these mice may show a potential perfusion-metabolism pattern that is different than wild-type mice. Future studies with these models aid in understanding how Trem2 plays a role in the progression of AD.

## 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:

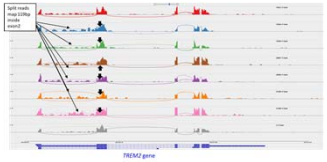
| | |
|---|---|
| R47H (JAX) | TGG GGG AGA CAC AAG GCC TGG |
| R47H (IU) | TGG GGG AGA CAC AAG GCT TGG |
| R47H (Haass) | TGG GGT CGA CAC AAA GCC TGG |

| | |
|---|---|
| Human | VSCPYDSMKHWGRRKAWCRQLGEK |
| Mouse | VSCTYDAL KHWGRRKAWCRQLGEE |

## Relevant models

| Common name | Genetic background | Strain Nomenclature | Availability | JAX # | Source |
|---|---|---|---|---|---|
| APOE4 Trem2*R47H | B6J | B6.Cg-Apoe$^{tm1.1(APOE*4)Adiu}$ Trem2$^{tm1.1(Adiu)}$/J | live | 28709 | MODEL-AD |
| Trem2*R47H | B6J | C57BL/6J-Trem2$^{tm1.1(Adiu)}$/J | live | 27918 | MODEL-AD |
| 5XFAD*TremR47H | B6J | B6.Cg-Tg(APPSwFiLon,PSEN1*M146L*L286V)6799Vas/Mmjax*C57BL/6J-Trem2$^{tm1.1(Adiu)}$/J | | | Landreth Lab |

## RNA-seq Data



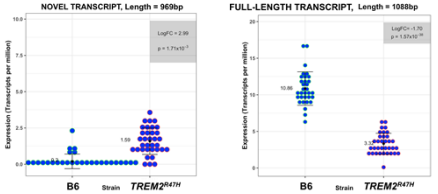

***TREM2$^{R47H}$*** **mice have a novel alternate splice junction at 119bp of Exon 2 in whole-brain.**

Differential Expression of Trem2 isoforms

| Transcript ID | Ensembl *TREM2$^{R47H}$* Base Mean | Ensembl *TREM2$^{R47H}$* Log2FC | Ensembl *TREM2$^{R47H}$* padj | Ensembl + Novel *TREM2$^{R47H}$* Base mean | Ensembl + Novel *TREM2$^{R47H}$* Log2FC | Ensembl + Novel *TREM2$^{R47H}$* padj |
|---|---|---|---|---|---|---|
| ENSMUST00000024791 | 104.2 | -1.25 | 2.36 x 10$^{-44}$ | 94.2 | -1.70 | 1.57 x 10$^{-59}$ |
| 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 x 10$^{-3}$ |

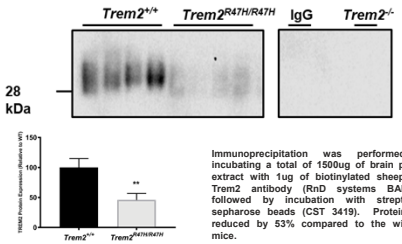
**Consistent 30-50% reduction in *TREM2* in IU, JAX, nanostring, RNA-seq data.**

## Reduced Trem2 Expression in Homozygous R47H Knock-In Mice



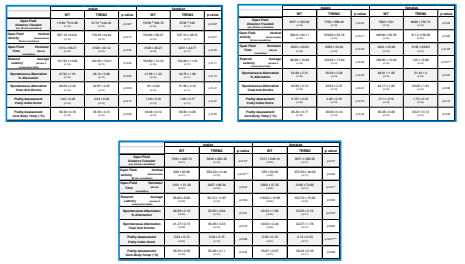

**Alternate splicing effect. Sashimi plot of TREM2$^{R47H}$ mice from IGV showing (A) all splice junction, (B) reads map 119 bp inside exon #2 of Trem2 primary isoform and map to novel exon 2 (selected in white bar) in custom isoform.**

## Reduced Trem2 Protein in Homozygous R47H Knock-In Mice



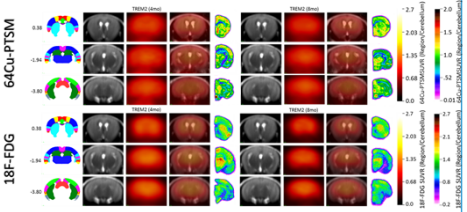

**Immunoprecipitation was performed by incubating a total of 1500ug of brain protein extract with 1ug of biotinylated sheep anti-Trem2 antibody (RnD systems BAF1729) followed by incubation with streptavidin sepharose beads (CST 3419). Protein was reduced by 53% compared to the wildtype mice.**

## 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 distance traveled (males), open field vertical activity (males), open fassessment (males only) at 6 months of age. Trem2R47H mice differ from wild-type control on the following tests: Open field ield perimeter time (females), spontaneous alternation (females) and frailty assessment (females) at 12 months of age.**

## PET/MRI/Autoradiography

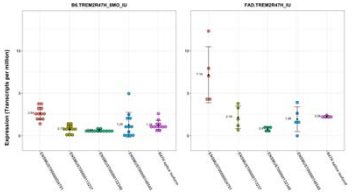



**Average MRI (left), PET (center-left), Fused (center-right), and Autoradiography (right) images at three bregma targets (0.38, -1.94, -3.80) as a function of time (top to bottom). 64Cu-PTSM PET/MRI labelling blood vessel perfusion. Images represent an average of 5 randomly selected male and females,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

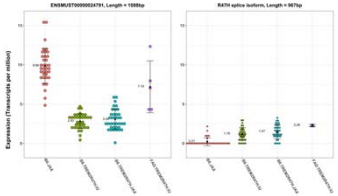
| Regions | APOE4 - Female PTSM 4 vs 8mo | APOE4 - Female FDG 4 vs 8mo | APOE4 - Female PTSM vs FDG Uncoupled | APOE4 - Male PTSM 4 vs 8mo | APOE4 - Male FDG 4 vs 8mo | APOE4 - Male PTSM vs FDG Uncoupled | P-Value |
|---|---|---|---|---|---|---|---|
| Agranular Insular Cortex (Dorsal-Ventral) | >0.9999 | 0.9379 | 0.9923 | 0.5965 | >0.9999 | 0.7473 | 0.05 |
| Auditory Cortex (Dorsal, Medial, Ventral) | 0.9883 | <0.0001 | 0.0261 | 0.7606 | 0.2967 | >0.9999 | 0.05-0.01 |
| | | | | | | | 0.01 |
| Caudate Putamen (Dorsal Striatum) | >0.9999 | 0.9938 | 0.999 | >0.9999 | 0.9972 | >0.9999 | 0.001 |
| Cerebellum | | | | | | | <0.001 |
| Cingulate Cortex | 0.4834 | >0.9999 | 0.1185 | >0.9999 | 0.9789 | 0.0253 | |
| Corpus Callosum | 0.5699 | 0.9344 | 0.1837 | >0.9999 | 0.9994 | 0.3407 | |
| Dorosolateral Orbital Cortex | 0.9999 | >0.9999 | >0.9999 | 0.987 | >0.9999 | 0.0394 | |
| Dorsimterned Entorhinal Cortex | >0.9999 | 0.2258 | 0.9864 | >0.9999 | >0.9999 | >0.9999 | |
| Dysgranular Insular Cortex | >0.9999 | 0.4528 | 0.8572 | 0.4019 | >0.9999 | 0.89 | |
| Ectorhinal Cortex | >0.9999 | 0.0322 | 0.1884 | 0.0683 | 0.8998 | >0.9999 | |
| Fornix | >0.9999 | 0.9995 | >0.9999 | >0.9999 | >0.9999 | >0.9999 | |
| Frontal Association Cortex | 0.4938 | >0.9999 | 0.197 | >0.9999 | 0.5329 | 0.081 | |
| Hippocampus (CA1-CA3) | 0.9997 | >0.9999 | 0.541 | >0.9999 | 0.9234 | 0.7166 | |
| Lateral Orbital Cortex | 0.9635 | >0.9999 | 0.5647 | >0.9999 | >0.9999 | 0.9995 | |
| Medial Orbital Cortex | 0.7831 | >0.9999 | 0.5173 | 0.8698 | >0.9999 | 0.1867 | |
| Parietal Cortex (Post-Rostral) | 0.4873 | 0.2143 | 0.0043 | >0.9999 | 0.3853 | 0.5871 | |
| Parietal Association Cortex (Lateral-Medial) | 0.4959 | 0.1859 | 0.0043 | >0.9999 | 0.3914 | 0.3068 | |
| Perirhinal Cortex | >0.9999 | 0.1033 | 0.6638 | 0.183 | 0.9059 | >0.9999 | |
| Prelimbic Cortex | 0.174 | >0.9999 | 0.4227 | >0.9999 | >0.9999 | >0.9999 | |
| Primary Motor Cortex | 0.3939 | 0.9998 | 0.0243 | >0.9999 | >0.9999 | >0.9999 | |
| Primary Somatosensory Cortex | 0.5423 | 0.9256 | 0.0341 | >0.9999 | >0.9999 | >0.9999 | |
| Retrosplenial Dysgranular Cortex | 0.3637 | 0.0606 | 0.0036 | >0.9999 | 0.1427 | 0.0573 | |
| Secondary Motor Cortex | 0.2321 | 0.9575 | 0.0036 | >0.9999 | >0.9999 | >0.9999 | |
| Secondary Somatosensory Cortex | >0.9999 | 0.0429 | 0.146 | 0.6245 | >0.9999 | 0.9955 | |
| Temporal Association Cortex | >0.9999 | <0.0001 | 0.0082 | 0.0615 | 0.5693 | >0.9999 | |
| Thalamus | >0.9999 | 0.8659 | 0.0986 | >0.9999 | 0.9998 | 0.984 | |
| Ventral Orbital Cortex | 0.6099 | >0.9999 | 0.7682 | 0.9907 | >0.9999 | 0.2296 | |
| Visual Cortex (Primary and Secondary ) | 0.7175 | 0.0224 | 0.913 | >0.9999 | 0.0772 | 0.3346 | |

**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.**

## Expression of each Trem2 isoform in 6 month old B6.TREM2$^{R47H}$ mice and 5XFAD.TRE2M$^{R47H}$ mice




## Expression of Trem2 primary isoform (ENSMUST00000024791 ) and R47H splice isoform




**Expression (Average TPM across all samples) of Trem2 isoforms enhance on 5XFAD background compare to B6 for 6 month old mice. Average expression of protein coding isoforms (ENSMUST00000024791, ENSMUST00000113237) increases more than other isoforms on 5XFAD background. Average expression of R47H splice isoform also increases (~1.8 times) on 5XFAD background but less than primary transcript (ENSMUST00000024791 , ~2.8 times). Average expression of primary transcript and R47H splice isoform are almost similar in B6.TREM2$^{R47H}$ mice from JAX and IU.**

## 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.

## FURTHER INFORMATION

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

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