Different splice isoforms of peripheral TREM2 mRNA expressions are associated with cognitive decline in mild dementia due to Alzheimer’s disease and reflect central microglia activation

Yi-Kuan Chiang  
Division of General Neurology, Department of Neurology, Neurological Institute, Taipei Veterans General Hospital

Yung-Shuan Lin  
Division of General Neurology, Department of Neurology, Neurological Institute, Taipei Veterans General Hospital

Chun-Yu Chen  
Division of General Neurology, Department of Neurology, Neurological Institute, Taipei Veterans General Hospital

Jiing-Feng Lirng  
Department of Radiology, Taipei Veterans General Hospital

Yu-Hsiu Yang  
Neurological Institute, Taichung Veterans General Hospital

Wei-Ju Lee  
Neurological Institute, Taichung Veterans General Hospital

Jong-Ling Fuh (jlfuh@vghtpe.gov.tw)  
Division of General Neurology, Department of Neurology, Neurological Institute, Taipei Veterans General Hospital

Research Article

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Abstract

Background

Triggering receptor expressed on myeloid cells 2 (TREM2) is upregulated in activated microglia and may be related to cognitive decline in patients with Alzheimer’s disease (AD). There is conflicting evidence regarding the association of peripheral levels of TREM2 mRNA expression and soluble TREM2 (the extracellular domain of TREM2) with cognitive function in patients with AD. The correlation between peripheral TREM2 mRNA expression and neuroinflammation is unclear.

Methods

We recruited subjects with mild dementia due to AD (clinical dementia rating = 0.5 or 1) and healthy controls. Quantitative real-time polymerase chain reaction analysis was performed using two types of primers. One detects all peripheral TREM2 mRNA isoforms, and the other is specific for TREM2\textsubscript{alt}. In a subgroup of patients with AD, magnetic resonance spectroscopy (MRS) was used to measure the myoinositol (mI) level in the posterior cingulate cortex, which is considered a marker for microglial activation. We analyzed the difference in mRNA expression between the two groups and the association between mRNA expression and cognition and mI levels.

Results

We recruited 61 patients with AD and 51 healthy controls. A one-way analysis of covariance adjusted for covariates showed higher TREM2 and TREM2\textsubscript{alt} mRNA expression levels in the AD group than in the control group (\(p = 0.013\) and \(p = 0.001\), respectively). Correlation analysis and linear regression examining the association between the mRNA expression levels and mini-mental state examination score showed a positive correlation in patients with AD (TREM2, \(r_s = 0.305, p = 0.017,\) adjusted \(p = 0.001;\) TREM2\textsubscript{alt}, \(r_s = 0.302, p = 0.018,\) adjusted \(p = 0.009\)) but not in the control group. Subgroup analysis of 25 AD patients with MRS showed a negative correlation between mRNA expression and mI levels (TREM2, \(r_s = -0.426, p = 0.034,\) adjusted \(p = 0.032;\) TREM2\textsubscript{alt}, \(r_s = -0.447, p = 0.025,\) adjusted \(p = 0.028\)).

Conclusions

Increased TREM2 and TREM2\textsubscript{alt} mRNA expression is associated with AD pathogenesis at the mild dementia stage, thereby serving as a potential biomarker for the early symptomatic stage of AD. TREM2 may exert protective effects on both cognition and microglia-mediated neuroinflammation.

Background

Alzheimer’s disease (AD), the most common neurodegenerative disease and dementia syndrome, involves amyloid-\(\beta\) (A\(\beta\)) deposition, tau aggregation, mitochondrial dysfunction, and cerebral vascular injury [1]. Recent evidence suggests that neuroinflammation contributes to its pathogenesis [1–5].
Neuroinflammation in AD can be modulated by microglial activation in response to Aβ pathology. Microglia may exhibit neuroprotective or neurotoxic effects that signify the course of disease [6, 7]. Triggering receptor expressed on myeloid cells 2 (TREM2), a transmembrane glycoprotein expressed on the surface of microglia, promotes the association between microglia and Aβ plaques. This interaction facilitates the uptake and degradation of Aβ by microglia and appears to exhibit disease progression-dependent effects [8, 9]. Several rare variants of TREM2 have been shown to increase AD risk by 2-4-fold, which is similar to the effect of a single copy of apolipoprotein E (APOE) 4 [10, 11].

Due to the ectodomain shedding of TREM2, its extracellular domain, soluble TREM2 (sTREM2), can be detected in both the cerebrospinal fluid (CSF) and plasma. However, the relative levels of TREM2 or sTREM2 in the CSF or peripheral blood between healthy controls and patients with AD have been conflicting in previous studies [12–18]. In addition, the association between peripheral TREM2 mRNA expression and cognitive status in patients with AD also varied between studies. However, most studies reported a negative correlation between the TREM2 expression level and mini-mental state examination (MMSE) score in patients with AD and patients with amnestic mild cognitive impairment (aMCI) [12–14, 19]. Finally, the correlation between peripheral TREM2 mRNA expression and central microglia-mediated neuroinflammation is uncertain. Previous studies have shown that the level of microglial activation can be measured by brain magnetic resonance spectroscopy (MRS), a non-invasive method to measure metabolic, neurodegenerative changes, gliosis, and microgliosis by quantifying N-acetyl-aspartate and myo-inositol (mI) [20–22]. Elevated levels of mI could be a surrogate of neuroinflammation, an organic glial marker with selective expression of mRNA for sodium-dependent mI transporters in astrocytes [20, 21, 23, 24].

In this study, we compared the peripheral expression of all TREM2 mRNA isoforms and one specific alternative isoform (TREM2alt) that encodes sTREM2 [25] at the same time in patients with mild AD to healthy controls to examine the clinical significance of different mRNA isoforms. MRS was used to investigate the association between peripheral TREM2 expression and central microglial activation. Based on previous studies [12, 14, 19], we proposed that peripheral TREM2 or TREM2alt expression might be higher in patients with mild AD than in healthy controls and is associated with cognitive function and central microglia activation.

**Subjects And Methods**

**Participants**

We recruited patients with mild AD (clinical dementia rating (CDR) [26] = 0.5 or 1) and healthy controls at the outpatient clinics of two teaching hospitals in Taiwan. According to the National Institute on Aging-Alzheimer's Association clinical criteria for probable AD, an AD diagnosis was made during a multidisciplinary consensus meeting [27]. The age at onset of the disease needed to be 65 years or older, and a caregiver who could provide a collateral history was required to be present. Patients with AD
received a standardized evaluation that included clinical interviews, neuropsychological assessments, laboratory tests, and magnetic resonance imaging (MRI).

Patients with significant neurological diseases other than AD that affect cognition, medical history of chronic inflammatory conditions, and anti-inflammatory or immunosuppressive medication use were excluded from the study. This study was approved by the institutional review boards of the two hospitals. Informed consent was obtained from all the patients and their caregivers before participating in the study.

**Clinical evaluation and procedures**

During recruitment, cognitive function was assessed using standard procedures. The MMSE [28] was used to assess global cognition. The CDR was administered to determine the severity of dementia. The 12-item memory test [29], modified 15-item Boston naming test [30], category verbal fluency test [31], trail making test A [32], and forward and backward digit span tests were used to assess short-term memory, language, executive function, attention, and working memory, respectively. Depression was evaluated using the short form of the geriatric depression scale [33].

**TREM2 mRNA measurement**

Total ribonucleic acid (RNA) was extracted from the whole extraction of total RNA according to the standard protocol of the Tempus™ Blood RNA Systems (P/N4342792; P/N4380204, Applied Biosystems, CA, USA). The concentration and purity of the extracted total RNA were measured using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific). To quantitatively estimate TREM2 mRNA levels, complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a High Capacity cDNA Reverse Transcription Kit (P/N4368814, Applied Biosystems, CA, USA) in accordance with the manufacturer’s instructions.

Preamplification was performed using the TagMan® PreAmp Master Mix Kit (P/N4391128; Applied Biosystems, CA, USA). Following the manufacturer’s protocol, the pooled assay mixtures contained TagMan® Gene Expression Assays (P/N4331182, Applied Biosystems, CA, USA) and endogenous control, and Tris-ethylenediaminetetraacetic acid (EDTA) buffer was used to dilute the pooled assays to a final concentration (Assay ID: Hs00219132_m1 to capture all TREM2 transcripts; A189K96 to capture TREM2_{alt} transcript; glyceraldehyde 3-phosphate dehydrogenase, Hs99999905_m1). The preamplification reaction, including the TagMan® PreAmp Master Mix, pooled assay mix, and cDNA sample, was incubated for 10 min at 95°C, followed by 14 cycles of 95°C for 15 s and 60°C for 4 min, and held at 4°C in an Applied Biosystems Thermocycler (9700). The preamplification product was further diluted with Tris-EDTA buffer to serve as a template for real-time polymerase chain reaction (PCR) analysis.

For quantitative estimation of TREM2 and TREM2_{alt} mRNA levels, the StepOnePlus™ Real-Time PCR System (Applied Biosystems, CA, USA) was used in triplicate for each sample. The final reaction volume was 10 µl using the TaqMan Universal Master Mix with UNG (P/N4440044, Applied Biosystems, CA, USA). Melt curve analysis was performed immediately after the amplification step to ensure that only a single product was amplified. Relative mRNA levels were calculated using the 2-ΔΔCT method using StepOne
software 2.3 (Applied Biosystems, CA, USA) [34]. The median level of healthy controls was used for calibration in all experiments to correct for any observational errors.

**DNA analysis**

Genomic DNA was isolated from whole blood using the Gentra Puregene kit, according to the manufacturer's protocol (Qiagen, Hilden, Germany). The presence of the ε2, ε3, and ε4 alleles of the APOE gene was determined by genotyping the single nucleotide polymorphisms rs429358 and rs7412. An APOE ε4 carrier was defined as having at least one ε4 allele (including ε2/ε4, ε3ε4, and ε4/ε4). Genotyping of rs429358 and rs7412 was performed using the TaqMan genotyping assay (Applied Biosystems, Foster City, CA, USA). PCRs were performed in 96-well microplates using an ABI StepOnePlus™ real-time PCR machine (Applied Biosystems). Allele discrimination was achieved by detecting fluorescence using StepOne software 2.3 (Applied Biosystems, CA, USA).

**MRS**

The second half of the study involved brain MRS in 25 consecutive patients consecutively. Brain MRI studies were performed using a GE Discovery 3 Tesla MRI. A 3D T1 FSPGR image series (TR = 6.8 ms, TE = 2.5 ms, 24 cm² FOV; 1 mm³ isotropic voxels) was used for the localization of posterior cingulate cortex (PCC) (volume of interest = 2 × 2 × 2 cm³ in the midsagittal PCC region), which is commonly known the site of initial neuropathological changes in AD and has been investigated in many previous studies [35, 36]. Proton MR spectra were acquired using the PRESS localization sequence with TR = 2000 ms, TE = 35 ms, and 8 and 160 total numbers of scans for each acquisition. Brain MRI and proton MR spectra quality were visually assessed by an expert neuroradiologist (Lirng JF) blinded to the clinical condition. Peak areas of creatine (Cr) + phosphocreatine (PCr) and ml were calculated using LCModel. The glial marker for neuroinflammation was measured using the ml relative to Cr + PCr at the PCC.

**Statistical analysis**

The demographic and neuropsychiatric data of the AD and control groups were compared using the chi-square test or Mann-Whitney U test when appropriate. The skewness values of TREM2 and TREM2\textsubscript{alt} mRNA expression exceeded one. Thus, we log-transformed TREM2 and TREM2\textsubscript{alt} mRNA expression values for further analysis [37]. Differences in TREM2 and TREM2\textsubscript{alt} mRNA expression levels between the two groups were examined using a one-way analysis of covariance after adjusting for age, sex, and APOE 4 carrier status. The associations between MMSE and TREM2 and TREM2\textsubscript{alt} in patients with AD were examined using Spearman's correlation and linear regression after adjusting for age, sex, years of education, and APOE 4 carrier status. To further evaluate the association between TREM2 expression and ml level (ml relative to Cr + PCr) on MRS, Spearman's correlation and linear regression analyses were performed after adjusting for age, sex, and APOE 4 carrier status.

**Results**
Subjects and demographics

In total, 61 patients with AD (36 males/25 females; mean age = 75.5 ± 6.4 years; mean education = 9.6 ± 5.0 years; mean MMSE = 22.5 ± 2.4) and 51 controls (20 males/31 females; mean age = 72.2 ± 6.2 years; mean education = 11.9 ± 4.3 years; mean MMSE = 28.6 ± 1.3) were recruited for this study. Table 1 shows the demographic and neuropsychiatric data of all participants. There were no differences in the sex ratio ($p = 0.058$) and depression scale score ($p = 0.948$) between patients with AD and controls. However, patients with AD had fewer years of education ($p = 0.011$) and were older ($p = 0.004$) than controls. Patients with AD were found to have lower scores in all cognitive assessments compared to the controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls ($n = 51$)</th>
<th>Patients with AD ($n = 61$)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, $n$ (%)</td>
<td>31 (60.8)</td>
<td>25 (41.0)</td>
<td>0.058</td>
</tr>
<tr>
<td>Age (years, mean ± SD)</td>
<td>72.2 ± 6.2</td>
<td>75.5 ± 6.4</td>
<td>0.004</td>
</tr>
<tr>
<td>Education (years, mean ± SD)</td>
<td>11.9 ± 4.3</td>
<td>9.6 ± 5.0</td>
<td>0.011</td>
</tr>
<tr>
<td>APOE 4 carrier no. (%)</td>
<td>9 (17.6)</td>
<td>21 (34.4)</td>
<td>0.075</td>
</tr>
<tr>
<td>MMSE (mean ± SD)</td>
<td>28.6 ± 1.3</td>
<td>22.3 ± 2.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>12-item delayed recall (mean ± SD)</td>
<td>8.8 ± 2.2</td>
<td>2.6 ± 2.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Forward digit span (mean ± SD)</td>
<td>11.2 ± 2.1</td>
<td>9.3 ± 2.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Backward digit span (mean ± SD)</td>
<td>7.8 ± 2.6</td>
<td>4.9 ± 2.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Category verbal fluency (mean ± SD)</td>
<td>12.9 ± 3.0</td>
<td>8.0 ± 2.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Modified Boston naming test (mean ± SD)</td>
<td>14.2 ± 1.1</td>
<td>12.8 ± 1.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Trail making A (seconds, mean ± SD)</td>
<td>55.0 ± 24.3</td>
<td>122.9 ± 88.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Geriatric depression scale (mean ± SD)</td>
<td>2.7 ± 2.3</td>
<td>3.0 ± 3.2</td>
<td>0.948</td>
</tr>
</tbody>
</table>

$P$-values represent the comparison between groups using a chi-square or Mann-Whitney U test.

AD Alzheimer's disease, APOE apolipoprotein E, MMSE mini-mental state examination

TREM2 and TREM2$_{alt}$ mRNA expression levels

After log-transforming the TREM2 and TREM2$_{alt}$ mRNA expression values, patients with AD showed a significantly higher expression of TREM2 mRNA than the control group (1.28 ± 0.46 vs 1.05 ± 0.44, $p = 0.013$). Patients with AD also showed significantly higher expression of TREM2$_{alt}$ mRNA compared to the control group (1.52 ± 0.77 vs 1.03 ± 0.48, $p = 0.001$) (Fig. 1).
Associations between MMSE and TREM2, as well as TREM2\textsubscript{alt} mRNA expression in patients with AD

After log-transforming the TREM2 and TREM2\textsubscript{alt} mRNA expression values, there were significant positive correlations between the MMSE score and TREM2 mRNA expression (rs = 0.305, \( p = 0.017 \)) and TREM2\textsubscript{alt} mRNA expression (rs = 0.302, \( p = 0.018 \)) in patients with AD (Fig. 2), but not in controls. After adjusting for covariates, linear regression also showed that TREM2 and TREM2\textsubscript{alt} mRNA expression were independent predictors of the MMSE scores in patients with AD (TREM2, \( p = 0.001 \); TREM2\textsubscript{alt}, \( p = 0.009 \)) (Table 2).

Table 2
Results of linear regression showing that TREM2 and TREM2\textsubscript{alt} mRNA expression are independent predictors for the MMSE in patients with AD. MMSE as the dependent variable. \( R^2 = 0.357 \).

<table>
<thead>
<tr>
<th>Model 1</th>
<th>B</th>
<th>SE</th>
<th>( \beta )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.093</td>
<td>0.041</td>
<td>-0.250</td>
<td>0.028</td>
</tr>
<tr>
<td>Sex</td>
<td>-1.007</td>
<td>0.562</td>
<td>-0.208</td>
<td>0.079</td>
</tr>
<tr>
<td>APOE 4 carrier status</td>
<td>-0.978</td>
<td>0.563</td>
<td>-0.196</td>
<td>0.088</td>
</tr>
<tr>
<td>Years of education</td>
<td>0.151</td>
<td>0.057</td>
<td>2.647</td>
<td>0.011</td>
</tr>
<tr>
<td>TREM2 mRNA expression</td>
<td>6.288</td>
<td>1.789</td>
<td>0.384</td>
<td>0.001</td>
</tr>
</tbody>
</table>

MMSE as the dependent variable. \( R^2 = 0.357 \).

<table>
<thead>
<tr>
<th>Model 2</th>
<th>B</th>
<th>SE</th>
<th>( \beta )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.074</td>
<td>0.043</td>
<td>-0.198</td>
<td>0.093</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.700</td>
<td>0.587</td>
<td>-0.145</td>
<td>0.238</td>
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<tr>
<td>APOE 4 carrier status</td>
<td>-0.820</td>
<td>0.597</td>
<td>-0.164</td>
<td>0.173</td>
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<tr>
<td>Years of education</td>
<td>0.162</td>
<td>0.060</td>
<td>0.336</td>
<td>0.009</td>
</tr>
<tr>
<td>TREM2\textsubscript{alt} mRNA expression</td>
<td>3.474</td>
<td>1.274</td>
<td>0.319</td>
<td>0.009</td>
</tr>
</tbody>
</table>

MMSE as the dependent variable. \( R^2 = 0.306 \).

\textit{AD} Alzheimer’s disease, \textit{APOE} apolipoprotein E, \textit{MMSE} mini-mental state examination, \textit{TREM2} triggering receptor expressed on myeloid cells 2, \textit{TREM2\textsubscript{alt}} alternatively spliced TREM2

Associations between mI level at PCC on MRS and TREM2 as well as TREM2\textsubscript{alt} mRNA expression in patients with AD
In total, 25 patients underwent MRS. There were no differences in the sex ratio \((p = 0.51)\), age \((p = 0.82)\), years of education \((p = 0.96)\), the prevalence of APOE 4 carriers \((p = 1.00)\), and MMSE score \((p = 0.72)\) between this subgroup and the subgroup without MRS. After log-transforming the TREM2 and TREM2\textsubscript{alt} mRNA expression values, there were significant negative correlations between mI levels at the PCC on MRS and TREM2 mRNA expression \((rs = -0.426, p = 0.034)\) and TREM2\textsubscript{alt} mRNA expression \((rs = -0.447, p = 0.025)\) in patients with AD (Fig. 3), but not in controls. After adjusting for covariates, linear regression also showed that TREM2 and TREM2\textsubscript{alt} mRNA expression were independent predictors of mI levels at the PCC on MRS in patients with AD \((TREM2, p = 0.032; \text{TREM2}_{\text{alt}}, p = 0.028)\) (Table 3).

### Table 3
Results of linear regression showing that TREM2 and TREM2\textsubscript{alt} mRNA expression are independent predictors for the mI level at PCC on MRS in patients with AD mI/Cr + PCr as the dependent variable. \(R^2 = 0.306\).

<table>
<thead>
<tr>
<th>Model 1</th>
<th>B</th>
<th>SE</th>
<th>(\beta)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.001</td>
<td>0.002</td>
<td>0.02</td>
<td>0.920</td>
</tr>
<tr>
<td>Sex</td>
<td>0.059</td>
<td>0.027</td>
<td>0.470</td>
<td>0.041</td>
</tr>
<tr>
<td>APOE 4 carrier status</td>
<td>0.008</td>
<td>0.027</td>
<td>0.059</td>
<td>0.779</td>
</tr>
<tr>
<td>TREM2 mRNA expression</td>
<td>-0.193</td>
<td>0.084</td>
<td>-2.307</td>
<td>0.032</td>
</tr>
</tbody>
</table>

mI/Cr + PCr as the dependent variable. \(R^2 = 0.306\).

<table>
<thead>
<tr>
<th>Model 2</th>
<th>B</th>
<th>SE</th>
<th>(\beta)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.001</td>
<td>0.002</td>
<td>-0.092</td>
<td>0.646</td>
</tr>
<tr>
<td>Sex</td>
<td>0.044</td>
<td>0.026</td>
<td>0.354</td>
<td>0.105</td>
</tr>
<tr>
<td>APOE 4 carrier status</td>
<td>-0.003</td>
<td>0.027</td>
<td>-0.025</td>
<td>0.903</td>
</tr>
<tr>
<td>TREM2\textsubscript{alt} mRNA expression</td>
<td>-0.149</td>
<td>0.063</td>
<td>-0.451</td>
<td>0.028</td>
</tr>
</tbody>
</table>

mI/Cr + PCr as the dependent variable. \(R^2 = 0.314\).


**Discussion**

The present study showed higher peripheral blood TREM2 and TREM2\textsubscript{alt} mRNA expression levels in patients with mild AD than in the control group. In addition, TREM2 and TREM2\textsubscript{alt} mRNA expression was
positively associated with cognition status and negatively associated with the marker of central microglial activation. These findings suggest a protective function of TREM2 in cognition and neuroinflammation in the early symptomatic stages of AD. Furthermore, we observed increased peripheral TREM2 and TREM2$_{alt}$ mRNA expression levels in patients with mild AD. The results provide supplemental evidence of changes in TREM2 expression during the early symptomatic stage of AD. They indicate that peripheral TREM2 and TREM2$_{alt}$ mRNA levels could be potential biomarkers associated with neuroinflammation in the early stage of AD dementia.

The increased peripheral TREM2 mRNA expression in patients with AD echoed most of the findings of previous studies [12–14]. However, the present study is the first to demonstrate that peripheral TREM2$_{alt}$ mRNA expression is higher in patients with AD. A previous study showed that TREM2$_{alt}$ mRNA expression was also higher in the temporal cortex of patients with AD, but this difference was not significant after adjusting for covariates [25]. The findings of the present study suggest that the measurement of TREM2$_{alt}$ expression in the peripheral blood may reflect the activation of TREM2-related signaling.

Our study found a positive correlation between the MMSE score and TREM2 expression in patients with mild AD after adjusting for covariates, including APOE 4 carrier status, but not in controls. A recent study also showed that CSF sTREM2 expression was decreased in the earliest asymptomatic phase of AD with the presence of abnormal Aβ pathology but not tau pathology or neurodegeneration, and an increased sTREM2 level in the CSF in an early symptomatic stage of AD was associated with tau-related neurodegeneration instead of Aβ pathology [38]. Together, these findings suggest that TREM2 expression might be a response to tau pathology in the early symptomatic phase of AD and that TREM2 has a protective effect during the early stage. The cognitive protective function of TREM2 was also supported by other studies, although indirectly. In vivo studies have shown that functional TREM2 plays a role in the phagocytic clearance of amyloid seeds during early amyloidogenesis [39] and that TREM2-deficient microglia exhibit strong cerebral metabolic defects and increased stress markers [40]. However, the results of studies examining the correlation between mRNA expression levels of TREM2 and MMSE were contradictory. One study showed a significant negative correlation between TREM2 expression and MMSE scores in patients with moderate to severe AD (MMSE: 0–16) [12]. Another study reported that higher TREM2 levels were correlated with lower MMSE scores after correcting for APOE 4 carrier status [13]. Another study evaluating patients with AD and schizophrenia showed no correlation between TREM2 levels and MMSE scores [14]. These conflicting findings suggest dynamic changes or a disease stage-dependent effect of TREM2 expression during the disease course of AD [18, 41] and an interaction among cognitive status, APOE 4 carrier status, and TREM2 expression.

Our study had several limitations. First, the diagnoses of AD were made based on clinical criteria without biomarker evidence of Aβ and tau, which may have influenced diagnostic accuracy. Second, we did not measure the corresponding TREM2 protein level at the same time because previous studies have shown similar results between the protein level and mRNA expression [12]. Furthermore, one of the goals of this study was to examine the difference between TREM2 and sTREM2 expression simultaneously, which can
be better measured by mRNA expression. Third, while many pharmacological or metabolic factors may alter TREM2 mRNA expression levels in study participants, we only excluded subjects with infective or inflammatory conditions or anti-inflammatory or immunosuppressive medication use at the time of blood sampling. Finally, the relatively small sample size may have limited the statistical power of our study. Another independent cohort study with more participants should be conducted in the future to confirm the results.

Conclusion

Increased TREM2 and TREM2_{alt} mRNA expression is associated with AD pathogenesis in patients with mild dementia. The positive associations of both TREM2 and TREM2_{alt} mRNA expression with the MMSE score in mild AD suggested that TREM2 may exhibit protective effects on cognitive status. In contrast, the negative association of TREM2 mRNA levels with ml levels at the PCC in the subgroup of patients with AD might suggest the role of TREM2 in modulating microglia-mediated neuroinflammation. Peripheral TREM2 and TREM2_{alt} mRNA levels may be potential biomarkers associated with neuroinflammation in the early symptomatic stages of AD.

Declarations

Ethics approval and consent to participate

This research was approved by the local ethics committees at Taipei Veterans General Hospital (IRB number 2012-05-033B) and Taichung Veterans General Hospital (IRB number SF12171A). Informed consent was obtained from all patients and their caregivers before study participation.

Consent for publication

All the authors gave their approval for publication.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Chiang YK: interpretation of the data and drafting the manuscript.

Lin YS: study concept and design and critical revision of manuscript.

Chen CY: study concept and design and critical revision of manuscript.

Lirng JF: data acquisition, analysis and interpretation.

Yang YH: data acquisition, analysis and interpretation.

Lee WJ: study concept and design, acquisition of data, analysis and interpretation of the data and drafting the manuscript.

Fuh JL: study concept and design, acquisition of data, and critical revision of manuscript.

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References


**Figures**

**A**

![Graph A](image1)

**B**

![Graph B](image2)

**Figure 1**

Comparison of the peripheral TREM2 and TREM2$_{alt}$ mRNA expression levels between patients with AD and controls. The expression of TREM2 and TREM2$_{alt}$ mRNA showed significant differences between patients with AD and controls (TREM2, $p = 0.013$; TREM2$_{alt}$, $p = 0.001$).
Figure 2

Correlation between the MMSE score and TREM2 and TREM2_{alt} mRNA expression in patients with AD. Significant correlations between the MMSE score and TREM2 mRNA expression ($r_s = 0.305, p = 0.017$) and TREM2_{alt} mRNA expression ($r_s = 0.302, p = 0.018$).

Figure 3

Correlation between the MRS-measured ml level (ml/Cr + PCr) at PCC and TREM2 and TREM2_{alt} mRNA expression in patients with AD ($n = 25$). Significant correlations between the MMSE score and TREM2 mRNA expression ($r_s = -0.426, p = 0.034$) and TREM2_{alt} mRNA expression ($r_s = -0.447, p = 0.025$).