TREM2 modulates differential deposition of modified and non-modified Aβ species in extracellular plaques and intraneuronal deposits

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Abstract
Progressive accumulation of Amyloid-β (Aβ) deposits in the brain is a characteristic neuropathological hallmark of Alzheimer’s disease (AD). During disease progression, extracellular Aβ plaques undergo specific changes in their composition by the sequential deposition of different modified Aβ species. Microglia are implicated in the restriction of amyloid deposits and play a major role in internalization and degradation of Aβ. Recent studies showed that rare variants of the Triggering Receptor Expressed on Myeloid cells 2 (TREM2) are associated with an increased risk for AD. Post-translational modifications of Aβ could modulate the interaction with TREM2, and the uptake by microglia. Here, we demonstrate that genetic deletion of TREM2 or expression of a disease associated TREM2 variant in mice lead to differential accumulation of modified and non-modified Aβ species in extracellular plaques and intraneuronal deposits. Human brains with rare TREM2 AD risk variants also showed altered deposition of modified Aβ species in the different brain lesions as compared to cases with the common variant of TREM2. These findings indicate that TREM2 plays a critical role in the development and the composition of Aβ deposits, not only in extracellular plaques, but also intraneuronally, that both could contribute to the pathogenesis of AD.

Keywords: TREM2, Microglia, Post-translational modification, Aβ, Intraneuronal, Vascular deposits

Background
Alzheimer’s disease (AD) is characterized neuropathologically by the combined occurrence of extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) with abnormally phosphorylated tau (τ) protein in the brain [24, 65]. Aβ deposits in the human brain contain different Aβ species, including N-terminal truncated, pyroglutamated, phosphorylated and nitrated variants that show significant differences in aggregation, stability, and toxicity [1, 42]. In particular, Aβ peptides with pyroglutamate-modification at glutamate residue 3 (N3pE-Aβ) or phosphorylated serine residue 8 (pSer8-Aβ) have increased propensity to form aggregates with increased neurotoxicity [38, 40–42, 53]. Previous investigations revealed a specific sequential deposition starting with non-modified Aβ (nmAβ) peptides, followed by N3pE-Aβ and pSer8-Aβ species in extracellular plaques during the progression from pre-clinical to clinical phases of AD [1, 54]. A similar sequence for the deposition of these Aβ species was also found in cerebral amyloid angiopathy (CAA) [11].

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Genome-wide association studies (GWAS) and exome sequencing have revealed genetic loci related to inflammatory pathways to be associated with an increased risk for AD [5, 18, 71]. Among these subsets of genes, rare variants of the microglial transmembrane receptor, Triggering Receptor Expressed on Myeloid cells (TREM2), confer a high risk for the development of AD, comparable to the risk exerted by the Apolipoprotein E4 allele (ApoE4) [18, 30]. TREM2 is preferentially expressed in microglia and functions as a receptor for different ligands, including anionic lipids, ApoE, and Aβ [12, 47, 74, 82]. Activation of TREM2 regulates microglial functions, including phagocytosis, cytokine production, proliferation and migration [16, 69, 75]. TREM2 is proteolytically processed by ADAM proteases to generate soluble variants of TREM2 (sTREM2) [25, 34, 78], that can be detected in extracellular fluids. sTREM2 could act as a decoy receptor to negatively modulate TREM2 signaling and inflammatory responses of microglia, and also shows trophic activity to promote microglial survival [35, 83].

TREM2 positive microglia cluster around extracellular plaques in brains of human AD cases and amyloid precursor protein (APP) transgenic mice, and the deletion of TREM2 in APP mouse models results in altered morphology and seeding of plaques, as well as decrease in number of plaque associated microglia [28, 51, 67], indicating an involvement of TREM2 in the restriction of Aβ deposits [11, 75]. Here, we sought to characterize the role of TREM2 in accumulation and distribution of modified and non-modified Aβ species in the brain. Deletion of TREM2 or the expression of the disease associated TREM2T66M variant in different APP transgenic mouse models led to altered composition not only of extracellular plaques, but also of intraneuronal deposits containing modified and non-modified Aβ variants. Human cases with rare AD associated TREM2 variants also showed altered composition and morphology of the different Aβ pathological lesions as compared to AD cases with the common TREM2 variant. Together, the data indicate an important role of TREM2 in altering the composition of Aβ related brain lesions during the pathogenesis of AD.

Methods

Transgenic mice
APP695KM670/671NL; PS1L166P TREM2+/+ and APP695KM670/671NL; PS1L166P TREM2−/− transgenic mice, and 5xFAD TREM2+/+ and 5xFAD TREM2−/− transgenic mice were described previously [31, 51, 66, 75].

12 M old female APPKM670/671NL; PS1ΔE9 transgenic mice endogenously expressing TREM2 WT or the homozygous TREM2T66M knock-in (KI) mutation were obtained from Taconic Biosciences GmbH, Cologne, Germany [31]. The different mouse models are described in Table 1.

Immunofluorescence (IF) analysis of mouse brains

Mouse brains were processed as described previously [31, 36, 51]. In brief, 20–25 μm sections were sequentially collected in Phosphate-buffered saline (PBS), placed on charged slides, and stained. For IF staining, Reveal Decloaker (Biocare Medical, #RV1000) was used for antigen retrieval [31] at 95 °C for 30 min. After this, the sections were washed with PBS and subjected to permeabilization with 0.25% Triton X-100 for 20 min before blocking for 2 h in 5% NHS and 3% BSA prepared in 1xPBST (Triton X100-0.2%). Mouse on Mouse Blocking Reagent (Vector laboratories, #MKB-2213) was used for primary antibodies generated in mouse or rat at a dilution of 1 drop/1000 μl. Primary antibodies were diluted in 3% NHS and 1.5% BSA prepared in 1xPBST (Tween 20-0.1%), added on sections and incubated at 4 °C overnight. For TREM2 staining, sections were incubated in the primary antibody at 4 °C for 48 h [31, 51]. After washing steps, appropriate secondary antibodies diluted in 3% NHS and 1.5% BSA prepared in 1xPBST (TWEEN 20–0.1%) were added on the sections and incubated for 1 h at RT. The sections were then washed and mounted with VECTASHIELD® Hardset™ antifade mounting medium (Vector laboratories, #H-1400) or VECTASHIELD® antifade mounting medium with DAPI (Vector laboratories, #H-1200). Primary and secondary antibodies are summarized in Additional file 1: Table S1.

Brain protein extraction

Snap-frozen brain hemispheres were extracted as previously described [22, 70]. Briefly, hemispheres were homogenized in PBS, 1 mM EDTA, 1 mM EGTA, 3 μl/ml protease inhibitor mix (Sigma). Homogenates were extracted in RIPA buffer (25 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% NP40, 0.5% NaDOC, 0.1% SDS), centrifuged at 100,000 g for 30 min and the pellet containing insoluble Aβ was solubilized in 2% SDS, 25 mM Tris–HCl, pH 7.5. The final protein concentration was determined using Pierce™ BCA Protein Assay kit (Thermo Fisher) according to the user’s manual.

Immunoblotting

The brain extracts were separated on 4–12% NuPAGE gels and transferred to 0.45 μm nitrocellulose (NC) membranes as described previously [36]. For immunodetection of proteins, membranes were blocked for 1 h in 5% nonfat dry milk in TBST (TWEEN 20–0.1%), then incubated with the primary antibodies in TBST (TWEEN 20–0.1%) overnight at 4 °C, followed by three washing steps for 10 min with TBST (TWEEN 20–0.1%), and
| Sr. No. | Transgenic mouse models | Age (months) | Sex | Genotype | No. of animals (n) | Total number of Aβ plaques/ROI stained with various antibodies | Somatosensory cortex (SSC) | Retrospenial cortex (RSC) | Dentate gyrus (DG) |
|---------|-------------------------|--------------|-----|-----------|-------------------|---------------------------------------------------------------|-------------------------|------------------------|---------------------|
|         |                         |              |     |           |                   | pSer8-Aβ | N3p3-Aβ | nmAβ | Aβ (4G8) | pSer8-Aβ | N3p3-Aβ | nmAβ | Aβ (4G8) | pSer8-Aβ | N3p3-Aβ | nmAβ | Aβ (4G8) |
| 1       | 5xFAD                   | 5 M Male     | TREM2^{+/+} | 5 | Total plaque count/group | 1117 | 1664 | 1791 | 1847 | 1842 | 1353 | 1770 | 1724 | 1777 | 1815 | 933 | 1263 | 1932 | 1872 | 1857 |
|         |                         |              |     |           |                   | Plaque load (%) | 2.89 | 8.11 | 10.91 | 11.42 | 11.46 | 4.35 | 7.49 | 7.71 | 9.27 | 8.52 | 2.89 | 5.12 | 8.76 | 8.98 | 8.93 |
|         |                         |              |     |           |                   | 1631 | 1836 | 2247 | 2314 | 2305 | 1734 | 1899 | 2120 | 2169 | 2198 | 1257 | 1403 | 2258 | 2196 | 2183 |
|         |                         |              |     |           |                   | Plaque load (%) | 9.53 | 10.80 | 17.90 | 19.08 | 17.49 | 7.66 | 9.29 | 13.82 | 14.41 | 14.45 | 5.27 | 6.94 | 13.55 | 14.29 | 14.22 |
| 15 M    | Male TREM2^{+/+}       | 5 Total plaque count/group | 1565 | 2322 | 3616 | 3725 | 3833 | 2057 | 2559 | 3641 | 3802 | 3778 | 1530 | 2535 | 3426 | 3537 | 3461 |
|         |                         |              |     |           |                   | Plaque load (%) | 4.59 | 17.26 | 27.31 | 28.10 | 29.38 | 7.09 | 13.19 | 25.04 | 25.84 | 26.41 | 5.49 | 10.24 | 21.40 | 21.51 | 21.00 |
|         |                         |              |     |           |                   | 2250 | 3013 | 4414 | 4417 | 4523 | 2846 | 3285 | 4398 | 4520 | 4488 | 2153 | 3139 | 4033 | 4144 | 4096 |
|         |                         |              |     |           |                   | Plaque load (%) | 13.06 | 23.76 | 41.46 | 41.37 | 42.44 | 15.73 | 21.93 | 32.66 | 35.63 | 33.95 | 10.99 | 16.07 | 32.16 | 32.39 | 31.95 |
| 2       | APP/PS1L166P            | 4 M Male     | TREM2^{+/+} | 5 | Total plaque count/group | 422 | n.d. | 1027 | 967 | 982 | 420 | n.d. | 852 | 892 | 917 | 245 | n.d. | 626 | 760 | 776 |
|         |                         |              |     |           |                   | Plaque load (%) | 1.93 | n.d. | 5.51 | 4.96 | 5.25 | 2.46 | n.d. | 5.59 | 6.52 | 6.62 | 1.35 | n.d. | 4.14 | 5.03 | 4.63 |
|         |                         |              |     |           |                   | 907 | n.d. | 1215 | 1156 | 1193 | 631 | n.d. | 1062 | 1115 | 1132 | 446 | n.d. | 845 | 957 | 988 |
|         |                         |              |     |           |                   | Plaque load (%) | 2.93 | n.d. | 8.19 | 9.03 | 9.88 | 4.09 | n.d. | 7.64 | 9.14 | 9.18 | 2.46 | n.d. | 6.57 | 7.01 | 7.63 |
| Sr. No. Transgenic mouse models | Age (months) | Sex | Genotype | No. of animals (n) | Total number of Aβ plaques/ROI stained with various antibodies |
|-------------------------------|--------------|-----|----------|-------------------|---------------------------------------------------------------|
|                              |              |     |          |                   | Somatosensory cortex (SSC) | Retroplenial cortex (RSC) | Dentate gyrus (DG) |
|                              |              |     |          |                   | pSer8-Aβ | N3p3-Aβ | nmAβ | Aβ (4G8) | Aβ (2964) | pSer8-Aβ | N3p3-Aβ | nmAβ | Aβ (4G8) | Aβ (2964) |
| 12 M Female TREM2^{+/+}       | 5            |     |          |                   | 804      | 1935    | 4003 | 4090 | 3999 | 942 | 2096 | 3776 | 4050 | 3943 | 649 | 1584 | 2437 | 2482 | 2485 |
| TREM2^{-/-}                   | 5            |     |          |                   | 1424     | 2545    | 4655 | 4770 | 4571 | 1391 | 2562 | 4226 | 4518 | 4398 | 972 | 1951 | 2766 | 2835 | 2835 |
| APP/PS1ΔE9 12 M Female TREM2 | 3            |     |          |                   | 491      | 1068    | 2044 | 2333 | 2331 | 414  | 889  | 1489 | 1785 | 1685 | 183 | 548  | 717  | 874  | 965  |
| TREM2^{T66M}                  | 3            |     |          |                   | 757      | 1397    | 2570 | 2581 | 2605 | 735  | 1349 | 1975 | 2073 | 2060 | 255 | 770  | 1001 | 1119 | 1086 |
|                              |              |     |          |                   | 8.02     | 18.63   | 39.91 | 39.44 | 41.83 | 7.36 | 18.89 | 30.25 | 31.31 | 33.08 | 3.37 | 12.10 | 14.19 | 16.75 | 15.94 |
addition of appropriate secondary antibodies in TBST (TWEEN 20–0.1%). After an incubation period of 60 min at RT, membranes were washed three times for 10 min with TBST (TWEEN 20–0.1%), and once for 5 min with TBS. For signal detection, the enhanced chemiluminescence ECL imager (Bio-Rad laboratories, Inc.) or Odyssey CLx™ (LI-COR, Biosciences) were used. The quantification was done by using Image Studio-Lite (Ver. 5.2). Primary and secondary antibodies are summarized in Additional file 1: Table S1.

**Patient material**

Tissue samples of patient autopsy cases were provided by the Neurobiobank Munich, Ludwig-Maximilians-University (LMU) Munich. Detailed clinical characteristics were ascertained from an integrated autopsy database. Written informed consent for autopsy and analysis of tissue sample data was obtained for all patients, either from the patients themselves or their kin and the samples were collected according to the guidelines of the local ethics committee following all ethical regulations. Information regarding cases, clinical diagnosis, age at death, post-mortem delay, fixation time, AD Braak & Braak stage, Thal phase, TREM2 coding variant is given in Table 2. The genotyping and identification of TREM2 variant carriers were done as described before [51]. Sample sizes were based on availability of patient material. For all analyses, temporal neocortex was used which included cortex of medial temporal gyrus at the level of anterior hippocampus.

**Immunohistochemistry (IHC) on human post-mortem brain tissue**

IHC was done as described before [51]. In brief, 5 μm temporal neocortex sections were mounted on slides, deparaffinized and rehydrated in a series of xylene and graded ethanol. The sections were subjected to citric acid antigen retrieval (1 M sodium citrate in PBS, pH 6.0) and boiled in a microwave for 20 min. After cooling, endogenous peroxidase activity was quenched using 30% hydrogen peroxide for 20 min. Sections were blocked and incubated with primary antibodies (Additional file 1: Table S1) overnight at 4 °C. Primary antibodies were detected with biotinylated anti-mouse and anti-rat IgG secondary antibodies and visualized with avidin–biotin complex (ABC-Kit, Vector laboratories) followed by development with diaminobenzidine-HCl (DAB, Vector laboratories) for 5 min. Lastly, sections were counterstained with haematoxylin. Stainings were performed in serially cut sections to compare the same region of interest through all immunostainings. Brightfield images were taken with Axio Scan.Z1 (Carl Zeiss MicroImaging GmbH, Germany).

**Confocal imaging**

All IF images were acquired using VisiScope CSU-W1 spinning disc confocal microscope and VisiView Software (Visitron Systems GmbH, Germany). Laser and detector settings were maintained constant for the acquisition of each immunostaining. All stainings were repeated at least three times to ensure reproducibility in the staining protocol. Images were acquired at 10×, 20×, 40×W or 63×W (W-water immersion) objective at 2048 x 2048 pixels, with z-step size of 1 μm (for 10×, 20×, 40×W, 63×W images) or 4-5 μm (for 10× montage image).

**Data analysis of Aβ deposits in mouse and human brain**

Initial optimization of dilutions and incubation times for the different antibodies was carried out for detection of the different Aβ species as reported previously [31, 40, 41]. Sections or areas with folds or poor staining quality were excluded from quantifications. To represent and quantify plaque densities (number and size distribution) from three sections/mouse brains regions, the somatosensory cortex (SSC), the retrosplenial cortex (RSC), and the dentate gyrus (DG) (Additional file 1: Figure S1a), acquired images were imported into Fiji software and data channels were separated (image/color/split channels). All layers from a single image stack were projected on a single slice (stack/Z-projection) to test the feasibility of quantifying the area and number of plaques (Additional file 1: Figure S1b). The plaques were then segmented and quantified in Fiji using automatic thresholding methods. Due to the thickness of brain sections and the limited penetration of antibody to plaques underneath the sectioned face, lightly embedded stained plaques may or may not be detected depending on image adjustment values (Additional file 1: Figure S1c). Therefore, the plaques and their quantified combined area in this study constitute the “lower boundary” of the plaque number volume density. Furthermore, as demonstrated with digitalized plaques (SSC region stained with nmAβ as an example (Additional file 1: Figure S2a-b), the number of plaques assessed using an image analysis method in a given brain region varied depending upon the cut-off value of pixel size and the thresholding of plaques. Initial analysis of optical images revealed that plaque-like images that were digitalized often displayed plaque-like artifacts. Therefore, plaques > 10μm² were considered as reliable “digital plaques”. Adjustment of thresholding of certain digitalized plaques appeared to vary in number valuables due to irregularity in plaque shapes or depending upon value of image distribution. It was found that the staining intensity cut-off had a significant impact on the number of plaques. Interestingly, TREM2 knockout
Table 2  Demographic and clinical characteristics of the TREM2 coding variants and control (CV) groups. n.i.- no information, ROI- Region of interest. Case#3, with highest fixation time, was omitted for the plaque count and size quantification as it was difficult to define boundaries of extracellular plaques stained with 1E4E11 (pSer8-Aβ) antibody

| Case # number | Clinical diagnosis                        | Age at death (yr) | Sex | Post-mortem Delay (h) | Fixation time (days) | CERAD Braak & Braak AD stage | Thal phase | ApoE status | TREM2 variant | Total number of Aβ plaques counted/analyzed ROIs | Total number of pSer8-Aβ plaques counted/analyzed ROIs | Total nmAβ intracellular deposits counted/analyzed ROIs | Total pSer8-Aβ intracellular deposits counted/analyzed ROIs |
|---------------|------------------------------------------|-------------------|-----|-----------------------|----------------------|----------------------------|------------|-------------|--------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|
| #1            | Dementia (rapidly progressive; without further specification) | 75                | M   | 24                    | n.i                  | C                          | 6          | 5           | E3/E4 R62H (G > A) | 13,053                                           | 2286                                           | 2871                                           | 1663                                           |
| #2            | Dementia of the Alzheimer type            | 78                | F   | 21                    | 15                   | C                          | 6          | 5           | E3/E4 R62H (G > A) | 14,125                                           | 2270                                           | 2616                                           | 1496                                           |
| #3            | Dementia (without further specification)  | 81                | F   | 11–35                 | 613                  | C                          | 5          | 5           | E3/E4 R62H (G > A) | -                                                | -                                              | 2764                                           | 2740                                           |
| #4            | Frontotemporal dementia (FTD)            | 77                | M   | 5                     | 25                   | C                          | 5          | 5           | E3/E4 R62H (G > A) | 15,782                                           | 4894                                           | 4415                                           | 3825                                           |
| #5            | Dementia (without further specification)  | 86                | M   | 16–40                 | 156                  | C                          | 5          | 4           | E3/E4 R62H (G > A) | 14,296                                           | 1984                                           | 373                                           | 3675                                           |
| #6            | Frontotemporal dementia (FTD)            | 77                | M   | 26                    | 297                  | C                          | 5          | 4           | E3/E4 R62C (C > T) | 14,432                                           | 1857                                           | 3831                                           | 2190                                           |
| #7            | Dementia of the Alzheimer type            | 81                | F   | 60                    | 39                   | C                          | 6          | n.i         | E3/E4 None          | 9384                                             | 2057                                           | 3304                                           | 1052                                           |
| #13           | Dementia, probably of the Alzheimer type  | 82                | M   | 15                    | 104                  | C                          | 6          | 5           | E3/E4 None          | 9816                                             | 2759                                           | 1850                                           | 1841                                           |
### Table 2 (continued)

| Case # number | Clinical diagnosis                      | Age at death (yr) | Sex | Post-mortem Delay (h) | Fixation time (days) | CERAD Braak & Braak AD stage | Thal phase | ApoE status | TREM2 variant | Total number of Aβ plaques counted/analyzed ROIs | Total number of pSer8-Aβ plaques counted/analyzed ROIs | Total nmAβ intracellular deposits counted/analyzed ROIs | Total pSer8-Aβ intracellular deposits counted/analyzed ROIs |
|---------------|----------------------------------------|-------------------|-----|------------------------|----------------------|------------------------------|------------|-------------|----------------|-----------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| #14           | Dementia (without further specification) | 85                | M   | 27                     | 27                   | C                            | 6          | 5           | E3/E4          | 9892                                           | 2386                                            | 2157                                            | 1021                                            |
| #15           | Dementia (without further specification) | 85                | M   | 49                     | 136                  | C                            | 6          | 5           | E3/E4          | 16,160                                          | 4104                                            | 2404                                            | 2834                                            |
| #16           | Dementia (without further specification) | 88                | F   | 9                      | 76                   | C                            | 4          | 5           | E3/E4          | 14,698                                          | 3894                                            | 2891                                            | 1224                                            |
| #17           | Dementia of the Alzheimer type          | 75                | M   | 3–13                   | 141                  | C                            | 5          | 5           | E3/E4          | 8681                                           | 2256                                            | 2295                                            | 837                                             |
mouse brain, showed large size plaques as well as an increased total number of plaques > 10 µm² even at lower thresholding, confirming the validity of the analysis.

For the quantification of plaque ratio in the IF experiments, we considered 300 plaques/region/group (each for SSC and RSC) and 150 plaques/region/group (for DG) (so, SSC=60 plaques/mouse, RSC=60 plaques/mouse, DG=30 plaques/mouse) so making total of 750 plaques/group containing 5 animals or 450 plaques/group containing 3 animals (from at least 3 sections/animal). For this analysis, randomly selected plaques that were positive with all three antibodies detecting Aβ species/staining in 1000 × 1000 µm ROI were analyzed by manually drawing boundary around each plaque by using freehand draw tool in Fiji and determining the IntDen [8] of total Aβ-immunoreactive area for each plaque stained with various antibodies specific to Aβ species within each section for all channels (Additional file 1: Figure S3).

For quantification of microglia surrounding plaques, microglia surrounding 30 cortical plaques of similar plaque area were manually counted and represented as a mean of 30 plaque associated microglia/animal. The total number of neurons was manually counted in a 1000 × 1000 µm area of SSC, in 2 independently stained sections and represented as a ratio of pSer8-Aβ positive neurons/total neurons.

For representation and quantification of plaque densities of 4G8 and pSer8-Aβ stained plaques in the human brain’s sections, 10 cortical 2 × 2 mm regions of interest (ROIs) were randomly selected per case and manually quantified. ROIs were selected to allow analysis of the same region for all four consecutive brain sections stained with different antibodies without interference by cuts, folds, or other irregularities (Additional file 2: Source data 1). The total number of plaques along with area measurements were considered for the analysis. The border around plaques was manually drawn by using “spline contour” tool in the ZEN 3.2 software, for the area stained with different antibodies. With 4G8 antibody-stained sections, an area cutoff of 10 µm² was determined, while with pSer8-Aβ antibody-stained sections, an area cutoff of 30 µm² was determined, below which were considered as either artifacts or intracellular deposits (quantified separately). Case#3, with highest fixation time, was omitted for the plaque count and size quantification as it was difficult to define boundaries of extracellular plaques stained with 1E4E11 (pSer8-Aβ) antibody. Aβ plaque load was calculated by summing the areas of all counted plaques divided by the total area of all ROIs. The intracellular deposits of nmAβ and phosphorylated Aβ were manually counted from 10 cortical consecutive 2 × 2 mm randomly selected ROIs per case.

**Statistical analysis**

Statistical analyses were performed using GraphPad prism software. The plaque area distribution showed positively skewed distribution [58] and varied from the normal distribution confirmed by D’Agostino & Pearson and the Shapiro–Wilk normality test. Hence, for this analysis, we considered non-parametric, Mann Whitney test (comparing the distributions of ranks in two groups) and Kolmogorov–Smirnov test (comparing the cumulative distributions) for the frequency distribution of all values. Besides this, since the data were not formally tested, we assumed it followed Gaussian distribution and that the variance between groups was comparable [51]; thus, unless otherwise stated, two-sided, unpaired t-test with Welch’s correction was used to determine the statistical difference between groups in analyses requiring only single comparisons. The degree of significance between groups is represented as *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, and **p > 0.05.

**Randomization and blinding**

The immunohistochemical analysis of mouse and human brains was initially performed blinded with coded slides. However, complete randomization was not possible in the staining with a microglial marker or when stained with anti-TREM2 antibody [31, 51], depicting microglial clustering. No randomization procedure was performed for selecting patient material as case inclusion was largely based on availability. Following the completion of the analysis, the groups were unblinded to perform statistics.

**Data collection**

Confocal images were acquired by using VisiScope CSU-W1 spinning disk confocal microscope and VisiView Software (Visitron Systems GmbH, Germany). Human brain immunostaining data were acquired by Axio Scan. Z1 at Plan-Apochromat 20x/0.8M27 objective imaged by Hitachi HV-F202SCL with ZEN 3.2 software (Carl Zeiss MicroImaging GmbH, Germany). FIJI (ImageJ) or ZEN 3.2 software was used for all immunohistochemical analyses. Microsoft Excel was used to organize and to calculate the averages of each repeated experiment. GraphPad (Prism v7.0) software was used to build graphs and perform statistical analyses presented throughout the manuscript.

**Validation**

Phosphorylation-state specific antibodies were generated and validated as described previously [38, 41]. Antibody 1E4E11 is specific for pSer8-Aβ and does not cross-react with other post-translationally modified variants of Aβ, including N-terminally truncated (Aβ 3–42), nitrated (3NTyr10-Aβ), pyroglutamated (N3pE-Aβ), or Aβ...
phosphorylated at Ser 26 [41]. Rabbit polyclonal antibody 2964 was raised against aggregated Aβ [73]. TREM2 antibody was verified for immunostainings [28, 51]. 7H3D6 antibody specifically detects Aβ species with a non-modified N-terminus and does not recognize phosphorylated Ser8-Aβ, but also does not recognize other Aβ variants with N-terminal modifications, including pyroglutamated, nitrated, and N-terminally truncated Aβ species [41]. It is important to note that both antibodies 1E4E11, detecting pSer8-Aβ and 7H3D6 used for detection of N-terminally non-modified Aβ, do not cross-react with full-length APP or APP C-terminal fragments [40, 41]. All the other antibodies used in the study were verified for immunostaining and immunoblotting in mouse and human samples according to the company websites (Additional file 1: Table S1).

Results

Selective accumulation of Ser8-phosphorylated Aβ species upon loss of TREM2 function in brains of transgenic mice

Phosphorylated Aβ variants were previously detected in brains of transgenic mouse models and human AD cases, and shown to exert increased toxicity in Drosophila models and human neurons derived from embryonic stem or induced pluripotent stem cells [11, 13, 38, 40, 41, 54]. To assess the role of TREM2 in the deposition of modified Aβ species in-vivo, 5xFAD transgenic mice were crossed with TREM2+/− or TREM2−/− mice as described previously [31, 51] (Table 1), and the deposition of different Aβ species was analyzed using several antibodies selectively detecting modified and non-modified variants of Aβ. Monoclonal antibody 1E4E11 specifically detects pSer8-Aβ species, while monoclonal antibody 7H3D6 selectively recognizes Aβ with Ser8 in non-phosphorylated state. Antibody 7H3D6 also does not recognize other Aβ variants with N-terminal modifications, including pyroglutamated, nitrated, and N-terminally truncated Aβ species [41].

Triple staining with mouse monoclonal 1E4E11, rat monoclonal 7H3D6, and rabbit polyclonal 2964 antibodies revealed that TREM2−/− mice at 15 months (15 M) of age had significantly more plaques detected by all three antibodies. Elevated plaque deposition, as measured by the plaque number (Fig. 1a–b, Additional file 1: Figure S4) and plaque load (Table 1), was detected in the three different brain regions analyzed, the somatosensory cortex (SSC), the retrosplenial cortex (RSC), and the dentate gyrus (DG). Consistent with a preferential deposition in the core of plaques, pSer8-Aβ positive deposits are of smaller size than those containing nmAβ that is also deposited in the corona of plaques (Fig. 1c, Additional file 2: Source data 2a-c). These findings are consistent with the function of TREM2 to restrict plaque size or growth [75, 81], and also demonstrate the importance of TREM2 to limit accumulation of pSer8-Aβ in the core of plaques. A selective increase in pSer8-Aβ was detected by analyzing the mean fluorescence signal intensities within Aβ deposits (Fig. 1d–e). Furthermore, increased number and size of pSer8-Aβ and nmAβ positive plaques in 5xFAD TREM2+/− compared to TREM2++/+ mouse brains was already observed in 5 M old mice (Fig. 2a–b, Additional file 2: Source data 2). Analysis of plaque size distribution at both ages revealed overall increased numbers of deposits of various sizes in all three analyzed brain regions of TREM2−/− mice (Additional file 2: Source data 2). In particular, the number of deposits >1200 µm² that are also positive for pSer8-Aβ were strongly increased in TREM2−/− mice already at 5 M of age, suggesting that TREM2 decreases the formation and growth of plaques already at early stages of deposition. However, the ratio of the mean fluorescence intensity for pSer8-Aβ and nmAβ was similar in TREM2−/− and TREM2+/+ at this young age (Fig. 2c–d), indicating that TREM2 deficiency promotes co-deposition of different Aβ species. Very similar findings were obtained with an independent double-transgenic APP/PS1L166P mouse model (Table 1, Additional file 1: Figure S5, Additional file 2: Source data 3).

Pyroglutamate-modified Aβ (N3pE-Aβ) also accumulates during the pathogenesis of AD in the cortex and hippocampus [52, 55]. Immunohistochemical analyses showed that TREM2−/− mice had significantly more N3pE-Aβ positive plaques at 15 M of age in the SSC, RSC, and the DG as compared to TREM2++/+ mice. Consistent
Fig. 1 (See legend on previous page.)
with data shown in Fig. 1, TREM2−/− mice showed higher number of plaques, (Fig. 3a–b), increased size of plaques (Fig. 3c, Additional file 2: Source data 2a–c) and elevated plaque load (Table 1) in all three regions as revealed by co-staining with antibodies detecting N-terminally non-modified Aβ and total Aβ species at 15 M of age. In contrast to pSer8-Aβ, N3pE-Aβ was not selectively increased in plaques as compared to nmAβ at 15 M of age, as evidenced by similar ratios of N3pE-Aβ/nmAβ in all analyzed brain regions (Fig. 3d). At 5 M of age, the total number of N3pE-Aβ deposits were only slightly, but not significantly increased in 5xFAD TREM2−/− mice as compared to TREM2+/+ 5xFAD mice (Fig. 3e–f). However, as observed with 15 M old mice, there was a significant increase in the size of N3pE-Aβ positive plaques especially for larger deposits >1500 µm² already at 5 M in 5xFAD TREM2−/− mice (Fig. 3g, Additional file 2: Source data 2d–f). Analysis of plaque size distribution revealed that there was an increase in the number of N3pE-Aβ deposits at both age groups, again particularly pronounced for deposits >1500 µm². An increase of pSer8-Aβ and N3pE-Aβ deposits <600 µm² in the three analyzed brain regions of TREM2−/− mice as compared to TREM2+/+ mice was only observed at 15 M of age (Additional file 2: Source data 2). The N3pE-Aβ/nmAβ intensity ratio in the analyzed brain regions was not different between TREM2−/− and TREM2+/+ mice (Fig. 3d, h). Very similar findings were obtained with the independent double-transgenic APP/PS1L166P mouse model (Additional file 1: Figure S6, Additional file 2: Source data 3). These findings indicate that N3pE-Aβ species showed increased deposition upon deletion of TREM2. However, N3pE-Aβ, in contrast to pSer8-Aβ, did not selectively accumulate upon deletion of TREM2 in relation to nmAβ.
Fig. 3  TREM2 deletion leads to increased N3pE-Ab deposits in 5xFAD transgenic mouse brains. a Representative images showing deposition of N3pE-Ab, nmAb, and Ab (4G8) in SSC of male 15 M old-5xFAD-TREM2−/− and TREM2+/+ mice (scale bar = 200 µm, 20x). b Dot plots representing number of plaques/mm². c Box and whiskers plots showing plaque size (µm²) stained with N3pE-Ab and Ab(4G8) antibodies in the SSC, RSC, and DG of the male 15 M-5xFAD-TREM2−/− and TREM2+/+ mice. d Ratio of N3pE-Ab/nmAβ in the SSC, RSC, and DG of male 15 M-5xFAD-TREM2−/− compared with TREM2+/+ mice. e Representative images showing deposition of N3pE-Ab, nmAb, and Ab (4G8) in SSC of male 5 M-5xFAD-TREM2−/− and TREM2+/+ mice (scale bar = 200 µm, 20x). f Dot plots representing number of plaques/mm². g Box and whiskers plots showing plaque size (µm²) stained with N3pE-Ab and Ab (4G8) antibodies in the SSC, RSC, and DG of male 5 M-5xFAD-TREM2−/− and TREM2+/+ mice. h Ratio of N3pE-Ab/nmAβ in the SSC, RSC, and DG of male 5 M-5xFAD-TREM2−/− compared with TREM2+/+ mice. Each dot represents average value of the number of plaques or the ratio of N3pE-Ab/nmAβ per animal. The box and whiskers plots represent min/max values of distribution of plaque size with the median (shown by the line dividing the box) and the dot plots represent mean ± SEM (n = 5 animals, color- blue (5xFAD-TREM2+/+) and green (5xFAD-TREM2−/−), unpaired t-test with Welch’s correction for analysis of the number and ratio while Mann–Whitney test for plaque size, /p > 0.05, *p < 0.05, **p < 0.01 or ****p < 0.0001)
TREM2<sup>T66M</sup> mutation leads to increased deposition of pSer8-Aβ in transgenic mouse brain

To assess potential effects of a disease associated TREM2 mutant, we used knock-in (KI) mice that carry the TREM2<sup>T66M</sup> variant (Table 1). The TREM2<sup>T66M</sup> variant is associated with Nasu–Hakola disease (NHD) and frontal lobe degeneration [17, 19, 49]. Importantly, the genetic modification at this site in the mouse genome does not result in aberrant splicing and lower mRNA levels as observed previously for knock-in mice expressing the Alzheimer-associated R47H variant [79]. TREM2<sup>T66M</sup> KI mice were crossed with APP/PS1ΔE9 double transgenic mice. Mice homozygous for endogenous TREM2 or the TREM2<sup>T66M</sup> KI mutation were analyzed at 12 M of age. Homozygous TREM2<sup>T66M</sup> KI mice showed an increase in the number and size of plaques (Fig. 4a–c, Additional file 2: Source data 4) as well as increased plaque load (Table 1). TREM2<sup>T66M</sup> KI mice also showed a selective accumulation of pSer8-Aβ in plaques as compared to nmAβ (Fig. 4d–e). Moreover, there was an increase in the number of larger sized (> 1500 µm<sup>2</sup>) as well as smaller sized (< 600 µm<sup>2</sup>) deposits of N3pE-Aβ along with pSer8-Aβ, nmAβ and total Aβ species in the analyzed brain regions of TREM2<sup>T66M</sup> mice (Fig. 4f–h, Additional file 2: Source data 4). Again, no selective accumulation of N3pE-Aβ as compared to that of nmAβ was observed in brains of TREM2<sup>T66M</sup> expressing mice (Fig. 4i–j). These findings further support a selective increase of pSer8-Aβ in parenchymal plaques in mice with impaired TREM2 function.

Loss of TREM2 function increases intraneuronal and vascular deposition of Aβ species

Clustering of Iba1 positive microglia around plaques was apparent in the different APP transgenic mouse models expressing endogenous TREM2 at advanced and earlier stages of Aβ deposition, but was strongly reduced in brains of the respective TREM2<sup>+/−</sup> (Additional file 1: Figure S7a-b) and TREM2<sup>T66M</sup> KI mice (Fig. 5a–b). Consistent with previous reports [8, 28, 51], TREM2<sup>−/−</sup> mice showed much less compact Aβ deposits as compared to TREM2<sup>+/+</sup> mice. Similar observations were made with the APP/PS1ΔE9-TREM2<sup>T66M</sup> KI mice as compared to APP/PS1ΔE9-TREM2<sup>−/−</sup> mice, indicating that impaired barrier function of microglia caused by dysfunctional TREM2 promotes the deposition of smaller plaques containing pSer8-Aβ. In addition, brains of APP transgenic TREM2<sup>−/−</sup> and TREM2<sup>T66M</sup> KI mice showed strongly elevated deposition of pSer8-Aβ within neurons as compared to brains of APP transgenic mice expressing endogenous functional TREM2 (Fig. 5c–d, Additional file 1: Figure S7c-d).

We also performed western immunoblotting analyses with different fractions of brain extracts from APP/PS1ΔE9-TREM2 and APP/PS1ΔE9-TREM2<sup>T66M</sup> KI mice. Levels of pSer8-Aβ, nmAβ, and total Aβ (immunostained with 4G8 or 2964 antibody) were significantly increased in the RIPA extracts with low detergent concentration that could contain extracellular and membrane-associated monomeric and oligomeric Aβ species (Fig. 6a–h). Consistent with the higher aggregation propensity of pSer8-Aβ, pSer8-Aβ reactivity was also observed in the upper region of the blot that likely represent SDS-stable oligomers. These species were also detected by antibodies 4G8 or 2964 that detect Aβ independently of the modification state. In contrast, non-modified Aβ was almost exclusively detected as monomers. However, levels of monomeric non-modified Aβ were also increased in RIPA extracts of APP/PS1ΔE9-TREM2<sup>T66M</sup> KI as compared to that of APP/PS1ΔE9-TREM2 WT mouse brains. In the SDS fractions that were obtained subsequently after extraction with RIPA buffer, and could also contain intracellular Aβ, levels of pSer8-Aβ migrating in the monomeric band were similar between TREM2 WT and TREM2<sup>T66M</sup> brains. Notably, levels of oligomeric pSer8-Aβ were elevated in TREM2<sup>T66M</sup> brains, indicating higher levels of pSer8-Aβ containing oligomers upon loss of TREM2 function. As observed for the RIPA extracts, nmAβ was also not detected as oligomers in the SDS fraction. Levels of monomeric nmAβ were decreased in the SDS fraction of TREM2<sup>T66M</sup> mice as compared to that of TREM2 WT mouse (Fig. 6i–l). Increased levels of oligomeric Aβ in brains of TREM2<sup>T66M</sup> mice were confirmed with antibodies 4G8 and 2964 in both, RIPA and SDS extracts (Fig. 6m–p). Together these data indicate that TREM2 could limit the accumulation of oligomeric Aβ assemblies which contain Ser8-Aβ. These oligomers might further aggregate to form Aβ deposits consistent with the increased plaque deposition observed in brains of mice with TREM2 deletion or expression of the dysfunctional TREM2<sup>T66M</sup> variant. Notably, increased deposition of pSer8-Aβ and nmAβ was not only observed in form of extracellular plaques, but also in brain vessels of APP/PS1L166P-TREM2<sup>−/−</sup> mice as compared to APP/PS1L166P-TREM2<sup>+/+</sup> mice (Additional file 1: Figure S7e). This vascular deposition of Aβ resembles cerebral amyloid angiopathy (CAA) observed in different APP transgenic mouse models, and very commonly in human AD brains [11, 37, 65].

TREM2 variants are associated with quantitative and qualitative differences in the deposition of distinct Aβ species in human brains

To assess the effect of rare disease associated TREM2 variants in human brain tissue, AD cases with...
Fig. 4  TREM2<sup>T66M</sup> causes increased deposition of pSer8-Aβ and N3pE-Aβ in APP/PS1 transgenic mouse brains. a Representative pSer8-Aβ stained female 12 M old-APP/PS1ΔE9-TREM2 and APP/PS1ΔE9-TREM2<sup>T66M</sup> mouse brain sections (color scale bar = 35 mm, represents min/max pixel intensities, 10x). b Dot plots representing number of plaques/mm<sup>2</sup>. c Box and whiskers plots showing sizes of plaques (µm<sup>2</sup>) stained with N3pE-Aβ, nmAβ, and Aβ (2964) antibodies in the SSC, RSC, and DG of female 12 M APP/PS1ΔE9-TREM2<sup>T66M</sup> and APP/PS1ΔE9-TREM2 mice. d Representative images showing deposition of pSer8-Aβ, nmAβ, and Aβ (2964) in SSC of female 12 M APP/PS1ΔE9-TREM2<sup>T66M</sup> and APP/PS1ΔE9-TREM2 mice. e Representative images showing deposition of pSer8-Aβ, nmAβ, and Aβ (2964) in SSC of female 12 M APP/PS1ΔE9-TREM2<sup>T66M</sup> and APP/PS1ΔE9-TREM2 mice. f Representative N3pE-Aβ stained female 12 M-APP/PS1ΔE9-TREM2 and APP/PS1ΔE9-TREM2<sup>T66M</sup> mouse brain sections (color scale bar = 35 mm, represents min/max pixel intensities, 10x). g Dot plots representing number of plaques/mm<sup>2</sup>. h Box and whiskers plots representing size of plaques (µm<sup>2</sup>) stained with N3pE-Aβ and Aβ (4G8) antibodies in the SSC, RSC, and DG of the female 12 M-APP/PS1ΔE9-TREM2 and APP/PS1ΔE9-TREM2<sup>T66M</sup> mice. i Representative images showing deposition of N3pE-Aβ, nmAβ, and Aβ (4G8) in SSC of female 12 M-APP/PS1ΔE9-TREM2 and APP/PS1ΔE9-TREM2<sup>T66M</sup> mice. j Ratio of N3pE-Aβ/nmAβ in SSC, RSC, and DG of female 12 M APP/PS1ΔE9-TREM2 and APP/PS1ΔE9-TREM2<sup>T66M</sup> mice. Each dot represents average value of number of plaques or ratio/animal. The box and whiskers plots represent min/max values of distribution of plaque size with the median (shown by the line dividing the box) and the dot plots represent mean ± SEM (n = 3 animals, color- blue (APP/PS1ΔE9-TREM2) and orange (APP/PS1ΔE9-TREM2<sup>T66M</sup>), unpaired t-test with Welch's correction for analysis of the number and ratio while Mann-Whitney test for plaque size, *p < 0.05, **p < 0.01, ***p < 0.001 or ****p < 0.0001)
TREM2R62H/R62C variants and the clinical diagnosis for dementia were compared to cases with the TREM2 common variant (CV) also diagnosed with dementia. All cases fulfilled the criteria for neuropathological diagnosis of AD (Table 2). Sequential temporal neocortical sections were stained separately with the phosphorylation-state specific antibodies 7H3D6 (nm\(\text{A}\beta\)), 1E4E11 (p\(\text{Ser8-}\text{A}\beta\)), and with 4G8 that detects A\(\beta\) independently of the phosphorylation state. In addition to extracellular plaques, all cases showed A\(\beta\) depositions in the wall of cerebral blood vessels, and inside of neurons (Fig. 7a, Additional file 1: Figure S8a).

TREM2R62H/R62C variant cases had increased number and size of 4G8 positive plaques resulting in a higher plaque load (% area) as compared to cases with the TREM2 common variant. Further, we also observed a higher number of smaller extracellular deposits stained with 4G8 antibody in TREM2 CV cases (Fig. 7a–d,

![Fig. 5](image_url)
Increased pSer8-Aβ in brain extracts of APP/PS1 ΔE9-TREM2ΔE9-TREM2 transgenic mouse brains. a–h Immunoblots and quantification showing levels of monomeric and oligomeric Aβ variants in RIPA extracts of brains of female 12 M old-APP/PS1ΔE9-TREM2ΔE9-TREM2 as compared to APP/ PS1ΔE9-TREM2 mice; pSer8-Aβ immunostained with antibody 1E4E11, (monomer: \(t(7.993) = 2.391, \*p = 0.0438\); oligomer: \(t(7.074) = 3.925, **p = 0.0056\), nmAβ detected with antibody 7H3D6 (monomer: \(t(7.608) = 3.849, **p = 0.0054\)), Aβ immunostained with antibody 4G8 (monomer: \(t(7.847) = 2.454, \*p = 0.0408\); oligomer: \(t(7.81) = 0.708, \text{ns} p = 0.4995\)), or antibody 2964 (monomer: \(t(7.607) = 2.449, \*p = 0.045\); oligomer: \(t(7.153) = 0.939, \text{ns} p = 0.3784\) antibodies). Each dot represents ratio of Aβ signal to β-actin per animal. All data represent mean ± SEM (n = 5 animals, monomer: orange arrowheads and oligomer: blue arrowheads, color—blue (APP/PS1ΔE9-TREM2) and orange (APP/PS1ΔE9-TREM2ΔE9-TREM2), unpaired t-test with Welch’s correction). Original uncropped immunoblots are provided in the Additional file 2: Source data 5.
Additional file 1: Figure S8b-c). Interestingly, only two of the six TREM2R62H/R62C cases showed very few extracellular nmAβ positive deposits, while five out of six cases with the TREM2 CV showed abundant deposition of nmAβ (detected by antibody 7H3D6) in extracellular plaques, indicating that deposition of N-terminally non-modified Aβ species is reduced in cases with disease associated TREM2 variants. In contrast, pSer8-Aβ was present in extracellular plaques in TREM2R62H/R62C and TREM2 CV cases, and prominently detected in the core of plaques. The number of pSer8-Aβ positive extracellular plaques and plaque load (% area) was not significantly different between TREM2R62H/R62C and TREM2 CV cases (Fig. 7a-f). pSer8-Aβ deposits stained with 1E4E11 were overall smaller as deposits stained with antibody 4G8 that detects total Aβ with positively skewed plaque size distribution with both antibodies and in all cases (Fig. 7g, Additional file 1: Figure S8d-e). However, analysis of plaque size distribution revealed that the number and size of pSer8-Aβ positive extracellular deposits were decreased in TREM2R62H/R62C cases as compared to TREM2 CV cases (Additional file 1: Figure S8d-e).

pSer8-Aβ and nmAβ was also detected in vessels in TREM2R62H/R62C and TREM2 CV cases. However, further analyses on the regional and quantitative deposition of phosphorylated Aβ species in different disease stages would be required to assess a potential effect of TREM2 genotypes on CAA in mouse and human brains (Additional file 1: Figures S7e, S8a; Fig. 7a).

We also observed intraneuronal deposition of nmAβ and pSer8-Aβ species. Interestingly, the number of neurons with nmAβ and pSer8-Aβ positive intracellular deposits was significantly higher in TREM2R62H/R62C carriers as compared to TREM2 CV cases (Fig. 7h–i).

**Discussion**

Here, we show that deletion of TREM2 or TREM2 disease associated variants lead to specific quantitative and qualitative changes of Aβ deposits in brains of APP transgenic mice and human cases with the diagnosis of dementia, and that TREM2 not only modulates the composition of extracellular plaques, but also of intraneuronal deposits.

TREM2 plays a fundamental role in the regulation of microglial activity and the deposition of Aβ in extracellular plaques [28, 29, 31, 68]. Aβ exists in multiple variants with different lengths and post-translational modifications which differ in their aggregation behavior, biostability, deposition and neurotoxic properties [1, 42]. It was shown recently that phosphorylation of Aβ modulates the direct interaction with TREM2 and the internalization by microglia [31]. Phosphorylation at Ser8 also decreases the degradation of Aβ by the insulin degrading enzyme that can be secreted by microglia [39, 61].

To assess the role of TREM2 in the deposition of modified Aβ species in-vivo, we analyzed Aβ pathology in three different mouse models of AD. Consistent with previous reports [28, 76], TREM2 deletion was associated with increased Aβ deposition at younger (4–5 month) and older ages (12–15 month), and impaired microglial clustering around extracellular Aβ plaques [31]. Importantly, the phosphorylated Aβ species, pSer8-Aβ, selectively increased as compared to other Aβ species in brains of 5xFAD and APP/PS1L166P transgenic mice upon deletion of TREM2. Increased accumulation of phosphorylated Aβ species was also observed in brains of knock-in (KI) mice with the TREM2T66M mutation that causes FTD-like syndrome and NHD in humans. In this regard,
Fig. 7 (See legend on previous page.)
it is interesting to note that NHD cases could show AD characteristic neuropathological lesions, including senile plaques and neurofibrillary tangles [4]. Consistent with previous findings [31, 36, 40, 41], pSer8-Ab is prominently detected in the core of extracellular plaques and intraneuronally. The deposition of pSer8-Ab in both lesions was increased in brains of 12 M old TREM2KO−/− as compared with TREM2WT+/− in 5xFAD as well as APP/PS1L166P transgenic mice. Very similar alterations in the deposition of the different Ab species were also observed in TREM2R66M KI mice as compared to mice expressing function mutations also modulate the differential deposition of distinct Ab variants.

Biochemical analysis of brain extracts revealed that TREM2 deficiency or the expression of the NHD associated T66M in APP transgenic mice increased the levels of pSer8-Ab in form of oligomeric assemblies. Ab oligomers exert neurotoxicity, and oligomer levels correlate with neuronal dysfunction in AD [3, 21, 50, 56, 57, 62, 80]. Ab oligomers also seed fibrillization and promote the deposition in form of extracellular plaques [6, 10, 20, 32]. In addition, oligomers can be internalized by neurons resulting in Ab accumulation within neurons [2, 9, 13, 56, 60]. Since TREM2 has been shown to selectively bind oligomeric assemblies of Ab and promote microglial uptake [31, 44, 72, 82], the loss of TREM2 function could result in decreased clearance of oligomers and thereby lead to increased uptake by neurons, and deposition in extracellular plaques and the vasculature. Indeed, altered plaque development and CAA related pathology has recently been observed in mouse brain upon depletion of microglia [23, 59], showing that functional microglia are important to shape extracellular plaques and to restrict the deposition of Ab in the brain vasculature. We also detected pSer8-Ab and nmAb in vessels of transgenic mouse brains, and it will be interesting the further investigate the role of TREM2 in the deposition and the potential pathophysiological implications of modified Ab species in the vasculature.

The analysis of human cases with rare TREM2R62H or TREM2R62C variants showed significantly increased accumulation of phosphorylated Ab species inside of neurons and alterations in plaque size, load and number, further indicating that impairment of TREM2 function mediates the formation and composition of AD characteristic lesions.

Thus, in addition to the previously described effects of TREM2 on plaque morphology, our data provide evidence for a critical role of TREM2 in restriction of intraneuronal Ab deposits that are highly correlated with clinical presentation and disease progression [2, 7, 14, 15, 27, 63, 77]. Further, we show here that TREM2 also modulates the composition of these lesions. This is interesting, because changes in the composition of extracellular plaques and CAA, in particular the occurrence of phosphorylated Ab, are associated with clinical manifestation and progression of AD [11, 46, 54, 64].

Although TREM2 loss of function mutations could also contribute to neurodegeneration independently of Ab by impairment of brain energy metabolism [33], synaptic dynamics [26, 48], and formation of neurofibrillary tangles [45], a recent study suggests an important role of Ab pathology in the TREM2 dependent formation of tau pathology and brain atrophy [43]. Thus, the differential interaction of TREM2 with modified Ab species might not only be important for the deposition and composition of different Ab lesions, but also contribute to the development of tau pathology that together determine onset and progression of AD.
Authors’ contributions
PI and JW conceived the study. PI performed most of the experiments and analyzed data. SK, FR, and ST contributed to biochemical and immunohistochemical experiments and interpretation. SP, CH, MC, NV, and MTH provided mouse brains. PI, TA, and JH selected and analyzed human brains. PI and JW wrote the manuscript with help all co-authors. All of the authors read, edited and approved the final version of the manuscript.

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Availability of data and materials
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Declarations

Competing interests
The authors declare no competing financial interests.

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References
1. Barykin EP, Mitkevich VA, Kozin SA, Makarov AA (2017) Amyloid beta modification: a key to the Sporadic Alzheimer’s Disease? Front Genet 8:58. https://doi.org/10.3389/fgen.2017.00058
2. Bayer TA, Wirths O (2010) Intracellular accumulation of amyloid-beta—a predictor for synaptic dysfunction and neuron loss in Alzheimer’s disease. Front Aging Neurosci 2.8. https://doi.org/10.3389/fnagi.2010.00008
3. Benilova I, Karran E, De Strooper B (2012) The toxic Abeta oligomer and Alzheimer’s disease: an emperor in need of clothes. Nat Neurosci 15:349–357. https://doi.org/10.1038/nn.3028
4. Bird TD, Koerker RM, Lebard B, Vlcek BW, Thorning DR (1983) Lipomembranous polyctytic osteodysplasia (brain, bone, and fat disease): a genetic cause of presenile dementia. Neurology 33:81–86. https://doi.org/10.1212/wnl.33.1.81
5. Bis JC, Jian X, Kunkle BW, Chen Y, Hamilton-Nelson KL, Bush WS, Salerno WJ, Lancour D, Ma Y, Renton AE et al (2020) Whole exome sequencing study identifies novel rare and common Alzheimer’s-Associated variants involved in immune response and transcriptional regulation. Mol Psychiatry 35:1859–1875. https://doi.org/10.1038/s41380-018-0112-7
6. Chen GF, Xu TH, Yan Y, Zhou YR, Jiang Y, Melcher K, Xu HE (2017) Amyloid beta: structure, biology and structure-based therapeutic development. Acta Pharmacol Sin 38:1205–1235. https://doi.org/10.1038/aps.2017.38
7. Condello C, Lemmin T, Stohr J, Nick M, Wu Y, Maxwell AM, Watts JC, Caro CD, Oehler A, Keene CD et al (2018) Structural heterogeneity and inter-subject variability of Abeta in familial and sporadic Alzheimer’s disease. Proc Natl Acad Sci USA 115:E782–E791. https://doi.org/10.1073/pnas. 1714966115
8. Condello C, Yuan P, Schain A, Gutzendier J (2015) Microglia constitute a barrier that prevents neurotoxic proBenzodiazepin ABeta2 hotspots around plaques. Nat Commun 6:6176. https://doi.org/10.1038/ncomms7176
9. Friedrich RP, Tepper K, Ronice R, Soom M, Westermann M, Reymann K, Kaether C, Fandrich M (2010) Mechanism of amyloid plaque formation suggests an intracellular basis of Abeta pathogenesis. Proc Natl Acad Sci USA 107:1942–1947. https://doi.org/10.1073/pnas.0904552106
10. Friesen M, Meyer-Luehmann M (2019) Abeta seeding as a tool to study cerebral amyloidosis and associated pathology. Front Mol Neurosci 12:233. https://doi.org/10.3389/fnmol.2019.00233
11. Gerth J, Kumar S, Rijal Upadhaya A, Ghebremedhin E, von Arnim CAF, Thal DR, Walter J (2018) Modified amyloid variants in pathological subgroups of beta-amyloidosis. Ann Clin Transl Neurol 5:815–831. https://doi.org/10.1002/acn3.5377
12. Gelbow K, Wunderlich P, Karaca I, Walter J (2016) Functional involvement of gamma-secretase in signaling of the triggering receptor expressed on myeloid cells-2 (TREM2). J Neuroinflammation 13:17. https://doi.org/10.1186/s12974-016-0479-9
13. Gouras GK, Almeida CG, Takahashi RH (2005) Intraneuronal Abeta accumulation and origin of plaques in Alzheimer’s disease. Neurobiol Aging 26:1235–1244. https://doi.org/10.1016/j.neurobiolaging.2005.05.022
14. Gouras GK, Tampellini D, Takahashi RH, Capetillo-Zarate E (2010) Intraneuronal beta-amyloid accumulation and synapse pathology in Alzheimer’s disease. Acta Neuropathol 119:523–541. https://doi.org/10.1007/ s00401-010-0679-9
15. Gouras GK, Tsai J, Naslund T, Vincent B, Edgar M, Chefer F, Greenfield JP, Haroutunian V, Buxbaum JD, Xu H et al (2000) Intraneuronal Abeta42 accumulation in human brain. Am J Pathol 156:15–20. https://doi.org/10.1016/s0002-9440(00)64700-1
16. Gratzeu M, Leyns CEG, Holtzman DM (2018) New insights into the role of TREM2 in Alzheimer’s disease. Mol Neurodegener 13:66. https://doi.org/10.1186/s13024-018-0298-9
17. Guerreiro R, Biligic B, Guven G, Bras R, Roher J, Lohmann E, Hanagasi H, Gurvit H, Emte M (2013) Novel compound heterozygous mutation in TREM2 found in a Turkish frontotemporal dementia-like family. Neurobiol Aging 34(2890):e2891-2895. https://doi.org/10.1016/j.neurobiolaging.2013.06.005
18. Guerreiro R, Wojtas A, Bras J, Carraquillo M, Rogaeva E, Majoeune E, Cruchaga C, Sass J, Younkin S et al (2013) TREM2 variants in Alzheimer’s disease. N Engl J Med 368:117–127. https://doi.org/10.1056/NEJMoa1211851
19. Guerreiro RL, Lohmann E, Bras JM, Gibbs JR, Roher JD, Gurulian N, Dur sun B, Biligic B, Hanagasi H, Gurvit H et al (2013) Using exome sequencing to reveal mutations in TREM2 presenting as a frontotemporal dementia-like syndrome without bone involvement. JAMA Neurol 70:78–84. https://doi.org/10.1001/jamaneurol.2013.638
20. Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer’s disease: progress and problems on the road to therapeutics. Science 297:353–356. https://doi.org/10.1126/science.1072994
21. He Y, Wei M, Wu Y, Qin H, Li W, Ma X, Cheng J, Ren J, Shen Y, Chen Z et al (2019) Amyloid beta oligomers suppress excitatory transmitter release by presynaptic depletion of phosphorylcholine-4.5-bisphosphate. Nat Commun 10:11193. https://doi.org/10.1038/s41467-019-09114-z
22. Heneka MT, Kummer MP, Stutz A, Deleate A, Schwartz S, Veira-Saekker A, Grieb A, Axt D, Remus A, Zheng TC et al (2013) NLRP3 is activated in Alzheimer’s disease and contributes to pathology in APP/PS1 mice. Nature 493:674–678. https://doi.org/10.1038/nature11729
23. Huang Y, Happanen KE, Bunrola PG, O’Connor C, Hahn N, Huang L, Nim mjerjahn A, Lemke G (2021) Microglia use TAMP receptors to detect and engulf amyloid beta plaques. Nat Immunol. https://doi.org/10.1038/s41590-021-00913-5
24. Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, Dickson DW, Duycentsa C, Fosch MP, Masliah E et al (2012) National Institute on Aging-Alzheimer’s Association guidelines for the neuropathologic assessment of Alzheimer’s disease. Alzheimers Dement 8:58. https://doi.org/10.1016/j.alz.2011.01.007
25. Ibach M, Mathews M, Linnartz-Gerlach B, Theil S, Kumar S, Feederle R, Brustle O, Neumann H, Walter J (2021) A reporter cell system for the triggering receptor expressed on myeloid cells-2 reveals differential effects of disease-associated variants on receptor signaling and activation by antibodies against the stalk region. Glia 69:1126–1139. https://doi.org/10.1002/glia.23953
26. Jadhav VS, Lin PBC, Pennington T, Di Prisco GV, Jannu AJ, Xu G, Moutinho M, Zhang J, Atwood BK, Puntambekar SS et al (2020) Trem2 Y38C
mutation and loss of Trem2 impairs neuronal synapses in adult mice. Mol Neurodegener 15:62. https://doi.org/10.1186/s13241-020-00409-0

27. Jawhari S, Trawick A, Jenneskens C, Bayer TA, Wirths O (2012) Motor deficits, neuron loss, and reduced anxiety coinciding with axonal degeneration and intraneuronal Abeta aggregation in the SYPAD mouse model of Alzheimer’s disease. Neurobiol Aging 33(196):e129–140. https://doi.org/10.1016/j.neurobiolaging.2010.05.027

28. Jay TR, Miller CM, Cheng PJ, Graham LC, Bemiller S, Broihier ML, Xu G, Margevicius D, Karlo JC, Sousa GL et al (2015) Trem2 deficiency eliminates Trem2β inflammatory macrophages and ameliorates pathology in Alzheimer’s disease mouse models. J Exp Med 212:287–295. https://doi.org/10.1084/jem.20142322

29. Jiang T, Tan L, Zhu XC, Zhang QQ, Cao L, Tan MS, Wang HF, Ding ZZ, Zhang YD et al (2014) Upregulation of Trem2 ameliorates neurontopathy and rescues spatial cognitive impairment in a transgenic model of Alzheimer’s disease. Neuropsychopharmacology 39:2949–2962. https://doi.org/10.1038/npp.2014.166

30. Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, Nuscher B, Ingvarsdottir A, Thobaben T, Wrang P et al (2020) TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. Sci Transl Med 12:eaaz286. https://doi.org/10.1126/scitranslmed.3002993

31. Joshi P, Riffel F, Satoh K, Enomoto M, Qamar S, Scheiblich H, Villacampa C, Nuscher B, Deussing M, Focke C, Nuscher B, Xiong M, Ghasemigharagoz A, Katzmarski N et al (2019) Loss of Trem2 function increases amyloid deposition but reduces plaque-associated ApoE. Nat Neurosci 22:191–204. https://doi.org/10.1038/s41593-018-0296-9

32. Katzmarski N, Ziegler-Waldkirch S, Scheffler N, Witt C, Abou-Ajram C, Nuscher B, Ingvarsdottir A, Thobaben T, Wrang P, Ingvarsdottir A, Thobaben T, Wrang P et al (2020) TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. Sci Transl Med 12:eaaz286. https://doi.org/10.1126/scitranslmed.3002993

33. Kleinberger G, Tarrabian Y, Suzuki-Calvet M, Czirr E, Lohmann E, Cuyvers K, Duyckaerts C, Nuscher B, Prinz M, Haass C, Meyer-Luehmann M (2020) Abeta oligomers trigger and accelerate Abeta seeding. Brain Pathol 30:35–46. https://doi.org/10.1111/bpa.12734

34. Lee SH, Meilandt WJ, Xie L, Gandham VD, Ng Yu H, Back KH, Rezzonico MG, Imperico J, Lalehzadeh G, Huntley MA et al (2021) Trem2 restrains the enhancement of tau accumulation and neurodegeneration by beta-amyloid pathology. Neuron 109:1283–1301.e1286. https://doi.org/10.1016/j.neuron.2021.02.010

35. LESSARD CB, MALNICK SL, ZHOU Y, LADD TB, CRUZ PE, RAN Y, MAHAN TE, CHAKRAPANY H, ZOLTAN DM, ULRICH JD et al (2018) High-affinity interactions and signal transduction between Abeta oligomers and TREM2. EMBO Mol Med. https://doi.org/10.15252/emmm.201809027

36. Leyns CEG, Ulrich JD, Finn MB, Stewart FR, Koscal LJ, Remolina Serrano J, Robinson GO, Anderson E, Colonna M, Holtzman DM (2017) Trem2 deficiency attenuates neuroinflammation and protects against neurodegeneration in a mouse model of tauopathy. Proc Natl Acad Sci USA 114:11524–11529. https://doi.org/10.1073/pnas.171311114

37. Libyan S, Walter J, Alafozzi F (2021) In vivo characterization of biochemical variants of amyloid-beta in subjects with idiopathic normal pressure hydrocephalus and Alzheimer’s disease neuropathological change. J Alzheimers Dis 58:1001–1012. https://doi.org/10.3233/JAD-201469

38. Linnartz-Gerlach B, Bodea LG, Klaus C, Gcinloch A, Halder R, Skinkonen L, Walter J, Colonna M, Neumann H (2019) Trem2 triggers microglial density and age-related neuronal loss. Glia 67:539–550. https://doi.org/10.1002/glia.20563

39. Maizhafi E, Snaidero N, Kleinberger G, Madore C, Daria A, Werner G, Kraesemann S, Capell A, Trumbach D, Wurst W et al (2017) TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury. EMBO Rep 18:1186–1198. https://doi.org/10.15252/embr.201743922

40. Poloneva J, Autti T, Raininko R, Partanen J, Salonen O, Puranan M, Hakola P, Halta M (2011) CNS manifestations of Nasu–Hakola disease: a frontal dementia with bone cysts. Neurology 65:1552–1558. https://doi.org/10.1212/wnl.0b013e31822d1f52

41. Palop JI, Mucke L (2010) Amyloid-beta-induced neuronal dysfunction in Alzheimer’s disease: from synapses toward neural networks. Nat Neurosci 13:812–818. https://doi.org/10.1038/nn.2583

42. Parhizkar S, Arzberger T, Brendel M, Kleinberger G, Deussing M, Focke C, Nuscher B, Xiong M, Ghasemigharagoz A, Katzmarski N et al (2019) TREM2 function promotes amyloid deposition and ameliorates pathology in a mouse model of Alzheimer’s disease. J Neurochem 36:1837–1853. https://doi.org/10.1002/jnc.25556

43. Paulsen JS, Gronvall K, Alperovitch A, Partanen J, Lah J, Nilsen MT, Pedersen L, Svarer C, Holmberg E, Nordberg A et al (2017) Longitudinal study of cerebral 

44. Peltier E, Voidolinion H, Valtonen M, Kostip J, Kaila K, Jarvelin I, Jorma J, Rezaei-Ghaileh N, Samsa H, Mihech A, Czabat P, Hoyer W, Wietz M, Ghasemigharagoz A, Katzmarski N et al (2019) Detection of brain amyloid beta isoform signatures in familial and sporadic Alzheimer’s disease. Acta Neuropathol 120:185–193. https://doi.org/10.1007/s00404-010-0690-10

45. Rezaei-Ghaileh N, Amininasab M, Kumar S, Walter J, Zweckstetter M (2016) Phosphorylation modifies the molecular stability of beta-amyloid deposits. Nat Commun 7:11359. https://doi.org/10.1038/ncomms11359

46. Rijal Upadhaya A, Kosterin I, Kumar S, von Arnim CA, Yamaguchi H, Fandrich M, Walter J, Thai DR (2014) Biochemical stages of amyloid-beta peptide aggregation and accumulation in the human brain and their association with symptomatic and pathologically preclinical Alzheimer’s disease. Brain 137:887–903. https://doi.org/10.1093/brain/awt362

47. Saito TC, Ivatsubo T, Mann DM, Shimada H, Ihsa Y, Kawashima S (1995) Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(Ep), in senile plaques. Neurology 44:457–466. https://doi.org/10.1212/01.wnl.44.3.457

48. Schultzmann MP, Hassecke F, Bachmann S, Zielinski M, Hansch S, Schroder GF, Zempel H, Hoyer W (2011) Endo-lysosomal Abeta concentration and pH trigger formation of Abeta oligomers that potently induce Tau misfolding. Nat Commun 12:4634. https://doi.org/10.1038/s41467-021-24900-4

49. Sen Gupta U, Nilsson AN, Kayed R (2016) The role of amyloid-beta oligomers in toxicity, propagation, and immunotherapy. EBiomedicine 6:42–49. https://doi.org/10.1016/j.ebiom.2016.03.035

50. Serrano-Pozo A, Mielke ML, Muzitisky A, Gomez-Isla T, Gorton-HJ, Backskii B, Betensky RA, Fosch MP, Hyman BT (2012) Stable size distribution of amyloid plaques over the course of Alzheimer disease. J Neuropathol Exp Neurol 71:694–701. https://doi.org/10.1097/NEN.0b013e31825277de

51. Shiobara E, Severson PL, Hofsheid LA, Cramer J, Jhang J, Burton EA, Spakw, Lin J, Phan NY et al (2019) Sustained microglial deplet}
an Alzheimer’s disease model. Nat Commun 10:3758. https://doi.org/10.1038/s41467-019-11674-z

60. Takahashi RH, Almeida CG, Kearney PF, Yu F, Lin MT, Milner TA, Gouras GK (2004) Oligomerization of Alzheimer’s beta-amyloid within processes and synapses of cultured neurons and brain. J Neurosci 24:3592–3599. https://doi.org/10.1523/JNEUROSCI.1676-03.2004

61. Tamboli IY, Barth E, Christian L, Siepmann M, Kumar S, Singh S, Tolksdorf K, Heneka MT, Luithjohand D, Wunderlich P et al (2010) Statins promote the degradation of extracellular amyloid (beta)-peptide by microglia via stimulation of exosome-associated insulin-degrading enzyme (IDE) secretion. J Biol Chem 285:37405–37414. https://doi.org/10.1074/jbc.M110.149468

62. Tanokashira D, Mamada N, Yamamoto F, Taniguchi K, Tamaoka A, Lashmanna MK, Araki W (2017) The neurotoxicity of amyloid beta-protein oligomers is reversible in a primary neuron model. Mol Brain 10.4. https://doi.org/10.1186/s13024-018-0284-5

63. Thal DR, Griffin WS, Braak H (2008) Parenchymal and vascular Abeta-deposition and its effects on the degeneration of neurons and cognition in Alzheimer’s disease. J Cell Mol Med 12:1848–1862. https://doi.org/10.1111/j.1529-0100.2008.00411.x

64. Thal DR, Ronisz A, Toussay T, Rijal Upadhyaya A, Balakrishnan K, Vandenberghe R, Vandenbulcke M, von Aminn Caf, Otto M, Beach T G et al (2019) Different aspects of Alzheimer’s disease-related amyloid beta-peptide pathology and their relationship to amyloid positron emission tomography imaging and dementia. Acta Neuropathol Commun 7:178. https://doi.org/10.1186/s40478-019-0837-9

65. Thal DR, Walter J, Saido TC, Fandrich M (2015) Neuropathology and biochemistry of Abeta and its aggregates in Alzheimer’s disease. Acta Neuropathol 129:167–182. https://doi.org/10.1007/s00401-014-1375-y

66. Turnbull IR, Gilfillan S, Cella M, Aoshi T, Miller M, Piccio L, Hernandez M, Welikovitch LA, Do Carmo S, Magloczky Z, Malcolm JC, Loke J, Klein WL, Freund T, Cuello AC (2020) Early intraneuronal amyloid triggers neuron-derived inflammatory signaling in APP transgenic rats and human brain. Proc Natl Acad Sci USA 117:6844–6854. https://doi.org/10.1073/pnas.1914511117

67. Ulland TK, Song WM, Huang SC, Ulrich JD, Sergushichev A, Beatty WL, Wang Y, Cella M, Colonna M, Holtzman DM (2014) Altered microglial response to Abeta via stimulation of exosome-associated insulin-degrading enzyme (IDE) and neurodegeneration. ACS Chem Neurosci 7:420–427. https://doi.org/10.1021/acschemneuro.5b00313

68. Ulland TK, Song WM, Huang SC, Ulrich JD, Sergushichev A, Beatty WL, Wang Y, Cella M, Colonna M, Holtzman DM (2014) Altered microglial response to Abeta plaques in APPPS1-21 mice heterozygous for TREM2. Mol Neurodegener 9:20. https://doi.org/10.1186/1750-1326-9-20

69. Ulrich JD, Holtzman DM (2016) TREM2 in the interface between genetics and transcriptomics. Trends Genet 33:434–447. https://doi.org/10.1016/j.tig.2018.02.007

70. Vilača A, Zhou Y, Sevalle J, Griffin JK, Satokh S, Allendorf DH, De S, Puigdel-livol M, Bruzas A, Burguillo MA et al (2021) Wild-type sTREM2 blocks Abeta aggregation and neurotoxicity, but the Alzheimer’s R47H mutant increases Abeta aggregation. J Biol Chem 296:100631. https://doi.org/10.1016/j.jbc.2021.100631

71. Wahlé T, Thal DR, Sastre M, Rentmeister A, Bogdanovic N, Fumulok M, Heneka MT, Walter J (2006) GGA1 is expressed in the human brain and affects the generation of amyloid beta-peptide. J Neurosci 26:12838–12846. https://doi.org/10.1523/JNEUROSCI.1982-06.2006

72. Walter J (2016) The triggering receptor expressed on myeloid cells 2: a molecular link of neuroinflammation and neurodegenerative diseases. J Biol Chem 291:4334–4341. https://doi.org/10.1074/jbc.R115.704981

73. Wang Y, Cella M, Mallinson K, Ulrich JD, Young KL, Robinette ML, Griffilfan S, Krishnan GM, Sudhakar S, Zinselmeyer BH et al (2015) TREM2 lipid sensing maintains the microglial response in an Alzheimer’s disease model. Cell 160:1061–1071. https://doi.org/10.1016/j.cell.2015.01.049

74. Wang Y, Ulland TK, Ulrich JD, Song W, Tzaferis JA, Hole JT, Yuan P, Mahan TE, Shi Y, Griffilfan S et al (2016) TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. J Exp Med 213:667–675. https://doi.org/10.1084/jem.20151948

75. Wang Y, Cella M, Mallinson K, Ulrich JD, Young KL, Robinette ML, Kleinerberger G, Song W, Colonna M, Hemms J et al (2018) The Trem2 R47H Alzheimer’s risk variant impacts splicing and reduces Trem2 mRNA and protein in mice but not in humans. Mol Neurodegener 13:49. https://doi.org/10.1186/s13024-018-0280-6

76. Wunderlich P, Glebov K, Kemmerling N, Tien NT, Neumann H, Walter J (2013) Sequential proteolytic processing of the triggering receptor expressed on myeloid cells-2 (TREM2) protein by ectodomain shedding and gamma-secretase-dependent intramembrane cleavage. J Biol Chem 288:33027–33036. https://doi.org/10.1074/jbc.M113.517540

77. Wang F, Piers TM, Wefers B, Zou K, Mallauch A, Brunner B, Kleinerberger G, Song W, Colonna M, Hemms J et al (2018) The Trem2 R47H Alzheimer’s risk variant impairs splicing and reduces TREM2 mRNA and protein in mice but not in humans. Mol Neurodegener 13:49. https://doi.org/10.1186/s13024-018-0280-6

78. Wahlé T, Thal DR, Sastre M, Rentmeister A, Bogdanovic N, Fumulok M, Heneka MT, Walter J (2006) GGA1 is expressed in the human brain and affects the generation of amyloid beta-peptide. J Neurosci 26:12838–12846. https://doi.org/10.1523/JNEUROSCI.1982-06.2006

79. Wahlé T, Thal DR, Sastre M, Rentmeister A, Bogdanovic N, Fumulok M, Heneka MT, Walter J (2006) GGA1 is expressed in the human brain and affects the generation of amyloid beta-peptide. J Neurosci 26:12838–12846. https://doi.org/10.1523/JNEUROSCI.1982-06.2006

80. Yoo SJ, Son G, Bae J, Kim SY, Yoo YK, Park D, Baek SY, Chang KA, Suh YH, Lee YB et al (2020) Longitudinal profiling of oligomeric Abeta in human nasal discharge reflecting cognitive decline in probable Alzheimer’s disease. Sci Rep 10:11234. https://doi.org/10.1038/s41598-020-68148-2

81. Yuan P, Cendolico C, Keene CD, Wang Y, Bird TD, Paul SM, Luo W, Colonna M, Baddeley D, Gruezendel J (2016) TREM2 haploinsufficiency in mice and humans impairs the microglia barrier function leading to decreased amyloid compaction and severe axonal dystrophy. Neuron 90:724–739. https://doi.org/10.1016/j.neuron.201605.003

82. Zhao Y, Wu X, Li X, Jiang LL, Gai X, Liu Y, Sun Y, Zhu B, Pina-Crespo JC, Zhang M et al (2018) TREM2 is a receptor for beta-Amyloid that mediates microglial function. Neuron 97:1023-1031 e1027. https://doi.org/10.1016/j.neuron.2018.01.031

83. Zhong L, Chen XF, Wang T, Wang Z, Liao C, Wang Z, Huang R, Wang D, Li X, Wu L et al (2017) Soluble TREM2 induces inflammatory responses and enhances microglial survival. J Exp Med 214:597–607. https://doi.org/10.1084/jem.20160844

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