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Neocortical and hippocampal TREM2 protein levels during the progression of Alzheimer's disease

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Abstract

Heterozygous TREM2 mutations are an Alzheimer's disease (AD) risk factor. Non-mutated TREM2 dysregulation occurs in AD brain. Whether TREM2 is altered in prodromal AD remains unknown. Western blotting was used to determine levels of TREM2 (~ 25 kDa) and Iba1 in the frontal cortex and TREM2 in the hippocampus, two structures early affected by amyloid plaque and neurofibrillary tangle (NFT) pathology, respectively, from people who died with an ante-mortem clinical diagnosis of non- and mild-cognitive impairment, mild/moderate AD (mAD) and severe AD (sAD). Immunohistochemistry defined the relationship between amyloid and Iba1 profiles. PCR analysis revealed that all subjects did not carry the most common R47H TREM2 variant. TREM2 was significantly upregulated in sAD frontal cortex, but stable in hippocampus. Frontal TREM2 mRNA and protein level patterns were similar, but not significantly different. Iba1 immunopositive microglia counts increased significantly in frontal cortex containing plaques in sAD. TREM2 and Iba1 levels were not associated with plaques, tangles, neuropathological criteria or cognitive performance. Frontal cortex TREM2 upregulation is a late event and may not play a major role early in the pathogenesis of the disease.

Keywords

Alzheimer's disease; mild cognitive impairment; TREM2; microglia; tau; amyloid; immunohistochemistry; western-blot

1. INTRODUCTION

Amyloid precursor protein (APP), presenilin 1, and presenilin 2 gene mutations are linked to familial AD (Bertram et al., 2010). By contrast, genetic mutations related to late-onset AD are less well characterized (Piaceri et al., 2013; Rezazadeh et al., 2014; Rosenthal and

Kamboh, 2014). Recently, mutations in the triggering receptor expressed on myeloid cells (TREM2) gene or its adaptor DNAX-activating protein of 12 kDa (DAP12 or TYROBP) were identified as risk factors for dementia in several degenerative diseases (Painter et al., 2015). The homozygous loss of TREM2 or DAP12 gene function cause the rare autosomal recessive Nasu-Hakola disease (Hakola, 1972; Nasu et al., 1973), which is associated with a form of neurodegeneration leading to dementia (Bianchin et al., 2004; Verloes et al., 1997) and a form of frontotemporal dementia (Chouery et al., 2008; Giraldo et al., 2013; Guerreiro et al., 2013a; Le Ber et al., 2014). Individuals harboring the heterozygous missense mutation (rs75932628) in exon 2 of TREM2 in which arginine substitutes for histidine at residue 47 (R47H) display an increased risk for AD (Benitez et al., 2013; Guerreiro et al., 2013b; Jonsson et al., 2013; Pottier et al., 2013; Slattery et al., 2014). This variant is very rare and occurs with less frequency than the ApoE ϵ 4 genotype (Guerreiro et al., 2013b; Jonsson et al., 2013). However, the role of the TREM2 in the onset of AD remains unclear.

TREM2 is a ~25.4 kDa cell surface Type-1 transmembrane protein (Jones et al., 2014; Schmid, et al., 2002; Sessa et al., 2004), showing a heterogeneous glycosylation or mature (~ 33–50 kDa) forms (Lue et al., 2015; Ma et al., 2016). TREM2 functions as an innate immune receptor and is expressed exclusively in brain microglia (Colonna, 2003). With no intrinsic signaling capacity, TREM2 requires the presence of DAP12 (Klesney-Tait et al., 2006) to mediate phagocytosis and clearance of apoptotic neural tissue and downregulates inflammation by suppression of microglial-mediated cytokine production and secretion (Takahashi et al., 2005). Several studies indicate that TREM2 is highly expressed in brain regions that develop amyloid plaque pathology in transgenic mouse models of AD (Frank et al., 2008; Jiang et al., 2014; Matarin et al., 2005; Melchoir et al., 2010; Yuan et al., 2016). Recently, it was shown that TREM2 mediates the clearance of A β and cell damage by lipid recognition (Wang et al., 2015), which seems to be impaired in the TREM2 R47H variant (Wang et al., 2015; Yeh et al., 2016). Interestingly, AD post-mortem temporal cortex displays TREM2 in microglia and amyloid plaques (Lue et al., 2015) suggesting that this protein regulates a microglial response to A β . TREM2 mRNA expression and protein levels for the glycosylated forms (~ 33–50 kDa) were reported higher in hippocampus and temporal cortex obtained from late stage AD compared to control cases, respectively (Celarain et al., 2016; Lue et al., 2015; Ma et al., 2016). By contrast, a downregulation of TREM2 protein (~28–48 kDa) levels was reported in sporadic AD hippocampus compared to controls (Zhao et al., 2013). Despite these contrasting reports little is known about neocortical and hippocampal non-glycosylated TREM2 protein and mRNA levels during the clinical progression of AD. Since glycosylated TREM2 forms have been quantified in AD (Lue et al., 2015; Ma et al., 2016), we focused on the immature/non-glycosylated ~ 25 kDa TREM2 form.

2. METHODS

2.1. Brain Tissue

Samples were obtained from European American descendants who died with an ante-mortem clinical diagnosis of NCI, MCI, and mild to moderate AD from the Rush Religious Orders Study (RROS) and cases with a clinical diagnosis of severe AD (sAD) from the Rush

Alzheimer's Center (see Tables 1 and 2). Human Research Committees of Rush University Medical Center approved this study and written informed consent for research and autopsy was obtained from participants or their family/guardians.

2.2. Clinical and neuropathological evaluation

Clinical evaluation and neuropathological criteria applied to the RROS have been published elsewhere (Mufson et al., 2012; Perez et al., 2012). Average time from the last clinical evaluation to death was ~8 months. Neuropsychological testing included Mini Mental State Examination (MMSE), global cognitive score (GCS, a composite z-score compiled from a battery of 19 cognitive tests) (Mufson et al., 2012), and z-scores from episodic memory, semantic memory, working memory, perceptual speed and visuospatial tests. Seven MCI cases were amnesic MCI (aMCI). Neuropathological diagnosis was based on Braak staging (Braak and Braak, 1991), NIA-Reagan criteria (Newell et al., 1999), and the Consortium to Establish a Registry for Alzheimer's disease (CERAD) (Mirra, 1997). Cases with other pathologies (e.g., stroke, Parkinson's disease, Lewy body or vascular dementia and hippocampal sclerosis) were excluded. Individuals were not treated with acetylcholinesterase inhibitors. Recent neuropathological guidelines for AD require the transactive response DNA-binding protein of 43 kDa (TDP-43) inclusions (Montine et al., 2012). The RROS neuropathology core is currently analyzing TDP-43 inclusions. Clinical, demographic and neuropathological characteristics of the RROS hippocampal and frontal cortex cases are presented in Tables 1 and 2, respectively

2.3. Pathological quantification

For the RROS cases, counts of the number of neuritic plaques (NPs), diffuse plaques (DPs), and NFTs were performed in a one square mm area (100x magnification) per neocortical region by a board-certified neuropathologist or trained technician blinded to all clinical data (Bennett et al., 2004, 2006; Mufson et al., 2012). Bielschowsky stain was used to visualize and count NPs and DPs. Paired helical filament tau (AT8, 1:800, Covance, WI) and APP/A β (4G8, 1:9000, Covance, WI) immunohistochemistry was used to visualize and measure NFT and A β load in the RROS cases, respectively (Mufson et al., 2016). Since the distribution of plaques and NFTs count values did not follow a normal distribution, standardized plaque and tangle counts from each area were converted to standard scores by dividing the standard deviation of mean raw counts per marker and region from the entire deceased cohort. Quantitation of amyloid load was performed by capturing images of A β stained sections as described previously (Mitchell et al., 2000). Calculation of percent area occupied by A β positive pixels used Object-Image 1.62p15 (Mitchell et al., 2000; Mufson et al., 2016). The non-RROS sAD cases were evaluated for NFTs using AT8 and thioflavin-S to determine Braak stage, but not for amyloid load, NPs, DPs and NFTs scores

2.4. Tissue samples

Frontal cortex (Brodmann's area 10) and hippocampal tissue were dissected free of white matter on dry ice and frozen at -80 °C. Samples were homogenized (150 mg/ml) in a phosphate buffered containing protease inhibitors (Sigma, St. Louis, MO). In addition, 4% paraformaldehyde fixed tissue was cut into 40-micron-thick sections from 6 randomly

chosen frontal cortex cases/per clinical group and immunostained to determine relative numbers of microglia in neocortical fields with and without amyloid plaques.

2.5. Polymerase Chain Reaction (PCR)

Briefly, cerebellar tissue was digested overnight at 55° C in a 200 ul 1X PBS, 10 ul 10% sodium dodecyl sulfate (SDS) and 15 ul proteinase K solution (Promega, Madison, WI). DNA was extracted using phenol:chloroform:isoamyl alcohol (25:24:1) (Sigma-Aldrich, St. Louis, MO) and quantitated using Infinite M200 Pro spectrometer (Tecan US, Morrisville, NC). Digested DNA fragments were separated through 2% agarose gel and imaged using Odyssey Fc (LI-COR, Lincoln, NE). One DNA fragment of 172-base-pair (bp) in the agarose gel indicates TREM2 rs75932628-T polymorphism caused by the loss of the HhaI enzyme restriction site (see Fig. 1).

2.6 Antibodies and Specificity Controls

For western-blots we used rabbit polyclonal antibodies against the synthetic peptide within 29–59 N-terminal aa' of human TREM2 (1:200, Abcam, ab175262, Cambridge, MA) (Ma et al., 2016; Raha et al., 2016) and against to the C-terminus of Iba1 (1:500, Wako, Osaka, Japan). TREM2 antibody recognized non-glycosylated/immature (~ 25 kDa) and glycosylated/mature (~ 50 kDa) form (Fig. S1). Loading control was a β -tubulin antibody (1:1000, Millipore, Billerica, MA) (Mufson et al., 2012; Perez et al., 2015). A β plaques and microglia quantitation employed a mouse monoclonal IgG antibody against human APP/A β (6E10) (1:2000, BioLegend, San Diego, CA) and a rabbit polyclonal antibody against Iba1 (1:1000, Wako), respectively.

2.7. Quantitative immunoblotting

Briefly, proteins were denatured in SDS loading buffer to a final concentration of 5 mg/ml. Proteins (50 μ g/sample) were separated by 4–20% SDS-PAGE (Lonza, Rockland, ME) and electrophoretically transferred to polyvinylidene fluoride membranes (Immobilon P, Millipore) (Perez et al., 2015). Membranes were blocked in Tris-buffered saline (TBS)/ 0.05% Tween-20/ 5% milk (1 h) at room temperature (RT). TREM2 or Iba1 antibodies were added to blocking buffer and membranes were incubated overnight (4° C), washed, incubated at RT (1 h) with horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (1:8000, Bio-Rad, Hercules, CA) or goat-anti rabbit IgG secondary antibody (1:5000, Bio-Rad, Hercules, CA), visualized by chemiluminescence on a Kodak Image Station 440CF (Perkin-Elmer, Wellesley, MA) and quantified with Kodak 1. The ~ 25 kDa TREM2 band was quantified across clinical groups. Protein signals were normalized to β -tubulin and analyzed in three independent experiments (Perez et al., 2015). Immunoblot controls consisted of pre-adsorption of the TREM2 antibody with an excess of the immunogenic TREM2 peptide (Abcam, ab217864), that blocks the anti TREM2 antibody-N-terminal used in the study (Abcam, ab175262) and deletion of the primary, both resulting in the absence of immunoreactive bands in the membranes (see Fig. S1).

2.8. TREM2 mRNA real time (rt)-qPCR

Total neocortical RNA (8 NCI, 8 MCI, 9 mAD and 8 sAD) samples were extracted using Trizol solution (Thermo Fisher Scientific, Waltham, MA) and quantitated by Infinite M200 Pro (Life Sciences-Tecan US, Morrisville, NC). Two-step TaqMan (rt)-qPCR was used to quantitate TREM2 expression. One microgram of total RNA per case was transcribed into cDNA using Superscript III reverse transcriptase and Oligo(dT)₂₀ (Thermo Fisher Scientific). Specific primer and probe (TREM2; Hs00219132_m1, Thermo Fisher Scientific) were employed and the relative expression was compared to the housekeeping gene GAPDH (Thermo Fisher Scientific). TaqMan Universal Master Mix (Thermo Fisher Scientific) was used for triplicate PCR reactions on QuantStudio 6 Flex (Applied Biosystems, Carlsbad, CA). Relative TREM2 mRNA expression were calculated using the 2^{-CT} method.

2.9. Immunohistochemistry

Frontal cortical sections were dual immunostained to visualize the coexistence of Iba1 microglia and APP/A β plaques. Three to 4 sections per case were incubated with a mouse anti-APP/A β (1:2000) over-night at RT in a TBS 0.25% Triton X-100/ 1% goat serum. After several TBS washes, tissue was incubated with a goat anti-mouse biotinylated secondary antibody (1:200; 1 h) (Vector Labs, Burlingame, CA), incubated in Vectastain ABC kit (1 h) (Vector Labs) and developed in acetate-imidazol buffer containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO). After visualizing APP/A β plaques, tissue was incubated with an avidin/biotin blocking kit (Vector Labs). Blocking buffer was reapplied (1 h) and incubated overnight at RT in a second antibody, rabbit anti-Iba1 (1:1000). The next day the appropriate biotinylated secondary antibody was applied (2 h, RT), incubated in ABC kit solution and processed with the Vector SG Substrate Kit (blue reaction product, Vector Labs) resulting in two-colored profiles: brown for plaques and dark blue/black for microglia. Immunohistochemical controls consisted of the omission of the primary antibodies resulting in the absence of immunoreactivity.

2.10. Microglia counts

Quantitation of Iba1 immunoreactive (-ir) microglia was performed in three frontal fields containing either amyloid plaques or none per section at 40X within an area of 0.07 mm² per counting field within three to four sections per case using a Nikon Eclipse 80i (Nikon Americas Inc, Melville, NY). Since plaque counts were not performed in this experiment, we used the 6E10 antibody, which recognizes both APP and A β for a more complete evaluation of plaque location. Iba1-ir counts/per field/per section/per case were averaged in each of the four clinical groups. A Nikon Eclipse 80i microscope coupled with NIS-Elements Imaging software acquired images and they were corrected for contrast and luminosity using Adobe Photoshop CS4 software (Adobe Systems Inc, San Jose, CA). Fiduciary landmarks were used to avoid counting the same area more than once. Hippocampal sections were not available for immunocytological evaluation.

2.11. Statistical Analysis

Data were evaluated across clinical groups using Mann-Whitney, Kruskal-Wallis, Chi-square and Wilcoxon signed-rank test followed by Conover-Inman, Holm-Sidak, Tukey, and

Dunn's post hoc test for multiple comparisons and Spearman rank for correlations (Sigma Plot 12.5, Systat Software, San Jose, CA). False discovery rate was used to adjust for multiple comparisons among the correlations. Statistical significance was set at $p < 0.05$ (two-tailed) and measurements were graphically represented using a Sigma Plot 12.5 software.

3. RESULTS

3.1. Case demographics

Frontal and hippocampal RROS cases did not differ by age, gender, education, post-mortem interval (PMI) or brain weight (see Tables 1 and 2). There were no significant differences in the number of cases carrying the ApoE $\epsilon 4$ allele between regions. MMSE, perceptual speed and GCS were significantly ($p < 0.001$) lower in mAD compared to MCI and NCI in hippocampal cases, while differences between the latter two groups did not reach significance. In the frontal cortex cases, MMSE, perceptual speed, working memory and visuospatial scores were significantly lower ($p < 0.001$) in mAD compared to the MCI and NCI groups, but no differences were found between MCI and NCI. GCS and episodic memory scores were significantly lower in neocortical cases in mAD than NCI and MCI, and MCI scores were also lower than NCI ($p < 0.001$). Semantic memory scores in the frontal cortex were significantly higher in NCI compared to mAD ($p < 0.02$), but not in hippocampus (see Tables 1 and 2). Braak scores, CERAD and NIA Reagan diagnosis were not significantly different among RROS clinical groups for frontal cortex and hippocampus. Neuropathology revealed 88% of hippocampal mAD compared to ~75–80% of NCI and MCI cases, were Braak stages III–VI, while in the frontal cortex 78% of mAD and MCI cases were Braak stages III–VI compared to 70% of NCI cases. Using NIA-Reagan criteria, 50% of NCI, 60% of MCI, and 88% of AD cases were classified as intermediate to high likelihood of AD in the hippocampal groups (Table 1), while in the frontal cortex 55% of NCI, 42% of MCI, and 78% of mAD cases were intermediate to high likelihood of AD. CERAD revealed 59% of NCI, 60% of MCI, and 100% of mAD cases were probable or definite AD for the hippocampal, while 55% of the NCI, 47% of the MCI and 84% of mAD cases were probable or definite AD for the frontal cases.

Demographics for sAD cases revealed the average age at death was 78.72 ± 4.28 years (range 71–86), PMI was 5.85 ± 2.07 h (range 2–12), brain weight was $1,107.94 \pm 128.25$ g (range 940–1350), MMSE score was 2.50 ± 3.92 (range 0–13), Braak staging was IV–V and, 63% were female. This group only had MMSE scores.

3.2. TREM2 rs75932628-T polymorphism genotype

PCR screening revealed that all cases examined did not carry the rs75932628-T TREM2 gene variant, but displayed the rs75932628 TREM2 normal genotype (Fig. 1).

3.3. TREM2 and Iba1 protein levels

Frontal TREM2 levels were significantly increased in sAD compared to NCI, MCI and mAD (Table 3, Fig. 2A, B; Kruskal-Wallis, $p = 0.009$), while hippocampal TREM2 levels were unchanged across the four groups (Table 3, Fig. 2C; Kruskal-Wallis, $p > 0.5$). Frontal

cortex TREM2 levels were significantly higher in NCI and mAD groups compared to that seen in the hippocampus (Fig. 2D; Wilcoxon Signed-Rank test, $p < 0.05$).

To examine whether the increase in TREM2 levels in the frontal cortex in severe AD was associated with activation of microglia, neocortical levels of Iba1 were quantified across groups by immunoblotting. Iba1 levels trended towards an increase in MCI and mAD and decrease in sAD, but failed to reach significance (Table 3, Fig. 3A, B; Kruskal-Wallis, $p > 0.05$). A significant upregulation in TREM2 protein levels was found in sAD (Mean $14.70 \pm$ SD 2.90) compared to MCI (Mean $6.00 \pm$ SD 5.40) and mAD (Mean $5.54 \pm$ SD 2.55) when TREM2 levels were corrected based on Iba1 levels (Kruskal-Wallis, $p < 0.05$).

Analysis of hippocampal TREM2 alone and frontal cortex TREM2 and Iba1 protein levels revealed no significant differences between groups when analyzed in relation to CERAD, NIA Reagan and Braak criteria (Fig. S2; Kruskal-Wallis, $p > 0.05$). Furthermore, there were no significant differences in hippocampal TREM2 protein, frontal cortex TREM 2 protein and Iba1 protein levels when the NCI cases were analyzed based on Braak scores 0 to III (low NFT pathology) compared to IV to VI (high NFT pathology) (Mann-Whitney, $p > 0.05$, data not shown). No significant differences in frontal and hippocampal TREM2 protein levels were found between aMCI and non-aMCI cases (Mann-Whitney, $p > 0.05$, data not shown).

3.4. TREM2 mRNA expression

There was a trend toward an increase in frontal cortex TREM2 mRNA expression in sAD similar to that seen for TREM2 protein levels, (Fig. 2E; Kruskal-Wallis, $p > 0.05$). The pattern of TREM2 mRNA expression in the frontal cortex was similar to TREM2 protein levels during disease progression (see Fig. 2D, E).

3.5. Iba1-ir microglia quantitation

A comparison across groups revealed that the number of Iba1-ir microglia in frontal cortex fields containing amyloid plaques (Fig. 4A–D) were significantly increased in sAD compared to NCI and mAD, but not MCI (Table 3, Fig. 4I; Kruskal-Wallis, $p = 0.001$). In neocortical fields without plaques (Fig. 4E–H) significant differences were found in the sAD group compared to mAD and MCI (Fig. 4J; Kruskal-Wallis, $p = 0.005$). A comparison of the number of Iba1-ir microglia found within frontal cortex fields with and without plaques revealed significantly higher numbers of microglia in areas containing plaques between the sAD, mAD and NCI (Wilcoxon Signed-Rank test, $p < 0.05$), but not in MCI (Fig. 4K).

3.6. Association between biochemical, clinical, and neuropathological measures

There were no associations between frontal and hippocampal TREM2 protein levels and neocortical Iba1 protein levels across the four clinical groups examined (Table S1). Iba1-ir microglia counts in regions of the frontal cortex containing plaques strongly correlated with Iba1-ir microglia counts in areas without plaques (Table S1; correlation coefficient (r) = 0.63, $p = 0.001$), but not with other biochemical measurements across the clinical groups evaluated (Table S1). Iba1-ir microglia counts in frontal cortex areas without amyloid plaques showed a negative correlation with age at death across the groups examined (Fig.

5A; $r = -0.56$, $p < 0.01$), while no correlation was found between neocortical areas containing plaques and age (Fig. 5B; $r = -0.36$, $p > 0.05$). There were no correlations between biochemical measurements and age at death, PMI, brain weight and years of education across groups (Spearman test, $p > 0.05$). None of the biochemical measurements and immunocytological counts correlated with A β load, NPs or DPs, NFTs or neuropathological criteria (Braak, CERAD and NIA Reagan) (Spearman test, $p > 0.05$) (Table S2) or cognitive domain scores during AD progression across groups (Spearman test, $p > 0.05$) (Table S3).

4. DISCUSSION

Studies examining TREM2 are mainly based on the presence of a rare TREM2 missense mutation (i.e., R47H (rs75932628-T) in humans (Guerreiro et al., 2013b; Jonsson et al., 2013; Benitez et al., 2013; Pottier et al., 2013; Slattery et al., 2014). Few clinical pathological investigations have evaluated this protein or its RNA in AD cohorts lacking this TREM2 polymorphism (Celarain et al., 2016; Lue et al., 2015; Strobel et al., 2015; Zhao et al., 2013) and only one in AD cohorts without and with R47H mutation (Ma et al., 2016); none used tissue obtained from cases that had a final premortem clinical diagnosis of NCI, MCI, mAD and sAD independent of postmortem neuropathological diagnosis. We found that frontal cortex non-glycosylated TREM2 protein levels were unchanged in MCI (prodromal AD) and mAD, but upregulated in sAD, but were not altered in the hippocampus. Unlike previous findings (Ma et al., 2016), mRNA expression levels did not reach significance in the sAD examined in the present study, although we found that the pattern of the neocortical TREM2 protein and mRNA levels were similar during AD progression. This discrepancy may be related to internal group variability, post-transcriptional changes, and/or methodologic detection differences between mRNA and protein or there is a mismatch between protein and mRNA levels. The upregulation of non-glycosylated (immature) TREM2 protein found in the frontal cortex in sAD cases is similar to the glycosylated (mature) TREM2 levels detected in advanced Braak stage V temporal cortex cases (Lue et al., 2015; Ma et al., 2016), suggesting that cortical post-translational TREM2 forms are affected similarly during AD progression (Ma et al., 2016). However, we did not find a correlation between frontal cortex TREM2 levels and plaque pathology during AD progression similar to that reported for the temporal cortex (Lue et al., 2015).

In AD, increased temporal cortex glycosylated TREM2 levels are reported to correlate with NFT scores, cell apoptosis, phospho-tau increases and synapse loss (Lue et al., 2015). Increased cerebrospinal fluid (CSF) soluble TREM2 concentration correlated with CSF levels of total and phospho-tau, but were not or weakly correlated with A β 42 in AD progression (Heslegrave et al., 2016; Piccio et al., 2016; Suarez-Calvet et al., 2016). Conversely, others have found a decrease in CSF soluble TREM2 in AD compared to controls (Kleinberger et al., 2014) or no differences in CSF soluble TREM2 levels between controls, MCI and AD cases (Henjum et al., 2016). The latter revealed a correlation with total and phospho-tau but not A β 42. These findings suggest an interaction between TREM2 and tangle pathology. However, we did not find an association between neocortical and hippocampal non-glycosylated/immature TREM2 levels and Braak staging or NFT scores in MCI, and mAD. Amyloid loads and NFT scores were not available for the sAD samples. It

is possible that frontal cortex and hippocampal non-glycosylated/immature TREM2 levels could be less responsive to tangle pathology in the early stages of AD progression compared to glycosylated or mature forms. Taken together, these data suggest that non-glycosylated/immature TREM2 protein may play less of a role early in the onset of NFT pathogenesis, but a greater involvement as neuronal apoptosis and/or NFTs occur in sAD resulting in an increase in phagocytosis (Lue et al., 2015).

Unlike the frontal cortex, hippocampal TREM2 protein levels remained stable during the progression of AD supporting a report showing no significant difference in hippocampal TREM2 mRNA expression in AD compared to controls (Strobel et al., 2015). However, there are reports of a downregulation of TREM2 protein levels (Zhao et al., 2013) and an upregulation of mRNA in the hippocampus in AD compared to controls (Celarain et al., 2016). The former investigation did not provide any clinical or neuropathological information about the cases examined. However, the latter study indicated that their AD cases had NFTs and amyloid plaques whereas the controls lacked these lesions and that only the CA1 region of the hippocampus was evaluated (Celarain et al., 2016) making direct comparisons between these studies and ours difficult. It is possible that some of the hippocampi from our NCI group were already inflammatory compromised as many cases displayed amyloid and tangle pathology. Nevertheless, when we subdivided our NCI cases into low and high Braak scores we found no differences in hippocampal TREM2 levels. The finding of greater TREM2 protein levels in the frontal cortex compared to the hippocampus suggests that TREM2 is differentially expressed across brain regions (Schmid et al., 2002) and may be involved in AD pathogenesis in a region-specific manner (Strobel et al., 2015). It is possible that the stability of hippocampal TREM2 levels is due to the ability of this area to exhibit compensatory/plasticity responses to the disease process (Mufson et al., 2015).

TREM2 is expressed exclusively in microglia (Colonna, 2003; Lue et al., 2015; Takahashi et al., 2005) and regulates inflammation and phagocytosis (Neumann et al., 2009). Here we found that Iba1-ir microglia numbers in frontal cortex areas containing A β plaques were significantly higher in sAD compared to mAD and NCI, a pattern mirrored by increased neocortical TREM2 protein levels (present findings). Similar changes in microglial-plaque envelopment were described in human AD cases carrying the R47H variant (Yan et al., 2016). Interestingly, in several mouse models of amyloidosis, TREM2 upregulation was associated with A β levels (Frank et al., 2008; Jiang et al., 2014; Melchior et al., 2010; Yuan et al., 2016) and the highest levels were observed in amyloid-associated microglia (Frank et al., 2008; Yuan et al., 2016), suggesting that TREM2 upregulation is a response to A β toxicity. In addition, TREM2 over expression ameliorates amyloid deposition, neuroinflammation, synaptic loss and cognitive defects in transgenic AD mice (Jiang et al., 2014; Melchoir et al., 2010). TREM2 levels show a positive correlation with amyloid phagocytosis in inactivated microglia (Melchoir et al., 2010), suggesting a neuro-protective function of TREM2 upregulation. TREM2 reduction in young APPS1–21 amyloid mice altered numbers and morphology of plaque-associated microglia, but not amyloid burden (Ulrich et al., 2014). TREM2 or DAP12 deficiency leads to an increase in plaques not fully enclosed by microglial and dystrophic profiles (Wang et al., 2016; Yuan et al., 2016). Together the findings suggest that increased TREM2 is a response aimed at regulating A β neurotoxicity in the disease process. In the present study, Iba1 protein levels showed a trend

towards an increase in the frontal cortex during disease progression, but did not reach significance. Lue and colleagues (2015) found an upregulation of Iba1 in association with glycosylated TREM2 levels in AD temporal cortex that correlated with NFT scores, but not with A β pathology. By contrast, there was no correlation between Iba1, immature TREM2 and AD pathology in the frontal cortex during the early clinical stages of AD (present findings). These discrepancies may be related to functional differences between post-translational TREM2 forms, and/or clinical and pathological characterization of the cases examined. These data indicate the need for more studies of the pathobiology TREM2 in non-mutated AD cases.

In summary, the present findings suggest that TREM2 upregulation in the frontal cortex in AD is a late event and may not play a role early in the development of AD pathogenesis and the onset of clinical dementia, but rather is a late stage response to the disease, perhaps as a last ditch effort to reduce A β toxicity. However, these data indicate also a need for more studies of the pathobiology TREM2 in non-mutated AD cases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

Iba1 ionized calcium-binding adaptor protein

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Figure 1.

Representative PCR gel showing the DNA products from several cerebellar autopsy samples (lanes 3 to 5) and a control sample provided for the company (lane 2), after the digestion by the enzyme of restriction HhaI. Lane 2 shows a DNA fragment at 172 bp revealing the loss of the HhaI restriction enzyme site and, the change from the nucleotide C to T present in TREM2 rs75932628-T variant. Note that the lanes 3 to 5 did not display DNA bands at 172 bp reflecting the absence of the TREM2 rs75932628-T polymorphism. Lane 1 shows the DNA ladder. bp, base-pairs.

Figure 2.

(A-C) Representative immunoblots and box plots of the frontal cortex and hippocampal levels of TREM2 from cases clinically diagnosed as NCI, MCI, mAD and sAD. β -tubulin was used to normalize the immunoreactive signal obtained by densitometry. (A). Levels of TREM2 protein in the frontal cortex (B) were significantly higher in sAD compared to NCI, MCI and mAD (* $p = 0.005$), whereas TREM2 protein levels in the hippocampus (C) remain stable across the four clinical groups ($p = 0.38$). (D) Frontal cortex TREM2 protein levels were significantly higher compared to hippocampal TREM2 in NCI and mAD (* $p < 0.05$). (E) Linear representation of the frontal cortex TREM2 mRNA levels in NCI, MCI, mAD and sAD. Statistical analysis revealed no changes in the TREM2 mRNA relative expression in the frontal cortex across the groups examined ($p = 0.3$). NCI, non-cognitive impairment, MCI, mild cognitive impairment, mAD, mild to moderate AD, sAD, severe AD. Circles in the box plots indicate outliers.

Figure 3.

(A) Representative immunoblot and box plot of frontal cortex Iba1 levels in cases clinically diagnosed as NCI, MCI, mAD and sAD. β-tubulin was used to normalize the immunoreactive signal obtained by densitometry (A). (B) Levels of Iba1 protein in the frontal cortex were stable across clinical groups ($p = 0.31$). NCI, non-cognitive impairment, MCI, mild cognitive impairment, mAD, mild to moderate AD, sAD, severe AD. Circles in the box plots indicate outliers.

Figure 4.

(A-H) Images of double immunolabeling for Iba1 (blue) and APP/A β (brown, arrows) in frontal cortex fields with plaques and without plaques in NCI, MCI, mAD and sAD. Note that many more APP/A β -ir plaques (arrows) are seen in sAD compared to NCI and MCI in the frontal cortex with plaques. (I, J) Box-plots showing significant increases in the number of Iba1-ir microglia in sAD compared to NCI and mAD in frontal cortex with plaques ($p = 0.001$) (I), while in frontal cortex without plaques these differences were significant between sAD and MCI and mAD ($p = 0.005$) (J). (K) Linear representation of the average number of Iba1-ir microglia in frontal cortex with and without plaques. Statistical analysis revealed that Iba1-ir microglia numbers were significantly higher in frontal cortex containing plaques compared to fields without plaques in sAD, MCI and NCI ($p < 0.05$), but not in mAD. Scale bar = 25 μ m.

Figure 5.

(**A**) Linear regression showing a significant negative correlation between numbers of Iba1 positive microglia in frontal cortex without plaques and age at death across clinical groups ($r = -0.56$, $p = 0.004$), (**B**) while in frontal cortex without plaques this correlation was no significant across clinical groups ($r = -0.36$, $p = 0.08$).

Table 1.
Demographic, Cognitive, and Neuropathological Characteristics for Hippocampus Cases

		NCI (N=12)	MCI (N=15)	mAD (N=8)	Total (N=35)	Overall p-value	Pair-wise comparisons
Age (years) at death	Mean ± SD Range	84.57 ± 5.01 (76 – 92)	85.86 ± 4.36 (79 – 93)	86.07 ± 4.78 (76 – 91)	84.05 ± 5.42 (76 – 93)	0.75 *	----
Number of males (%)		4 (33)	5 (33)	2 (25)	11 (31)	0.91 [‡]	----
Years of education	Mean ± SD Range	17.25 ± 4.31 (10 – 25)	17.67 ± 3.54 (10 – 25)	20.25 ± 3.49 (16 – 26)	18.11 ± 3.89 (10 – 26)	0.22 *	----
Number with ApoE ε4 allele (%)		0 (0)	5 (33)	2 (29)	7 (21)	0.09 [‡]	----
MMSE	Mean ± SD Range	27.92 ± 1.56 (26 – 30)	27.80 ± 2.43 (22 – 30)	23.37 ± 4.03 (15 – 28)	26.83 ± 3.19 (15 – 30)	0.01 *	NCI, MCI > mAD
GCS	Mean ± SD Range	-0.12 ± 0.26 (-0.55 – 0.36)	-0.35 ± 0.43 (-1.20 – 0.40)	-1.17 ± 0.28 (-1.54 – 0.76)	-0.46 ± 0.53 (-1.54 – 0.40)	<0.001 *	NCI, MCI > mAD
Episodic memory z-score	Mean ± SD Range	0.24 ± 0.43 (-0.61 – 0.91)	-0.20 ± 0.56 (-1.00 – 1.31)	-1.62 ± 0.75 (-3.11 – 0.79)	-0.37 ± 0.90 (-3.11 – 1.31)	<0.001 *	NCI > MCI > mAD
Semantic memory z-score	Mean ± SD Range	-0.41 ± 0.63 (-1.44 – 0.32)	-0.32 ± 0.67 (-1.58 – 0.49)	-0.40 ± 0.63 (-1.39 – 0.38)	-0.57 ± 0.93 (-1.58 – 0.49)	0.92 *	----
Working memory z-score	Mean ± SD Range	-0.17 ± 0.50 (-0.96 – 0.48)	-0.17 ± 0.64 (-1.32 – 1.08)	-0.59 ± 0.51 (-1.62 – 0.08)	-0.37 ± 0.63 (-1.62 – 1.08)	0.20 *	----
Perceptual speed z-score	Mean ± SD Range	-0.57 ± 0.60 (-1.63 – 0.45)	-0.81 ± 0.67 (-2.08 – 0.08)	-1.95 ± 0.92 (-3.35 – 0.26)	-0.99 ± 0.87 (-3.35 – 0.45)	0.01 *	NCI, MCI > mAD
Visuospatial z-score	Mean ± SD Range	-0.27 ± 0.55 (-1.06 – 0.76)	-0.79 ± 0.68 (-1.84 – 0.27)	-1.24 ± 0.47 (-2.12 – 0.72)	-0.71 ± 0.69 (-2.12 – 0.76)	0.01 *	NCI > MCI, mAD
Post-mortem interval (hours)	Mean ± SD Range	4.96 ± 2.44 (1 – 9)	5.55 ± 2.34 (2 – 10)	3.77 ± 1.45 (1 – 5)	4.94 ± 2.26 (1 – 10)	0.27 *	----
Brain weight (g)	Mean ± SD Range	1199.67 ± 95.72 (1000 – 1320)	1174.33 ± 164.35 (890 – 1480)	1167.63 ± 104.09 (962 – 1320)	1181.49 ± 128.46 (890 – 1480)	0.83 *	----
Distribution of Braak scores		0	0	0	0	0.88 [‡]	----
	I/II	3	3	1	5		
	III/IV	8	9	6	25		
	V/VI	1	3	1	5		
NIA Reagan (likelihood of AD)	No AD	0	0	0	0		
	Low	6	6	1	13		
	Intermediate	6	6	6	18		
	High	0	3	1	4		

	NCI (N=12)	MCI (N=15)	mAD (N=8)	Total (N=35)	Overall p-value	Pair-wise comparisons
CERAD	No AD	2	0	7	0.09 [‡]	
	Possible	3	0	4		
	Probable	7	5	18		
	Definite	0	3	6		

* Kruskal-Wallis test with Conover-Inman test for pair-wise comparisons;

[‡] Chi-square p-value; one AD individual did not have APOE ε4 genotyping; severe AD cases not shown in table

Table 2.

Demographic, Cognitive, and Neuropathological Characteristics for Frontal Cortex Cases.

		NCI (N=21)	MCI (N=20)	mAD (N=19)	Total (N=60)	Overall p-value	Pair-wise comparisons
Age (years) at death	Mean $\pm$ SD Range	84.43 $\pm$ 4.48 (78 – 94)	86.78 $\pm$ 4.64 (79 – 95)	88.29 $\pm$ 4.96 (76 – 95)	86.49 $\pm$ 5.27 (76 – 101)	0.05 *	-----
Number (%) of males		9 (45)	6 (30)	7 (37)	22 (37)	0.48 [†]	-----
Years of education	Mean $\pm$ SD Range	18.24 $\pm$ 3.96 (10 – 25)	18.60 $\pm$ 3.39 (10 – 25)	18.63 $\pm$ 3.74 (9 – 26)	18.48 $\pm$ 3.65 (9 – 26)	0.97 *	-----
Number (%) with ApoE ϵ 4 allele		1 (5)	5 (25)	2 (10)	8 (13)	0.29 [†]	-----
MMSE	Mean $\pm$ SD Range	28.00 $\pm$ 1.26 (26 – 30)	27.35 $\pm$ 2.43 (22 – 30)	20.16 $\pm$ 4.79 (14 – 28)	25.30 $\pm$ 4.69 (14 – 30)	<0.001 *	NCI, MCI > mAD
GCS	Mean $\pm$ SD Range	0.02 $\pm$ 0.25 (–0.36 – 0.42)	–0.39 $\pm$ 0.38 (–1.20 – 0.25)	–1.33 $\pm$ 0.68 (–2.80 – 0.08)	–0.55 $\pm$ 0.73 (–2.80 – 0.42)	<0.001 *	NCI > MCI > mAD
Episodic memory z-score	Mean $\pm$ SD Range	0.39 $\pm$ 0.36 (–0.41 – 1.02)	–0.26 $\pm$ 0.54 (–1.12 – 0.93)	–1.70 $\pm$ 0.98 (–3.75 – 0.08)	–0.49 $\pm$ 1.09 (–3.75 – 1.02)	<0.001 *	NCI > MCI > mAD
Semantic memory z-score	Mean $\pm$ SD Range	–0.17 $\pm$ 0.70 (–1.44 – –1.08)	–0.36 $\pm$ 0.57 (–1.58 – 0.36)	–0.88 $\pm$ 0.82 (–2.96 – 0.38)	–0.46 $\pm$ 0.75 (–2.96 – 1.08)	0.02 *	NCI > mAD
Working memory z-score	Mean $\pm$ SD Range	–0.08 $\pm$ 0.42 (–0.96 – 0.48)	–0.22 $\pm$ 0.74 (–1.32 – 1.27)	–0.89 $\pm$ 0.81 (–2.26 – 0.57)	–0.38 $\pm$ 0.75 (–2.26 – 1.27)	<0.001 *	NCI, MCI > mAD
Perceptual speed z-score	Mean $\pm$ SD Range	–0.38 $\pm$ 0.70 (–1.63 – 0.77)	–0.90 $\pm$ 0.66 (–2.45 – 0.04)	–2.19 $\pm$ 0.83 (–3.38 – 0.26)	–1.09 $\pm$ 1.01 (–3.38 – 0.77)	<0.001 *	NCI, MCI > mAD
Visuospatial z-score	Mean $\pm$ SD Range	–0.27 $\pm$ 0.55 (–1.24 – 0.76)	–0.74 $\pm$ 0.61 (–1.68 – 0.44)	–0.87 $\pm$ 0.59 (–2.41 – 0.75)	–0.66 $\pm$ 0.71 (–2.41 – 0.76)	<0.001 *	NCI, MCI > mAD
Post-mortem interval (hours)	Mean $\pm$ SD Range	5.55 $\pm$ 2.62 (1 – 12)	5.13 $\pm$ 2.18 (2 – 10)	4.85 $\pm$ 2.68 (1 – 12)	5.19 $\pm$ 2.48 (1 – 12)	0.36 *	
Brain weight (g)	Mean $\pm$ SD Range	1214.62 $\pm$ 106.01 (1000 – 1400)	1154.05 $\pm$ 147.86 (890 – 1480)	1156.42 $\pm$ 130.13 (962 – 1460)	1176.37 $\pm$ 129.38 (890 – 1480)	0.17 *	-----
Distribution of Braak scores	0 I/II III/IV V/VI	0 6 14 1	0 4 13 3	0 4 9 6	0 14 36 10	0.35 [†]	-----
NIA Reagan (likelihood of AD)	No AD Low Intermediate High	0 9 12 0	0 11 6 3	0 4 9 6	0 24 27 9	0.02 [†]	-----

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	NCI (N=21)	MCI (N=20)	mAD (N=19)	Total (N= 60)	Overall p-value	Pair-wise comparisons
CERAD	No AD	10	2	18	0.06 [‡]	-----
	Possible	1	1	6		
	Probable	5	7	20		
	Definite	4	9	16		

* Kruskal-Wallis test with Conover-Inman test for pair-wise comparisons;

[‡] Chi-square p-value; severe AD cases not shown in table

Table 3.

Summary of the TREM2 protein, TREM2 mRNA, Iba1 protein levels and Iba1-ir microglia counts by clinical category.

		NCI	MCI	mAD	sAD	Overall p-value*
Hippo TREM2	Mean ± SD (Range)	n=12 0.17 ± 0.12 (0.05 – 0.46)	N=15 0.27 ± 0.15 (0.05 – 0.59)	n=8 0.16 ± 0.09 (0.06 – 0.40)	n=8 0.23 ± 0.20 (0.09 – 0.64)	0.38
FC TREM2	Mean ± SD (Range)	n=14 0.45 ± 0.17 (0.23 – 0.73)	n=14 0.56 ± 0.44 (0.13 – 1.72)	N=12 0.47 ± 0.21 (0.14 – 0.89)	n=6 1.07 ± 0.20 (0.78 – 1.33)	0.005 NCI, MCI, mAD < sAD
FC TREM2 mRNA	Mean ± SD (Range)	n=8 2.23 ± 1.40 (0.75 – 4.40)	n=8 2.34 ± 1.60 (0.25 – 4.50)	n=9 1.90 ± 2.26 (0.46 – 7.55)	n=8 4.40 ± 3.98 (0.67 – 11.50)	0.30
FC Iba1	Mean ± SD (Range)	n=15 0.07 ± 0.02 (0.04 – 0.11)	n=14 0.08 ± 0.03 (0.03 – 0.15)	n=11 0.09 ± 0.04 (0.04 – 0.17)	n=6 0.07 ± 0.02 (0.04 – 0.11)	0.31
FC Iba1-ir counts with plaques	Mean ± SD (Range)	n=6 22.00 ± 2.03 (20.33 – 25.66)	n=6 25.03 ± 4.45 (20.33 – 33.17)	n=6 16.27 ± 6.54 (9.83 – 24.7)	n=6 38.50 ± 6.54 (28.75 – 45.72)	0.001 NCI, mAD < sAD
FC Iba1-ir counts without plaques	Mean ± SD (Range)	n=6 12.94 ± 2.16 (10.11 – 15.83)	n=6 11.21 ± 1.46 (10.00 – 13.83)	n=6 11.24 ± 1.81 (9.16 – 13.91)	n=6 17.67 ± 4.87 (14.00 – 26.66)	0.005 MCI, mAD < sAD

* Kruskal-Wallis test

Hippo, hippocampus; FC, Frontal Cortex,