의학박사 학위논문

알츠하이머병에 있어 sTREM2의 역할 규명

The role of sTREM2 in Alzheimer's continuum

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이 논문을 의학박사 학위 논문으로 제출함

2023년 8월

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Abstract

BACKGROUND Neuroinflammation has emerged as a vital player in the Alzheimer's disease (AD) pathogenesis. Microglial activation is considered the center of this theory. Triggering receptors expressed on myeloid cells 2 (TREM2) are known to increase the risk of AD and are related to other neurodegenerative diseases, implicating microglia and the innate immune system in the CNS as pivotal factors in the pathogenesis of AD. The soluble fragment of TREM2 (sTREM2) serves as a marker of microglial activation and changes dynamically during AD. sTREM2 is released into the cerebrospinal fluid (CSF) and plasma after proteolytic processes via shedding by ADAM proteases in the ectodomain of TREM2. CSF sTREM2 is selectively expressed in microglia. Plasma sTREM2 is thought to originate from peripheral blood mononuclear cells (PBMCs).

OBJECTIVES We aimed to elucidate the roles of cerebrospinal fluid (CSF) and plasma sTREM2 in the AD continuum by analyzing their relationships with conventional AD biomarkers. Therefore, we investigated the potential of CSF and plasma sTREM2 levels as biomarkers of AD.

METHODS Participants were consecutively recruited from Asan Medical Center from 2018 to 2020. The participants were stratified according to amyloid positivity and clinical status. Along with other AD biomarkers, sTREM2 levels were measured in plasma and CSF.

RESULTS CSF sTREM2 levels were positively correlated with pTau-181 levels ($\rho = 0.25$, $P = 0.019$). In contrast, plasma sTREM2 showed an inverse relationship and were negatively correlated with pTau-181 ($\rho = -0.33$, $P = 0.002$). Plasma sTREM2 were negatively correlated with amyloid deposition in basal ganglia ($\rho = -0.35$, $P = 0.01$, in AD continuum). Neither CSF nor plasma sTREM2 predicted amyloid positivity in the brain compared to plasma pTau-181. However, plasma sTREM2 were negatively correlated with basal ganglia amyloid deposition ($\rho = -0.33$, $P = 0.028$) in AD continuum. In terms of cognitive decline, the combination of plasma sTREM2 and $A\beta_{42}/A\beta_{40}$ ratio (AUC = 0.761, $P < 0.001$) showed statistical significance.

CONCLUSION The CSF sTREM2, in line with previous studies, was found to be related to tau biomarkers rather than amyloid. Plasma sTREM2 is a prognostic marker for predicting cognitive decline and reflects amyloid deposition in the basal ganglia in the AD continuum. In clinical practice, these findings might facilitate biomarker-supported diagnosis and prediction of fast decliners in patients with AD.

Key Words: Alzheimer's disease, Amyloid, Neuroinflammation, Dementia

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1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease and the leading cause of dementia[1]. According to the revised National Institute on Aging and Alzheimer's Associated (NIA-AA) research framework, not only cognitive status (cognitive unimpaired, mild cognitive impairment [MCI], dementia) but also confirmation of pathological mechanisms such as amyloid and tau are important processes in the diagnosis of AD[2]. In addition, since Aduhelm, an anti-amyloid immunotherapy, has been approved by the FDA for the first time, the demand for easy and accurate methods of identifying AD pathological change in the brain has gradually increased[3,4]. In particular, for the most important biomarkers, amyloid and tau, there are still no established test and standard cut-off values other than CSF study and positron emission tomography (PET)[5-7]. However, the aforementioned methods are difficult to repeat frequently because of its expensive and invasive nature.

The amyloid β_{42} plaques and abnormal hyperphosphorylated tau tangles are hallmarks of AD pathology[8]. Based on the amyloid cascade hypothesis, pathological changes in AD have been proposed as a series of processes from amyloid deposition to neurodegeneration[9]. However, accumulating evidence suggests that the amyloid cascade alone cannot explain the complex pathogenesis of AD, indicating the involvement of other processes[10]. Neuroinflammation, which was previously considered a secondary phenomenon, has now emerged as a vital player in the pathogenesis of AD[11-13]. Neuroinflammation is an inflammatory response in the central nervous system (CNS). This process is mediated by innate immune cells in the CNS, such as astrocytes and microglia, which release pro-inflammatory molecules[12]. The release of pro-inflammatory molecules can lead to neurodegenerative processes, including neuronal death, synaptic dysfunction, and inhibition of neurogenesis.

This theory is based on microglial activation[14,15]. Among the various functions of microglia, immune surveillance plays an important role in the development of AD[16]. The failure of activated microglial immune surveillance to remove amyloid plaques from surrounding neurons and phagocytose them leads to the formation and accumulation of amyloid plaques and tau tangles[17]. The membrane-bound triggering receptor expressed on myeloid cells 2 (TREM2) is one of the mediators of microglial activation[17]. In humans, the gene encoding TREM2 is located on chromosome 6p21.1 within a cluster of related *TREM* genes[18]. Whole-genome sequencing studies have revealed that rare variants of TREM2 increase the risk of developing AD two to three-folds, similar to a single copy of ApoE ε4 carriers. The activity of TREM2 can be measured using the levels of soluble fragments of TREM2 (sTREM2)[19]. sTREM2 is released into the CSF and plasma after proteolytic processes via shedding by ADAM proteases in the ectodomain of TREM2. CSF sTREM2 is selectively expressed in microglia. Plasma sTREM2 is thought to originate from peripheral blood mononuclear cells (PBMCs)[20]. Previous studies have reported that CSF sTREM2 levels change dynamically during the disease course; thus, its potential as a biomarker is expected[21,22]. Specifically, CSF sTREM2 reached its highest levels during the MCI stage. In the dementia state, this increase is attenuated[23]. Pathologically, CSF sTREM2 is associated with an increase in amyloid-related hyperphosphorylated tau and glucose hypermetabolism in the AD brain[24]. However, there are relatively few

studies on the role of plasma sTREM2; thus, little is known about its role in AD[25].

In this study, we aimed to elucidate the roles of CSF and plasma sTREM2 in the AD continuum. To this end, we hypothesized the role of sTREM2 in the following aspects: First, both CSF and plasma sTREM2 levels were associated with conventional AD biomarkers belonging to the AT(N) classification. Second, sTREM2 is a potential biomarker for predicting changes in cognitive status and amyloid deposition in the brain. Therefore, we investigated the potential clinical implications of CSF and plasma sTREM2 levels as biomarkers of AD.

2. Methods and Materials

2.1. Participants

This study consecutively and prospectively enrolled 104 participants who visited the Memory Clinic of Asan Medical Center, Seoul, Korea, from June 2018 to July 2020. Informed consent was obtained from all participants or their proxies. All the participants underwent brain magnetic resonance image (MRI), detailed neuropsychological testing, fluorine-18 [18F]-florbetaben amyloid PET, and CSF analysis. The participants who were included in the study met the following criteria: (1) age > 45 and < 90 years; (2) cognitive impairment, including subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia, and was defined by subjective memory impairment reported by the patients or caregivers, activities of daily living (ADL) as judged by a physician, and objective memory decline below the 16th percentile (-1 standard deviation) for demographically matched norms determined by neuropsychological testing; and (3) no evidence of structural lesions including extensive white matter disease that could influence the cognitive function in the brain MRI. All participants underwent blood tests for parameters including complete blood count; lipid profiles; erythrocyte sedimentation rate (ESR); vitamin B12, folate, and homocysteine serum levels; and thyroid function. The apoE genotype was identified after extracting genomic DNA from venous blood. All evaluation processes were performed for approximately three months based on the time of the neuropsychological test (Seoul Neuropsychological Screening Battery [SNSB]). Of the 104 participants, eight were excluded because of withdrawal of informed consent (three patients) or errors during image processing (five patients); therefore, the final sample set consisted of 96 patients (Figure 1). This study used 100 amyloid PET scans. The power calculation test suggested that each group (subjective cognitive decline, mild cognitive impairment, and dementia) required at least 25 subjects for statistical significance (alpha value = 0.05, power = 0.8). However, because our study was exploratory rather than confirmatory, we recruited a broad spectrum of subjects (both amyloid-positive and amyloid-negative) by screening patients using a cognitive function test.

Of the 96 participants, 75 completed a follow-up study within one year of baseline evaluation. Plasma sTREM2, pTau-181, neurogranin, neurofilament light chain (NfL), and telomere length with simple blood tests were included in the follow-up evaluation. Skilled neurologists conducted a cognitive screening test. Among these

subjects, those who showed decreased K-MMSE scores by more than four points and increased CDR-sum of box scores by one point within one year were classified as fast decliners[26].

2.2. Imaging acquisition

2.2.1. Brain Magnetic Resonance Image (MRI)

Of the 96 patients, 62 underwent three-dimensional (3D) volume imaging. T1-weighted and fluid-attenuated inversion recovery (FLAIR) MRI images were used to identify structural abnormalities.

2.2.2. Amyloid Positron Emission Tomography Image

All PET images were obtained using Discovery 690, 710, and 690 Elite PET/CT scanners (GE Healthcare, Chicago, IL, USA). Amyloid PET images were acquired for 20 min, beginning 90 min after the injection of 300 ± 30 MBq 18F-florbetaben. Two neurologists (H.J.K. and J.H.L) and two nuclear medicine physicians (J.S.K. and M.O) reviewed the PET scans according to predefined regional cortical tracer binding (RCTB) and brain amyloid plaque load (BAPL) scoring systems. The final score was reached by consensus, with a BAPL score of 1 regarded as Aβ- and BAPL scores of 2 and 3 considered to be Aβ+.

The standardized uptake ratio (SUVR) was calculated using a novel deep-learning (DL)-based spatial normalization (SN) of PET images, which does not require MRI or computed tomography (CT) images of the same patient[27]. The proposed deep neural network model, comprising cascaded U-Nets, generated local displacement fields for nonlinear registration as the input of an affine-registered amyloid PET image[28]. Only PET images in individual spaces were used to create the deformation fields. When the DNN model was trained, only the PET images in an individual space were fed into the DNN model to generate SN images in the template space. The ROIs included the frontal, lateral temporal, parietal, occipital, precuneus, posterior cingulate, and the basal ganglia.

2.3. Soluble Triggering Receptor Expressed on Myeloid cell 2 and solid-phase PLA (spPLA)

To detect sTREM2 in CSF and plasma, spPLA was performed according to the previous protocol[19]. spPLA can detect proteins at femtomolar concentrations of 1 nM or 10 nM. sTREM2 concentrations in the CSF and plasma were measured using a biotinylated anti-sTREM2 polyclonal antibody and recombinant TREM2 protein (R&D Systems). Briefly, 1 mg/mL Dynabeads MyOne Streptavidin T1 (Invitrogen) was incubated with 50 nM biotinylated anti-sTREM2 polyclonal antibody (R&D Systems) for 1 h at room temperature (RT) under rotation to immobilize the sTREM2 antibodies onto magnetic beads. The magnetic beads were washed twice with 0.05% Tween 20 in 1X PBS (washing buffer). During the reaction, plasma samples were diluted two-fold in PLA buffer,

and recombinant TREM2 protein (R&D Systems) was serially spiked in PLA buffer from 1 nM to 1 fM as a standard. Diluted plasma samples and serial dilutions of the standards were mixed with antibody-conjugated magnetic beads and incubated for 90 min with rotation. The beads were then washed twice with the washing buffer. PLA probes were formed by separately incubating 50 nM streptavidin–oligonucleotide conjugates SLC1 and SLC2 with 50 nM biotinylated anti-sTREM2 antibody for 1 h at RT. Prior to use, SLC1- and SLC2-anti-sTREM2 antibodies were mixed in an equal ratio and incubated for 5 min at RT. The final concentration of each probe was 500 pM. Finally, the magnetic beads were mixed with 1 nM PLA probe mix and incubated for another 90 min at RT followed by two washes with washing buffer. Real-time PCR mix (1X PCR buffer [QIAGEN] comprising 2.5 mM MgCl2 [QIAGEN], 0.22 µM Sybr Green [Life Technologies], 0.1 µM of each primer [reverse primer, forward primer, and splint primer] [IDT], 80 µM ATP [ThermoFisher], 0.2 mM dNTP with U [ThermoFisher], 0.03 U/µL Taq polymerase [QIAGEN], 0.01 U/µL T4 DNA ligase [ThermoFisher], 0.02 U/µL UNG [ThermoFisher], and nuclease-free water [QIAGEN]) was prepared as previously described. Before the cycling stage, a heat incubation step was performed for 15 min at 95 °C, and application steps were performed at 40 cycles for 30 s at 94 °C, 1 min at 50 °C, and 1 min at 72 °C. A StepOnePlus real-time PCR instrument (Applied Biosystems) was used for all experiments and analyses. To investigate the performance efficiency of sTREM2 spPLA, the LLOD, LLOQ, precision, and dilutional linearity were measured. The LLOD formula was CtLOD = CtN-2SN. CtN is the average Ct value obtained from the background noise, and SN is the standard deviation of this value. sTREM2 spPLA had an inter-assay precision of < 17.5% and intra-assay precision of < 2%. In terms of inter-assay precision, a previous study explained that relatively large coefficients of variation were the result of the PCR step, which can be highly variable at low target copy numbers. The sTREM2 spPLA showed LLOD 50.58 pg/mL, LLOQ 151.75 pg/mL and an average 95.9% dilutional linearity.

2.4. Plasma and CSF biomarkers

To measure biomarker levels, we conducted ELISA and SIMOA following the instructions provided by commercial suppliers[19]. The reaction was stopped with a stop solution and the absorbance was read at 450 nm. To measure total tau in the CSF, a human tau (Total) ELISA kit (Invitrogen) was used. The standards and samples were diluted with standard diluent buffer at a 1:1 ratio and added to wells coated with capture antibodies for 2 h at RT. After washing, human tau biotin-conjugated antibodies were added to each well for 1 h. Next, streptavidin-horseradish peroxidase solution was added to the wells for 30 min. Finally, the stabilized chromogen and stop solutions were added to each well. The total tau level in the CSF was measured at 450 nm. In case of $A\beta_{42}$ levels, the CSF was diluted threefold with sample diluent due to high endogenous levels. Standards and samples were added and the mixture was incubated for 2 h at 2–8 °C. After washing four times, human amyloid β (aa1-42) conjugate was added to each well and incubated for another 2 hr at at 2–8 °C. The wells were washed and the substrate was allowed to react for 30 min at RT. The level of $A\beta_{42}$ was determined using a microplate reader

(Biotek) set to 450 nm. For plasma neurogranin, Human Neurogranin ELISA (Lifespan Biosciences) was used. The samples were then added to the wells for 1 h. After washing, detection reagent A and B was added to the wells for 1 h each at 37 °C. The TMB substrate reaction was allowed to proceed for 15 min, and the optical density was determined at 450 nm. Finally, the NfL level in the CSF was detected using the Simoa NF-light Advantage Kit. CSF was diluted 4-folds and 100-folds, respectively. CSF NfL was measured at the DNA Link Laboratory, South Korea, on the Simoa-HD1 platform, as previously described.

Plasma pTau-181 levels were measured using the Simoa Human p-Tau181 Advantage V2 assay (Quanterix Corp, Boston, MA, USA, PN/103714), and plasma NfL levels were measured using the Simoa NF-Light Advantage assay (Quanterix Corp, Boston, MA, USA, PN/103186). For a typical run setup, each sample and control were transferred into 96-well Quanterix® plates for duplicate tests with an onboard 4× dilution by the instrument. Detailed instructions are provided in the Simoa Guide (Quanterix). The two-step immunoassay was performed using a Simoa HD-X instrument (Quanterix). Furthermore, the target antibody-coated paramagnetic beads were combined with the sample and biotinylated detector antibodies for the same incubation period. Target molecules present in the sample were captured using antibody-coated beads and simultaneously bound to a biotinylated antibody detector. Plasma Aβ40 and Aβ42 levels were measured using the Simoa Human $A\beta_{40}$ Advantage assay (Quanterix Corp, Boston, MA, USA, PN/101672) and the Simoa Human $A\beta_{42}$ Advantage assay (Quanterix Corp, Boston, MA, USA, PN/101664), respectively. For a typical run setup, each sample and control were transferred to 96-well Quanterix® plates for duplicate tests with onboard 4× dilution by the instrument; detailed instructions can be found in the Simoa Guide (Quanterix). The two-step immunoassay was performed using a Simoa HD-X instrument (Quanterix). The target antibody-coated paramagnetic beads were combined with the sample and biotinylated detector antibodies during incubation. Target molecules present in the sample were captured using antibody-coated beads and simultaneously bound to a biotinylated antibody detector.

2.5. Statistics

Data were analyzed using the Mann-Whitney U test, Kruskal-Wallis test, chi-square test, analysis of variance (ANOVA), Spearman's correlation analysis, analysis of covariance (ANCOVA), logistic regression analysis, and time-dependent receiver operating characteristic (ROC) curve analysis.

To compare the demographic profiles and biomarkers of the four groups (amyloid-positive pre-dementia/dementia and amyloid-negative pre-dementia/dementia), we used the Kruskal-Wallis test and ANOVA. For the two groups (amyloid-positive/negative), we used the Mann-Whitney U test. However, the variables were not normally distributed. To evaluate group differences in dichotomous variables, we used the chi-square test. Spearman's correlation analysis was used to demonstrate the correlation between CSF and plasma biomarkers.

To compare regional amyloid deposition by quantitative SUVRs, we used ANCOVA. Age was adjusted for in the

SUVR comparison. To determine the influence of each biomarker on the prediction of amyloid positivity and cognitive decline, we used a logistic regression model and ROC curve analysis for group differences. Biomarkers for each model were determined using a stepwise backward elimination process. Delong's test was performed to confirm a high-risk model for the prediction of amyloid positivity in the brain and fast cognitive decline.

All statistical analyses were performed using R v4.0.3 (Institute for Statistics and Mathematics, Vienna, Austria; www. R-project. org), which was also used to derive the estimates of 95% confidence intervals and standard error.

3. Results

3.1. Demographics

The clinical characteristics of the study participants are shown in Table (1). The amyloid-positive and amyloid-negative groups showed significant differences in age, duration (defined as the period from first symptom onset to diagnosis), presence of diabetes mellitus (DM), serum LDL levels, and the *APOE* genotype. Among the four groups, the mean age of the amyloid-positive dementia group indicated that it had the youngest patients, which included eight early-onset patients, followed by the amyloid-positive pre-dementia group ($P = 0.009$). The longest duration was seen in the amyloid-positive dementia group (43.3 ± 25.6 months), followed by amyloid-negative dementia (34.6 ± 39.5 months) ($P = 0.039$). The amyloid-positive groups had a higher frequency of DM patients than the amyloid-negative groups ($P = 0.006$), with 13 of the 16 patients in the amyloid-positive dementia group (82.2%) and 26 of the 23 patients (81.2%) in the amyloid-positive pre-dementia group having DM. The serum LDL levels were significantly higher in the amyloid-positive dementia group (140.94 ± 10.02, P = 0.039). The carrier of *APOE* ε4 allele was significantly higher in the amyloid-positive group than that in the amyloid-negative group ($P = 0.001$). However, there was no significant difference in the frequency of *APOE* ε4 allele between the amyloid-positive pre-dementia (19 of 32, 59.4%) and amyloid-positive dementia (10 of 16, 62.5%) ($P = 0.708$). In terms of the cognitive function tests, both the K-MMSE ($P < 0.001$) and CDR SOB scores ($P < 0.001$) showed the lowest performance in the amyloid-positive dementia group. Other demographic features, including sex distribution, frequency of hypertension and hyperlipidemia, and serum levels of BUN, Creatinine, and eGFR, did not show significant differences among the four groups.

3.2. Comparison of AD biomarkers in the cohort

The CSF (sTREM2, $A\beta_{42}$, $A\beta_{42}/A\beta_{40}$ ratio, pTau-181, total tau, and NfL) and plasma (sTREM2, $A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$ ratio, pTau-181, NfL, and telomere length) were compared among the four groups (Table 2). As expected, CSF $A\beta_{42}$ levels were significantly lower in the amyloid-positive dementia group ($P = 0.007$). CSF pTau-181 and total tau levels were significantly increased in the amyloid-positive dementia group ($P < 0.001$). In

the plasma, only pTau-181 levels were significantly different among the four groups ($P < 0.001$).

Differences in CSF and Plasma sTREM2 by cognitive status

There were no statistically significant differences in the sTREM2 levels among the four groups (P = 0.650 and P = 0.132, respectively). A tendency was observed of both the CSF and plasma sTREM2 levels to be higher for amyloid-positive and amyloid-negative in the dementia group (Figure 2).

3.3. Validity of imaging analysis and fluid biomarkers

The SUVR of all ROIs, including the deep nuclei (basal ganglia, caudate nucleus, putamen, and thalamus), were higher in the amyloid-positive group than in the amyloid-negative group (Table 3 and Figure 3). Global SUVR was significantly correlated with plasma p-Tau 181 levels ($\rho = 0.604$, $P < 0.001$). The CSF $A\beta_{42}$ ($\rho = -0.476$, $P < 0.001$) and p-Tau 181 ($\rho = 0.469$, $P < 0.001$) also correlated with the global SUVR (Figure 4). CSF and plasma NfL levels were positively correlated in the entire dataset ($\rho = 0.65$, $P < 0.001$) and amyloid-positive ($\rho = 0.56$, $P < 0.001$) and amyloid-negative ($\rho = 0.73$, $P < 0.001$) subgroups (Figure 5 and Supplementary 1).

3.4. Correlation of sTREM2 with conventional AD biomarkers

In the whole group (Figure 5A and supplementary 1A), the CSF sTREM2 positively correlated with CSF pTau-181 ($\rho = 0.25$, $P = 0.019$), total tau ($\rho = 0.44$, $P < 0.001$), plasma $A\beta_{42}$ ($\rho = 0.32$, $P = 0.003$) and $A\beta_{40}$ ($\rho = 0.28$, $P = 0.007$). The CSF sTREM2 and CSF $A\beta_{42}/A\beta_{40}$ ratio correlated negatively ($\rho = -0.22$, $P = 0.044$). The plasma sTREM2 negatively correlated with CSF pTau-181 ($\rho = -0.33$, $P = 0.002$) and total tau ($\rho = -0.26$, $P = 0.013$). In contrast, it positively correlated with plasma NfL levels ($\rho = 0.29$, $P = 0.007$). There was no significant correlation between the CSF and plasma sTREM2 ($\rho = -0.19$, $P = 0.07$).

In the AD continuum (Figure 5B and supplementary 1B), the CSF sTREM2 positively correlated with CSF total tau ($\rho = 0.51$, $P < 0.001$) and plasma $A\beta_{42}$ ($\rho = 0.35$, $P = 0.022$). The plasma sTREM2 was correlated with NfL positively only ($\rho = 0.33$, $P = 0.03$). There was no significant correlation between the CSF and plasma sTREM2 ($\rho = -0.16$, $P = 0.302$).

In the amyloid negative group (Figure 5C and supplementary 1C), the CSF sTREM2 positively correlated with CSF total tau ($\rho = 0.41$, $P = 0.006$), plasma $A\beta_{42}$ ($\rho = 0.33$, $P = 0.031$) and $A\beta_{40}$ ($\rho = 0.38$, $P = 0.012$). The plasma sTREM2 negatively correlated with telomere length ($\rho = -0.31$, $P = 0.041$). There was no significant correlation between the CSF and plasma sTREM2 ($\rho = -0.12$, $P = 0.448$).

3.5. Correlation among conventional AD biomarkers

In the whole group (Figure 5A and supplementary 1A), the CSF $A\beta_{42}$ positively correlated with CSF $A\beta_{42}/A\beta_{40}$ ratio ($\rho = 0.37$, $P < 0.001$) and negatively correlated with plasma pTau-181 ($\rho = -0.34$, $P = 0.001$). The CSF $A\beta_{42}/A\beta_{40}$ ratio negatively correlated with CSF pTau-181 ($\rho = -0.47$, $P < 0.001$), total tau ($\rho = -0.61$, $P < 0.001$), plasma $A\beta_{40}$ ($\rho = -0.25$, $P = 0.017$), pTau-181 ($\rho = -0.35$, $P = 0.001$) and neurogranin ($\rho = -0.23$, $P = 0.034$). CSF pTau-181 positively correlated with CSF total tau ($\rho = 0.82$, $P < 0.001$) and plasma pTau-181 ($\rho = 0.55$, $P < 0.001$). The CSF total tau positively correlated with plasma pTau-181 levels ($\rho = 0.45$, $P < 0.001$). Telomere length showed no significant correlation with any other AD fluid biomarkers.

In the AD continuum (Figure 5B and supplementary 1B), the CSF pTau-181 positively correlated with CSF $A\beta_{42}$ ($\rho = 0.32$, $P = 0.033$) and total tau ($\rho = 0.76$, $P < 0.001$). The CSF total tau positively correlated with plasma $A\beta_{42}$ ($\rho = 0.35$, $P = 0.02$) and negatively correlated with CSF $A\beta_{42}/A\beta_{40}$ ratio ($\rho = -0.36$, $P = 0.017$). The plasma $A\beta_{40}$ positively correlated with plasma neurogranin ($\rho = 0.35$, $P = 0.019$).

In the amyloid negative group (Figure 5C and supplementary 1C), The CSF $A\beta_{42}$ negatively correlated with plasma $A\beta_{40}$ ($\rho = -0.31$, $P = 0.041$). The CSF pTau-181 positively correlated with CSF $A\beta_{42}$ ($\rho = 0.59$, $P < 0.001$), total tau ($\rho = 0.76$, $P < 0.001$), and negatively correlated with CSF $A\beta_{42}/A\beta_{40}$ ratio ($\rho = -0.35$, $P = 0.021$). The CSF total tau positively correlated with CSF $A\beta_{42}$ ($\rho = 0.42$, $P = 0.004$). CSF NfL levels were positively correlated with plasma pTau-181 levels ($\rho = 0.33$, $P = 0.03$). The plasma pTau-181 levels were positively correlated with plasma NfL levels ($\rho = 0.43$, $P = 0.003$).

3.6. Prediction of amyloid positivity in the brain

ROC analysis revealed that plasma pTau-181 and CSF pTau-181 levels were significant predictors of amyloid deposition in the brain. Among the three biomarkers (CSF $A\beta_{42}/A\beta_{40}$ ratio, CSF and plasma pTau-181), plasma pTau-181 showed the highest AUC followed by CSF pTau-181 in prediction of amyloid deposition in the brain (cutoff value, 3.14 pg/ml, AUC = 0.861, $P < 0.001$; cutoff value, 44.753 pg/ml, AUC = 0.846, $P < 0.001$) (Supplementary 2). However, both CSF (cutoff value, 4388.49 pg/ml, AUC = 0.588) and plasma (cutoff value, 3485.84 pg/ml, AUC = 0.597) sTREM2 were not beneficial for predicting amyloid deposition in the brain compared to plasma pTau-181 (Figure 6).

Among all fluid biomarkers, the combination of CSF $A\beta_{42}$ (OR, 0.993; CI 0.988 – 0.998; $P = 0.007$), CSF pTau-181 (OR, 1.060; CI 1.023 - 1.099; $P = 0.0014$), and plasma pTau-181 (OR, 2.536; CI 1.358 – 4.736; $P = 0.004$) can accurately predict brain amyloid positivity (AUC = 0.909, $P = 0.703$ [Table 4A and Figure 7A]).

In the third model consisting of Sex, DM, HTN, HL, Age with plasma fluid biomarkers, the combination of plasma

sTREM2 (OR, 1.000; CI 1.000; $P = 0.100$), plasma pTau-181(OR, 2.40; CI 2.431 – 2.631; $P < 0.001$), *AOPE* ε4 carrier (OR, 4.59; CI 3.98 – 5.60; $P = 0.013$), and MMSE (OR, 0.883; CI 0.761 – 1.025; $P = 0.103$) could predict brain amyloid positivity accurately (AUC = 0.901, $P = 0.703$ [Table 4B and Figure 7B])

3.7. Regional amyloid burden and sTREM2 (plasma and CSF)

In the whole dataset (Figure 8 and supplementary 3), plasma sTREM2 negatively correlated with SUVR of lateral temporal ($\rho = -0.21$, $P = 0.038$), and basal ganglia ($\rho = -0.27$, $P = 0.008$). There was no significant correlation between the CSF sTREM2 levels and regional amyloid deposition (Figure 8A).

In the AD continuum, the plasma sTREM2 negatively correlated with SUVR of basal ganglia ($\rho = -0.35$, $P = 0.017$). There was no significant correlation between the CSF sTREM2 levels and regional amyloid deposition (Figure 8B).

In the amyloid-negative group, there was no significant correlation between sTREM2 levels and regional amyloid deposition (Figure 8C).

3.8. Prediction of Cognitive Decline in the AD continuum

In the whole dataset and the amyloid-negative group, there was no statistically significant variation in terms of changes in cognitive function. In the amyloid-positive group, plasma sTREM2 and $A\beta_{42}/A\beta_{40}$ ratio-remained after backward elimination (Table 5A). The results from the ROC analysis revealed that the combination of plasma sTREM2 and $A\beta_{42}/A\beta_{40}$ ratio (AUC = 0.761, $P < 0.001$) were not inferior to all plasma biomarkers with global SUVR and SVLT-delayed scores in predicting cognitive decline (AUC = 0.87, $P < 0.001$, DeLong's test P = 0.153) (Table 5B and Figure 9).

4. Discussion

In this study, five main findings emerged regarding the clinical implications of sTREM2 expression. First, the combination of plasma sTREM2 and $A\beta_{42}/A\beta_{40}$ ratio among plasma biomarkers could potentially identify rapidly progressive cognitive decliner in the AD continuum. This finding is supported by an association between plasma sTREM2 levels and amyloid deposition in the basal ganglia. Second, among the plasma biomarkers, the combination of plasma sTREM2, pTau-181, ApoE genotype, and baseline K-MMSE scores significantly predicted brain amyloid positivity. Third, both CSF and plasma sTREM2 levels were associated with tau (T) and neurodegenerative (N) biomarkers, rather than amyloid (A) biomarkers. Finally, there was no direct relationship

between the CSF and plasma sTREM2 levels.

Our findings suggest that both the CSF and plasma sTREM2 levels are associated with tau pathology and neurodegeneration, although their roles appear to be different. CSF sTREM2 originates from the cleavage of microglial cell surface proteins or alternative splicing of microglial mRNA; however, the exact origin of plasma sTREM2 is not yet fully understood[29,30]. In line with previous studies, the levels of sTREM2 in the CSF were associated with hyperphosphorylated tau and neurodegenerative markers, but not with amyloid[22,31]. CSF sTREM2 levels were assumed to reflect a corresponding change in microglial activation status[32]. The positive correlation between hyperphosphorylated and total tau in the CSF suggests that sTREM2 plays an important role in the development of AD pathology and neurodegeneration[19,21,22]. The increase in CSF sTREM2 levels represents the initial process of barrier function through microglial clustering around the amyloid plaques[12]. Failure of these processes can lead to the propagation of amyloid plaques and formation of neurofibrillary tangles[33]. Unlike CSF sTREM2, plasma sTREM2 levels were negatively associated with hyperphosphorylated tau and total tau levels. The reason why plasma and CSF sTREM2 levels show inverse correlation patterns has not been fully elucidated. However, several hypotheses have been proposed in previous studies. CSF sTREM2 levels are directly influenced by microglial activation in the central nervous system (CNS)[34,35]. In neurodegenerative diseases, homeostatic microglia become disease-associated microglia (DAM)[36,37]. DAM activation is divided into two stages: stage 1 is unrelated to TREM2, whereas stage 2 is related to TREM2 activation[38]. Stage 2 DAM is associated with neuronal injury as a downstream factor of amyloid deposition, which is temporally associated with the development and increase in hyperphosphorylated tau[39]. Unlike CSF sTREM2, the highest plasma sTREM2 concentration was observed during the early stages of neuroinflammation[29,39]. Furthermore, plasma sTREM2 levels may reflect altered peripheral pro-inflammatory cytokines in the early stages of AD pathogenesis, even before amyloid deposition in AD pathogenesis[20,40]. Plasma sTREM2 expression decreases when the neuroinflammatory process becomes chronic in overt AD[29]. These findings suggest that elevated plasma sTREM2 levels in the early stages of AD pathogenesis may be related to neuroinflammatory processes. However, controversial data exist regarding TREM2 expression in circulating cells and further research is warranted to confirm the role of plasma sTREM2 in the AD continuum[29,41,42].

With the increasing possibility of prescribing anti-amyloid therapies, there is a growing need for a plasma biomarker model that can predict brain amyloid deposition and cognitive status. In particular, plasma hyperphosphorylated tau species, such as plasma pTau-181, 217, and 231, have been confirmed to predict amyloid deposition in the brain well[43,44]. The results of our study confirmed that plasma pTau-181 levels could also predict amyloid deposition in the brains of the Korean population. We further conducted a logistic regression analysis to identify a model that could predict amyloid deposition in the brain using plasma biomarkers and cognitive function tests. After backward elimination, plasma sTREM2, pTau-181, and ApoE genotype tests and baseline MMSE scores were included in the model. The AUC value was 0.907, suggesting that amyloid deposition in the brain on the AD continuum could be predicted only by plasma biomarkers without expensive imaging studies or invasive

tests such as spinal puncture. However, when the ROC analysis was performed using CSF and plasma sTREM2 values to predict amyloid deposition, the ACU values were 0.588 and 0.597, respectively. These findings indicate that sTREM2, neither CSF nor plasma, is not related to amyloid deposition in the AD continuum[22,24].

Our major finding was that plasma sTREM2 could be a prognostic biomarker for predicting rapid cognitive decline in the AD continuum. After the backward elimination, the plasma sTREM2 and $A\beta_{42}/A\beta_{40}$ ratio remained in the final prediction model. Many studies predicting a faster decline in the AD continuum have been conducted using imaging biomarkers such as amyloid burden, hippocampal atrophy, and glucose hypometabolism[45,46]. However, little research has been conducted to determine whether rapid cognitive decline can be predicted using a combination of plasma biomarkers in amyloid-positive subjects. The reason why plasma sTREM2 levels are associated with more severe cognitive decline has not been extensively investigated[42]. In the present study, we found that plasma sTREM2 levels were associated with CSF pTau-181, total tau, and the quantitative amount of amyloid deposition in the basal ganglia. These findings suggest faster cognitive decline in these patient groups. Compared with the amyloid-negative subject groups, amyloid deposition in the basal ganglia was negatively correlated with plasma sTREM2 levels. These results suggested that low plasma sTREM2 levels in the AD continuum reflect striatal amyloid deposition. Several studies have suggested that subcortical involvement results in a greater cortical amyloid burden, hypometabolism and hippocampal atrophy[47,48]. Conventionally, CSF sTREM2 has been regarded as a reflecting tau biomarker rather than an amyloid biomarker, and our findings are consistent with those of previous studies. The association between plasma sTREM2 levels and amyloid deposition in the basal ganglia may reflect the physiological washout processes. The association between plasma sTREM2 levels and amyloid deposition in the basal ganglia may reflect the physiological washout processes. Peak levels of plasma sTREM2 were reached in the early stage of AD pathogenesis which then gradually decreased as the disease progressed[42]. This dynamic change in plasma sTREM2 levels may be associated with cognitive changes in the AD continuum[40,42]. Therefore, our results suggest that plasma sTREM2 levels reflect cognitive decline through several processes. However, the role of plasma sTREM2 levels depending on race and medical condition is still debated, and follow-up confirmatory studies are warranted.

The present study has three major strengths. First, to identify the role of sTREM2 in neurodegenerative disorders, we conducted a study using a well-constructed cohort comprising CSF, plasma, detailed cognitive function tests, and imaging data. Therefore, the clinical role and implications of sTREM2 in the pathogenesis of AD have been explored using various approaches. Second, conducting a follow-up observation, including a cognitive function test after 1 year, allowed us to build a model that could predict changes in cognitive status. Finally, only a small number of patients with diabetes were included in the AD continuum. In this study, we investigated several inflammatory biomarkers associated with AD in the CSF and plasma. AD is closely related with diabetes[49]. According to previous studies, the chronic inflammatory status represented by diabetes may affect the levels of inflammatory biomarkers[50]. Under chronic hyperglycemic conditions, plasma sTREM2 levels gradually decrease as the cognitive status worsens; however, this phenomenon disappears in non-diabetic subjects[51,52]. Therefore, the

results of our study may reflect the characteristics of the neuroinflammatory processes in patients with pure AD without diabetes. However, this study has some limitations. First, it was designed as an exploratory rather than a confirmatory study. Secondly, the younger mean age of the AD dementia group (amyloid-positive dementia) implied that patients with early onset AD were included in the study. Therefore, there is some limitation to considering that our result represents the characteristics of sporadic late-onset AD (LOAD). Third, there may have been heterogeneity in the diagnosis of the amyloid-negative groups because of insufficient follow-up periods. Fourth, the number of subjects corresponding to the AD continuum and subjective cognitive decline (SCD) was relatively small. Finally, we used AI programs with a deep learning-based SN method to calculate the SUVR because not all subjects underwent 3DT1 MRI scans. Therefore, amyloid positivity was determined by visual rating.

Perspective

Nevertheless, the potential use of sTREM2 as an AD biomarker is noteworthy. Our findings suggest that CSF and plasma sTREM2 are activated at different stages of AD pathogenesis, and are potential biomarkers for predicting the prognosis of patients with AD. These biomarkers could play a clinical role in screening patients for anti-amyloid therapy.

Our future studies will focus on the following aspects. First, to confirm temporal changes in sTREM2 with the progression of Alzheimer's disease and establish a model to predict fast cognitive decline. Second, genetic confirmatory studies, such as GWAS or single-nucleotide polymorphism analyses could be conducted. These confirmatory genetic studies provide detailed information on the activity and role of neuroinflammation in AD pathogenesis. Third, a comparison with other races or datasets, such as the Alzheimer's Disease and Neuroimaging Initiative (ADNI) is warranted. Finally, the relationship between other neuroinflammatory biomarkers such as glial fibrillary acidic protein (GFAP) and YKL40 (chitinase 3-like 1) needs to be further evaluated.

Conclusion

The CSF sTREM2, in line with previous studies, was related to tau biomarkers rather than amyloids. Plasma sTREM2 is a prognostic marker for predicting cognitive decline and reflects amyloid deposition in the basal ganglia in the AD continuum. In clinical practice, these findings might facilitate biomarker-supported diagnosis and prediction of fast decliners in patients with AD.

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TABLE

Table 1. Demographics of this study

	amyloid-negative		amyloid-positive		
	Pre-dementia (N=31)	Dementia (N=17)	Pre-dementia (N=32)	dementia (N=16)	P
Age (Y)	71.8 ± 9.0	71.5 ± 7.7	68.4 ± 10.0	63.0 ± 9.9	0.009*
Duration (M)	25.4 ± 19.8	34.6 ± 39.5	31.6 ± 27.6	43.3 ± 25.6	0.039*
Education (Y)	12.1 ± 4.9	8.4 ± 5.6	10.6 ± 5.4	10.7 ± 5.0	0.190
Sex					
female	14 (45.2%)	9 (52.9%)	19 (59.4%)	10 (62.5%)	0.611
male	17 (54.8%)	8 (47.1%)	13 (40.6%)	6 (37.5%)	
Hypertension					
NO	18 (58.1%)	8 (47.1%)	17 (53.1%)	7 (43.8%)	0.783
YES	13 (41.9%)	9 (52.9%)	15 (46.9%)	9 (56.2%)	
Diabetes					
NO	20 (64.5%)	6 (35.3%)	26 (81.2%)	13 (81.2%)	0.006*
YES	11 (35.5%)	11 (64.7%)	6 (18.8%)	3 (18.8%)	
Hyperlipidemia					
NO	20 (64.5%)	10 (58.8%)	13 (40.6%)	9 (56.2%)	0.275
YES	11 (35.5%)	7 (41.2%)	19 (59.4%)	7 (43.8%)	
LDL (mg/dl)	112.06 ± 25.03	88.63 ± 41.00	121.94 ± 37.70	140.90 ± 10.02	0.039*
BUN (mg/dl)	16.97 ± 6.795	13.94 ± 3.81	15.54 ± 2.94	16.71 ± 4.62	0.213
Creatinine (mg/dl)	0.91 ± 0.31	0.79 ± 0.18	0.84 ± 0.14	0.87 ± 0.25	0.354
eGFR	78.45 ± 15.84	84.24 ± 12.46	82.44 ± 14.19	80.13 ± 17.47	0.571
APOE genotype					0.001*
non-carrier	24 (77.4%)	14 (82.4%)	13 (40.6%)	6 (37.5%)	

carrier	7 (22.6%)	3 (17.6%)	19 (59.4%)	10 (62.5%)	
K-MMSE (base)	26.3 ± 2.4	19.2 ± 4.6	24.4 ± 3.5	18.5 ± 4.5	< 0.001*
CDR SOB (base)	1.4 ± 1.1	7.0 ± 3.7	2.0 ± 1.2	6.1 ± 3.1	< 0.001*

Demographics of this study. To compare the group differences, Kruskall-Wallis test and chi-square test were conducted.

Abbreviations: LDL, low density lipoprotein; BUN, blood urea nitrogen; eGFR, estimated glomerular filteration rate; K-MMSE, Korean version of mini-mental status examination; CDR SOB, clinical dementia rating scale sum of boxed scores.

* Significant at the level of $P < 0.05$

Table 2. Comparison of fluid biomarkers among four groups

		amyloid-negative		amyloid-positive		
		Pre-dementia (N=31)	Dementia (N=17)	Pre-dementia (N=32)	Dementia (N=16)	P
CSF						
	sTREM2	5868.2 ± 3689.0	6897.8 ± 5224.6	6443.2 ± 3258.9	7278.9 ± 3746.7	0.650
	$A\beta_{42}$	419.4 ± 219.4	410.2 ± 266.1	284.2 ± 124.3	274.4 ± 77.4	0.007*
	$A\beta_{42}/A\beta_{40}$	0.97 ± 0.06	0.12 ± 0.07	0.09 ± 0.18	0.05 ± 0.02	0.364
	pTau-181	40.6 ± 30.4	32.9 ± 19.8	64.0 ± 24.5	72.8 ± 28.9	< 0.001*
	Total tau	276.78 ± 212.23	209.18 ± 221.05	566.55 ± 338.41	628.69 ± 452.44	< 0.001*
	NfL	1871.2 ± 2330.9	1512.9 ± 877.4	1012.0 ± 736.4	1455.4 ± 1253.7	0.180
Plasma						
	sTREM2	5278.8 ± 2884.0	6953.3 ± 5223.0	4313.8 ± 3057.5	5553.2 ± 4320.3	0.132

$A\beta_{42}$	8.03 ± 2.65	7.21 ± 2.24	7.38 ± 1.97	7.82 ± 2.60	0.603
$A\beta_{42}/A\beta_{40}$	0.04 ±0.01	0.04 ±0.02	0.04 ±0.01	0.04 ±0.01	0.768
pTau-181	2.35 ± 1.25	2.19 ± 1.00	4.14 ± 1.73	5.26 ±1.69	<0.001*
Neurogranin	2381.4 ± 1762.3	1697.1 ± 797.6	2812.3 ± 1763.8	1855.8 ± 860.0	0.066
NfL	37.4 ± 35.4	39.9 ± 22.1	26.2 ± 10.7	33.6 ± 27.0	0.229
Telomere length	7.5 ± 1.9	7.4 ± 1.5	7.5 ± 1.6	7.6 ± 1.2	0.916

Comparison of AD biomarkers in the cohort. To compare among group differences, we conducted ANOVA test.

Abbreviations: NfL, neurofilament light chain

* Significant at the level of $P < 0.05$

Table 3. The comparison of all SUVR between the two groups.

Region of interest (ROI)	negative (N=47)	Positive (N=49)	p
Global	1.0 ± 0.1	1.3 ± 0.2	< 0.001
Frontal	1.0 ± 0.1	1.4 ± 0.2	< 0.001
Posterior cingulate pre-cuneus	1.0 ± 0.1	1.5 ± 0.2	< 0.001
Temporal	0.9 ± 0.1	1.3 ± 0.2	< 0.001
Parietal	1.0 ± 0.1	1.3 ± 0.2	< 0.001
Hippocampus	0.8 ± 0.1	1.0 ± 0.1	< 0.001
Basal ganglia	1.0 ± 0.1	1.3 ± 0.1	< 0.001
Caudate	0.7 ± 0.1	1.0 ± 0.2	< 0.001
Putamen	1.1 ± 0.1	1.5 ± 0.2	< 0.001
Thalamus	1.0 ± 0.1	1.2 ± 0.1	< 0.001

Group comparison of SUVR between visually amyloid positive and negative groups. Age adjusted ANCOVA was conducted.

Table 4. Models for amyloid prediction

A. All fluid biomarkers

		CI		
	Exp(B)	lower	upper	P
CSF $A\beta_{42}$	0.993	0.988	0.998	0.007
CSF pTau-181	1.060	1.023	1.099	0.0014
Plasma pTau-181	2.536	1.358	4.736	0.004

APOE genotype, CSF (sTREM2, $A\beta_{42}$, pTau-181, total Tau, and NfL), and plasma (sTREM2, $A\beta_{42}/A\beta_{40}$ ratio, pTau-181, Neurogranin, and telomere length) biomarkers were included in logistic regression analysis model. The backward elimination was performed.

Abbreviations: CI, confidence intervals

B. Plasma biomarkers and demographic factors

		CI		
	Exp(B)	lower	upper	P
Plasma sTREM2	1.000	1.000	1.000	0.100
Plasma pTau-181	2.40	2.43	2.63	<0.001
APOE ε4 carrier	4.59	3.98	5.60	0.013
MMSE	0.883	0.761	1.025	0.103

APOE genotype, Diabetes, Hypertension, Hyperlipidemia, Age, Sex, plasma (sTREM2, $A\beta_{42}/A\beta_{40}$ ratio, pTau-181, Neurogranin, and telomere length) biomarkers and baseline MMSE scores were included in logistic regression analysis model. The backward elimination was performed.

Table 5. Prediction of fast cognitive decline in AD continuum

A. Models for prediction of fast cognitive decline in AD continuum

		CI		
	Exp(B)	lower	upper	P
Plasma sTREM2	1.00	1.000	1.000	0.077
Plasma $A\beta_{42}/A\beta_{40}$ ratio	1.060	0.449	226511.2	0.081

plasma (sTREM2, $A\beta_{42}/A\beta_{40}$ ratio, pTau-181, Neurogranin, and telomere length) biomarkers, SVLT-delayed recall scores, and global SUVR were included in logistic regression analysis model. The backward elimination was performed.

B. Comparison between prediction models.

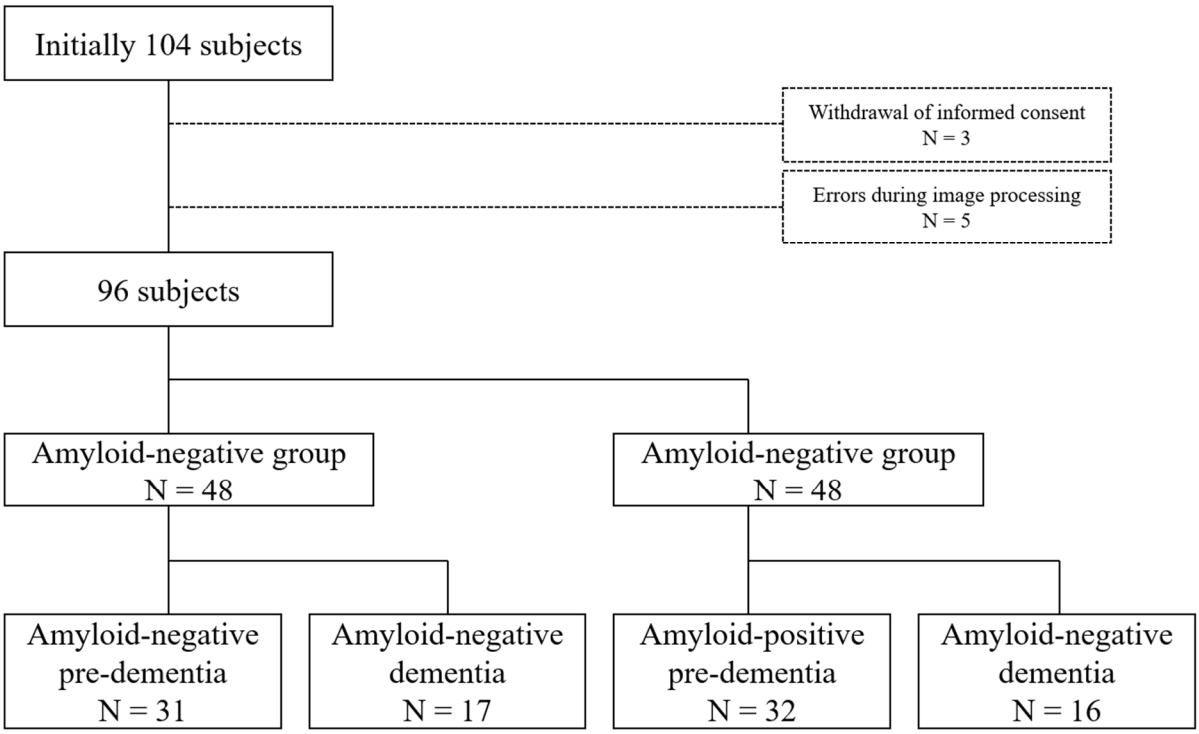
	AUC	sensitivity	specificity	P	DeLong's test P value
Model 1 plasma sTREM2 and plasma Aβ42/Aβ40 ratio	0.764 (0.603 - 0.925)	100	55.6	< 0.001	
Model 2 (All variations) plasma sTREM2, pTau-181, Aβ42/Aβ40 ratio, neurogranin, NfL, telomere length, SVLT-Delayed recall score, and global SUVR	0.87 (0.75 - 0.991)	100	66.7	< 0.001	0.153

The results from the ROC curve analysis revealed that the combination of plasma sTREM2 and $A\beta_{42}/A\beta_{40}$ ratio were not inferior to all plasma biomarkers with global SUVR and SVLT-delayed scores in predicting cognitive decline.

Abbreviations: ACU, area under curve; NfL, neurofilament light chain

FIGURE LEGENDS

Figure 1. Flow chart of this study



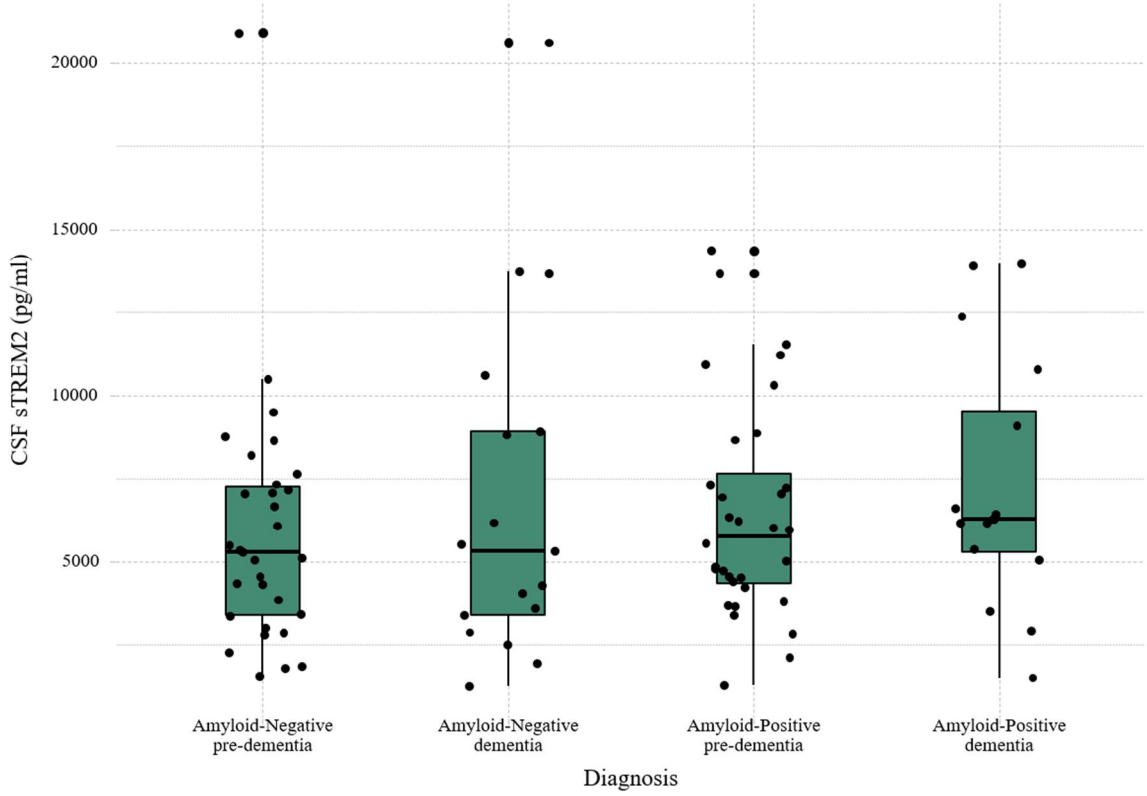
Flow chart of this study from initial screening to final analysis.

The solid outline squares represent the subjects that remained. The dash line squares represent

the excluded subjects.

Figure 2. Difference of CSF (A) and plasma (B) sTREM2 among four groups.

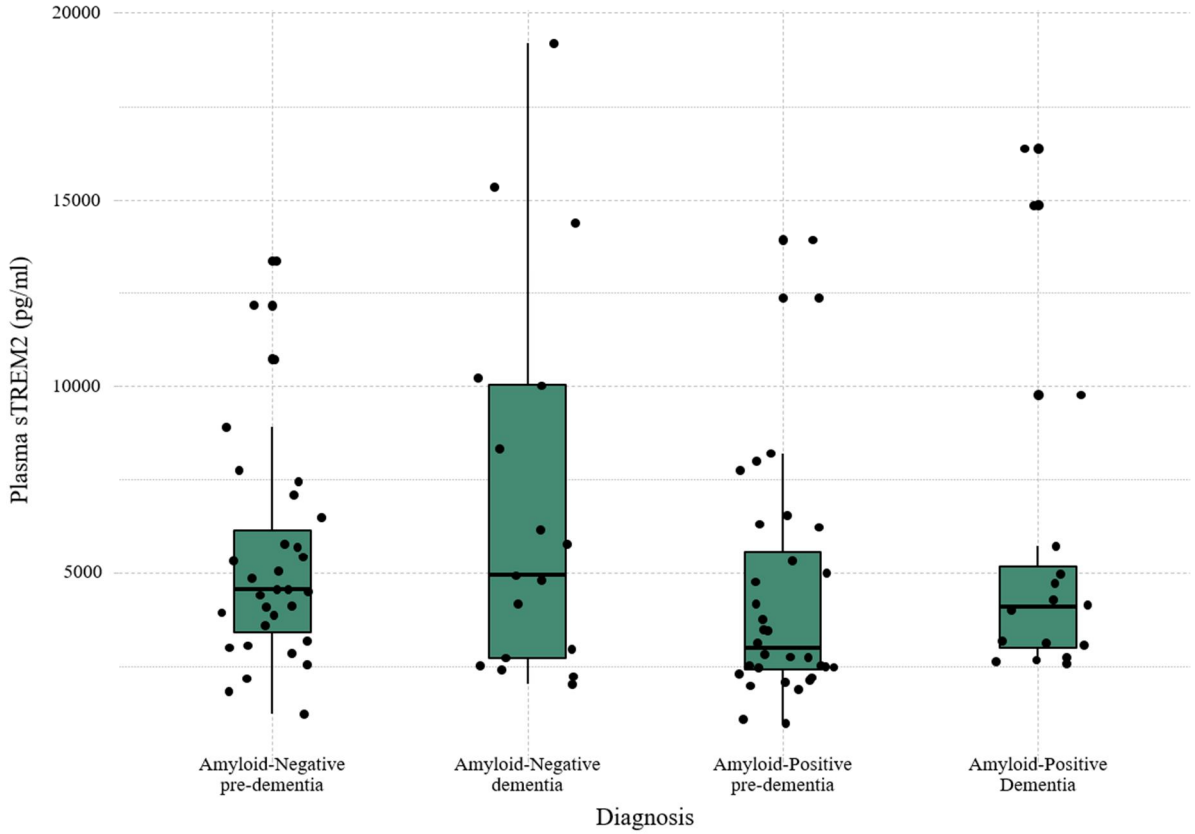
Figure 2A. Differences in CSF sTREM2 by cognitive status



Kruskal-Wallis test was performed to evaluate the difference of CSF among four groups.

CSF, Cerebrospinal fluid; sTREM2, soluble triggering receptor expressed on myeloid cell 2

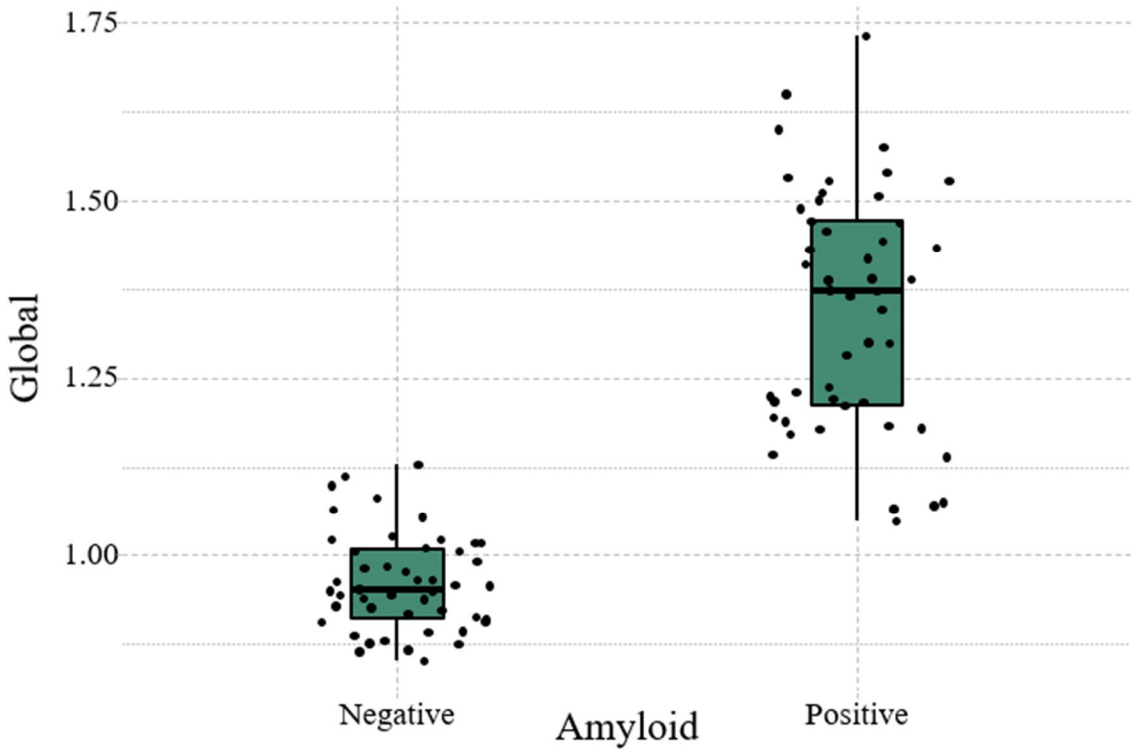
Figure 2B. Differences in plasma sTREM2 by cognitive status



Kruskal-Wallis test was performed to evaluate the difference of plasma among four groups.

sTREM2, soluble triggering receptor expressed on myeloid cell 2

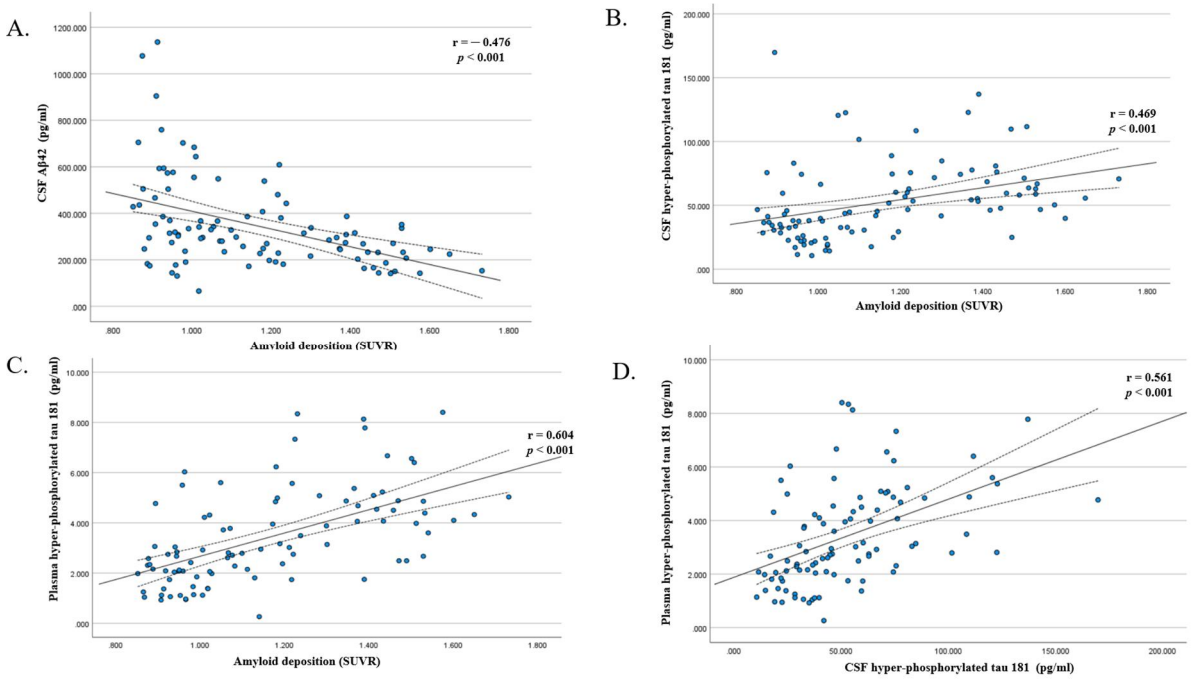
Figure 3. The comparison of global SUVR between two groups.



Group comparison of SUVR between visually amyloid positive and negative group. Age adjusted ANCOVA test was conducted.

SUVR, standardized uptake value ratio; ANCOVA, analysis of covariance

Figure 4. Relationship between conventional AD biomarkers.



The spearman's correlation analysis was conducted to confirm validity of imaging analysis and fluid biomarkers.

(A) Correlation between CSF amyloid beta 42 and global amyloid deposition.

(B) Correlation between CSF pTau-181 and global amyloid deposition

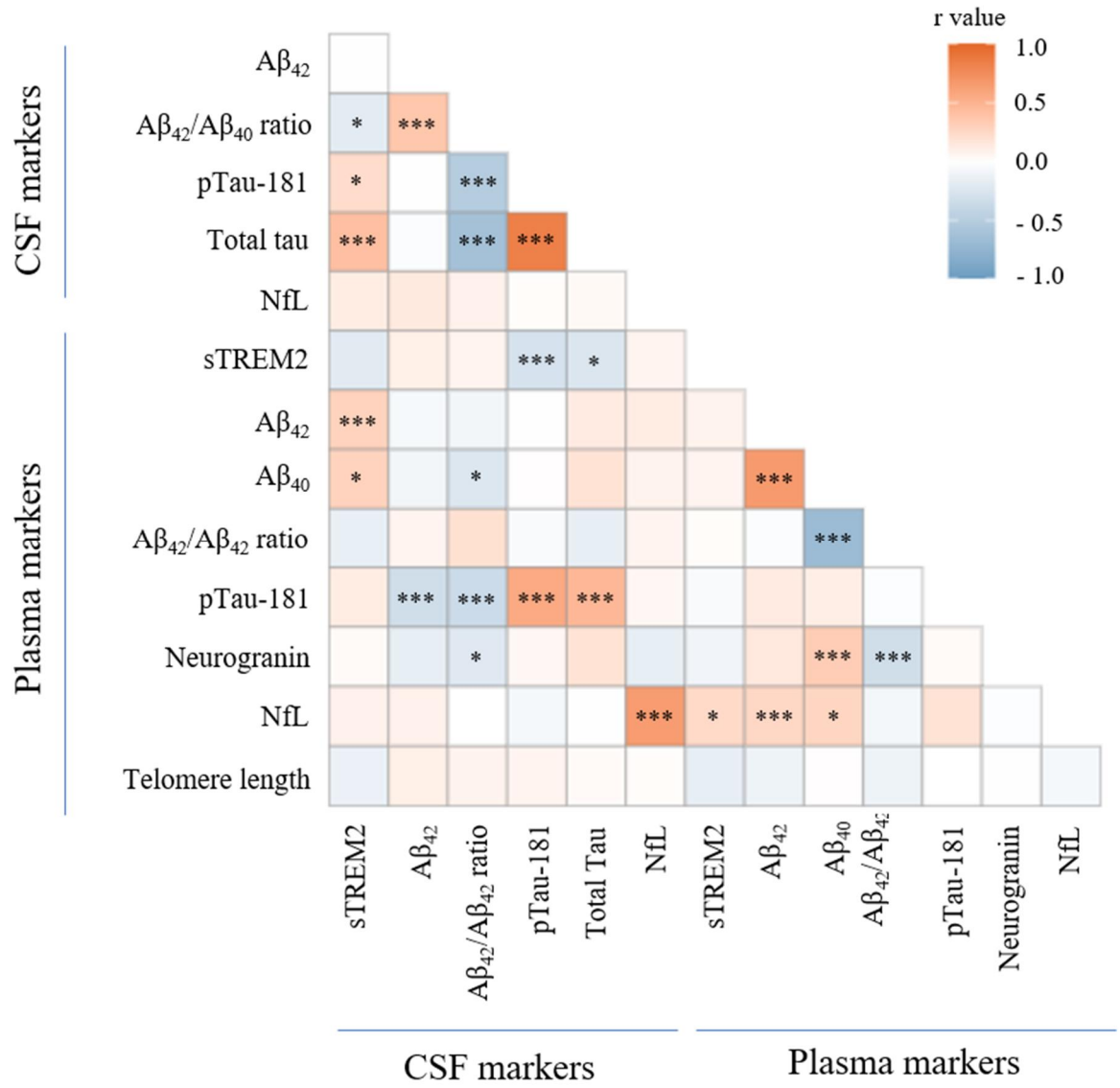
(C) Correlation between plasma pTau-181 and global amyloid deposition.

(D) Correlation between CSF and plasma pTau-181.

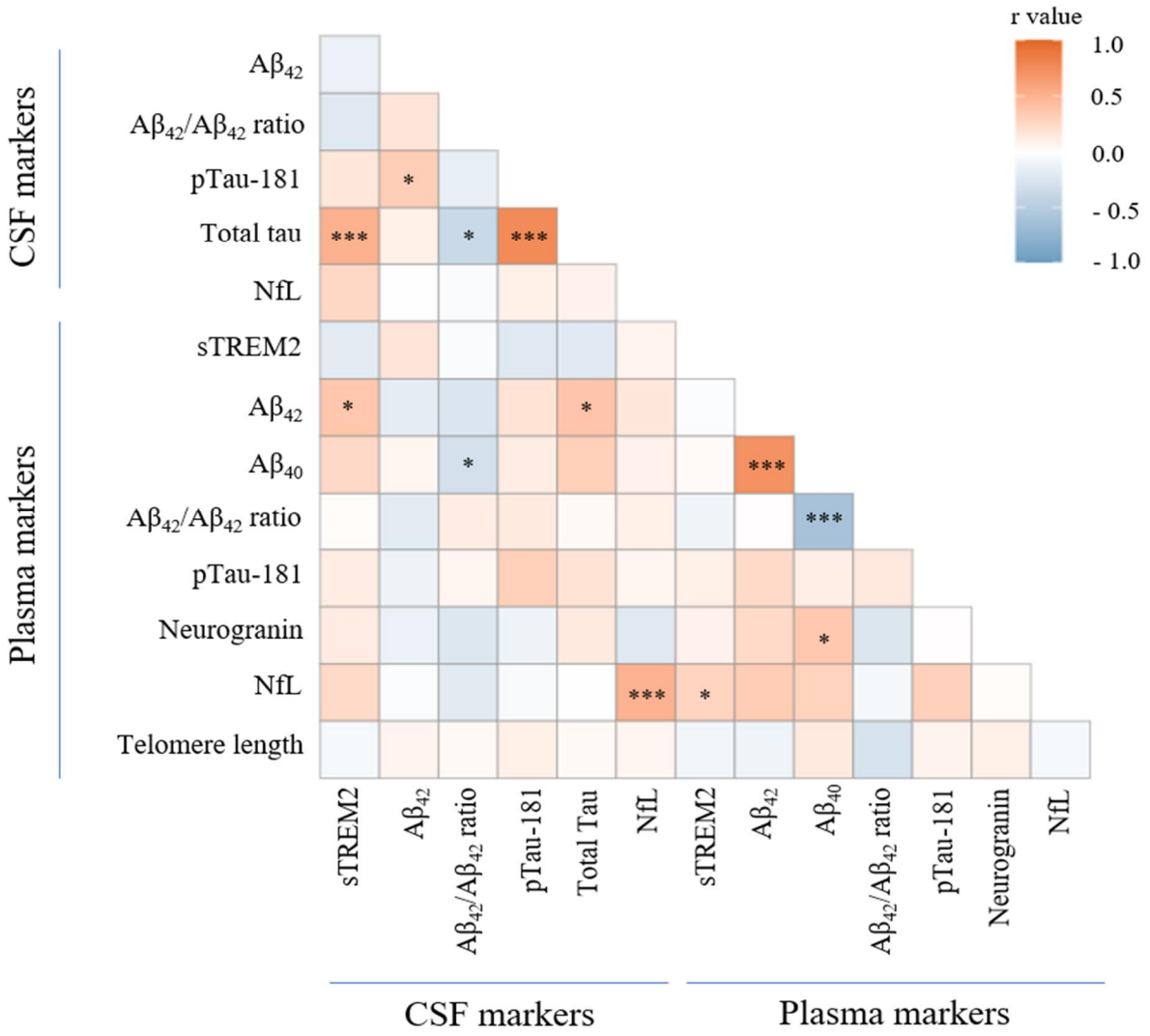
AD, Alzheimer's disease; SUVR, standardized uptake value ratio.

Figure 5. Correlation with sTREM2 and conventional AD biomarkers.

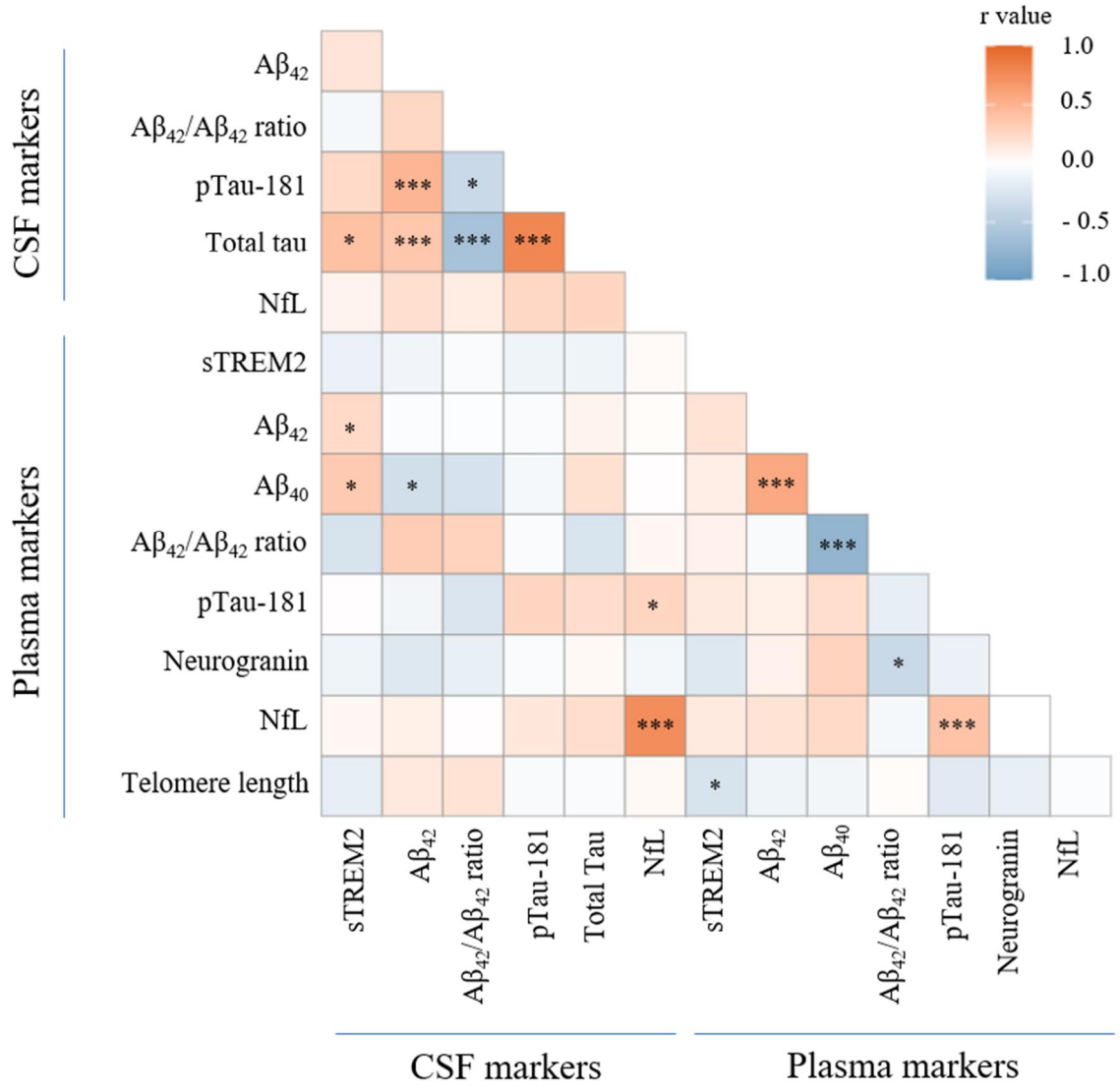
A. Whole dataset (N = 96)



B. Alzheimer's disease continuum (N = 48)



C. Suspected non-Alzheimer's pathologic change (N = 48)

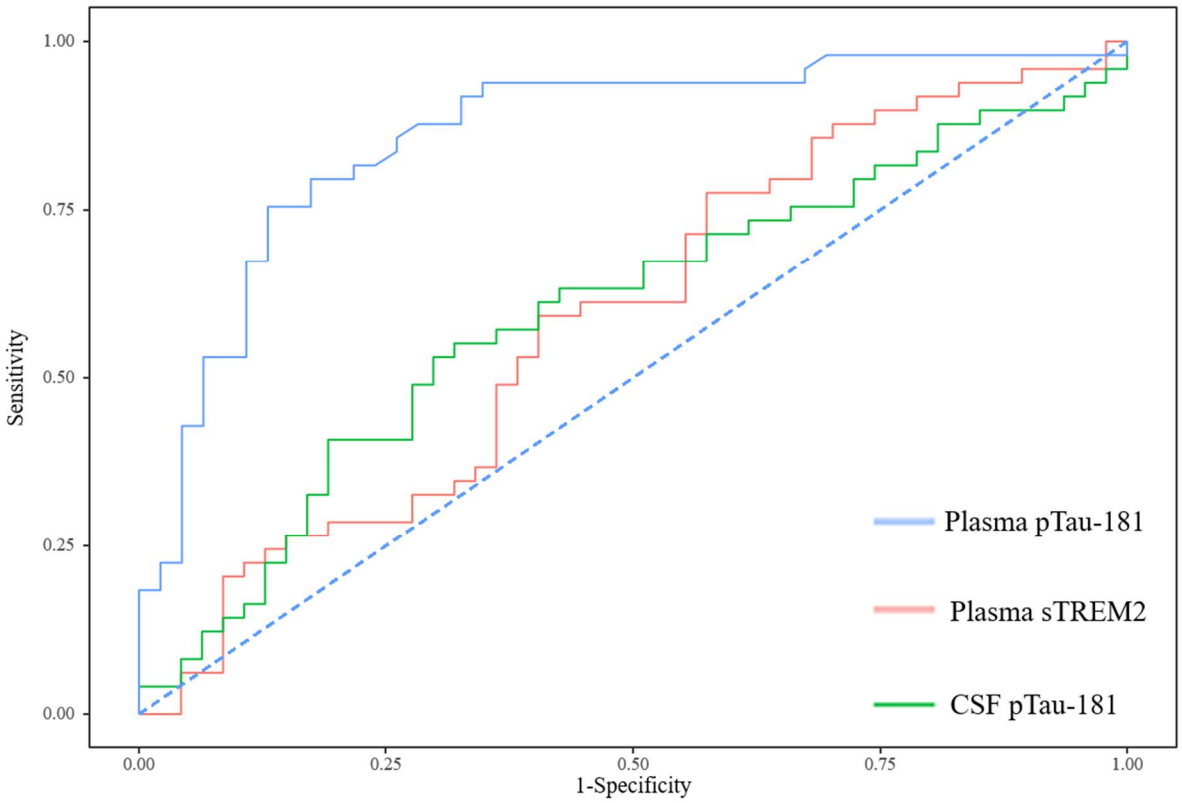


Spearman's correlation analysis was conducted.

AD, Alzheimer's disease; sTREM2, soluble triggering receptor expressed on myeloid cell 2;

CSF, cerebrospinal fluid; NfL, neurofilament light-chain

Figure 6. Prediction of amyloid positivity using sTREM2.

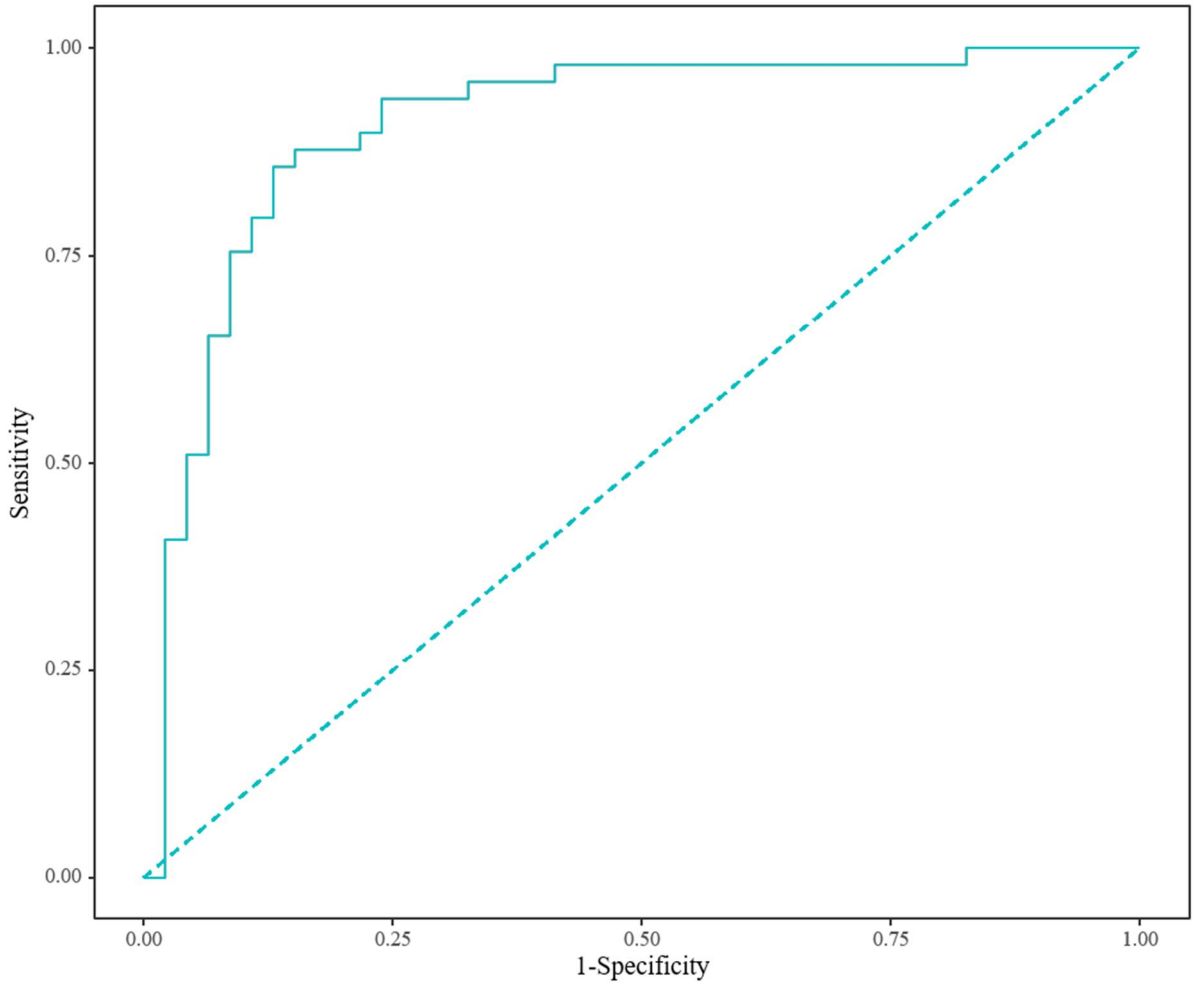


Prediction of amyloid positivity using sTREM2. There are no benefit of plasma and CSF sTREM2 compared to plasma hyperphosphorylated tau 181 in prediction of brain amyloid deposition. ROC comparison was conducted to compare the power of prediction in brain amyloid deposition.

ROC, receiver operating characteristic; sTREM2, soluble triggering receptor expressed on myeloid cell 2; CSF, cerebrospinal fluid

Figure 7. Prediction of amyloid deposition in the brain.

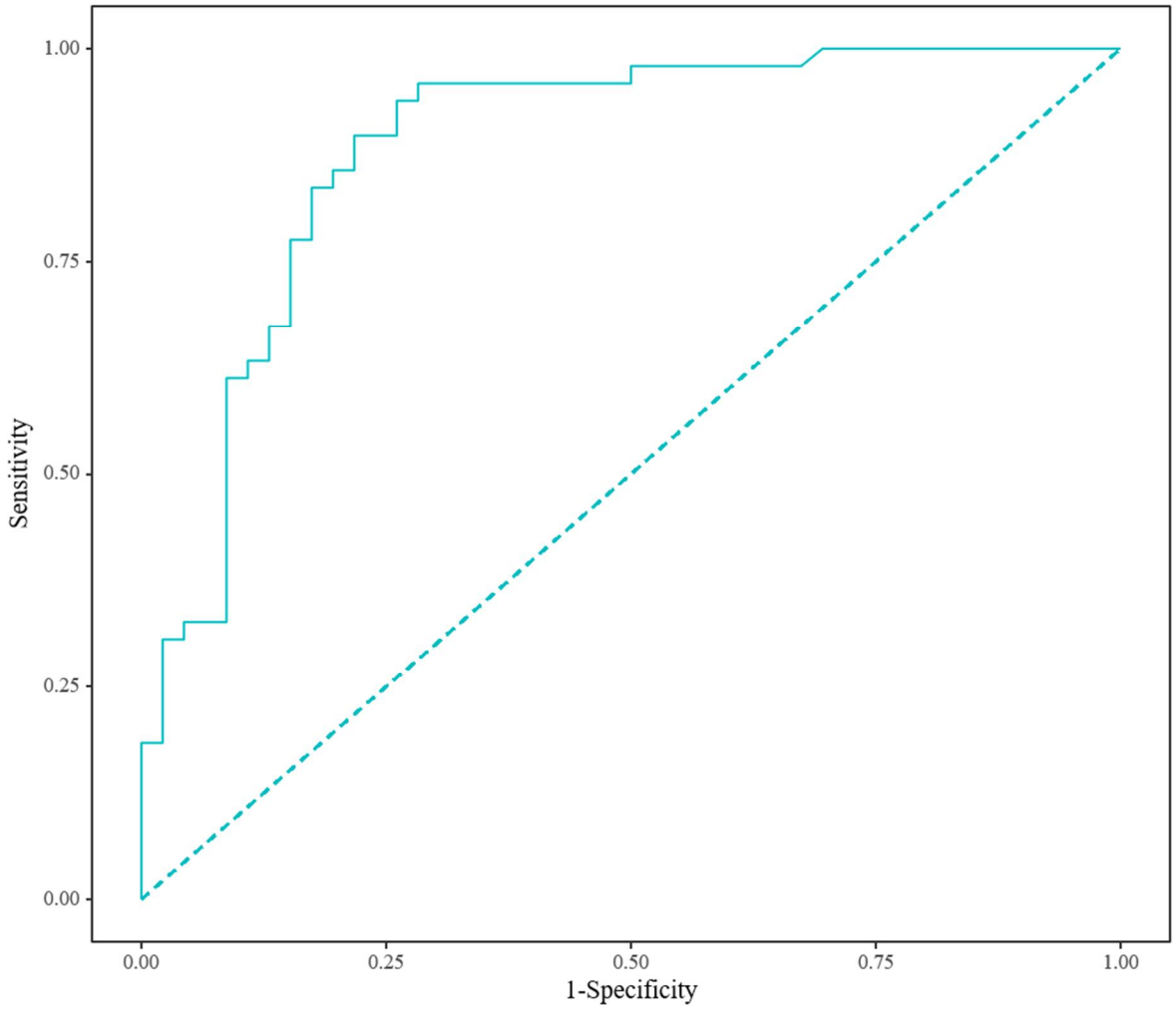
Figure 7A. ROC curves for all fluid biomarkers.



Model I. Combination of all fluid biomarkers, combined of CSF $A\beta_{42}$ (OR, 0.993; CI 0.988 – 0.998; P = 0.007), CSF pTau-181 (OR, 1.060; CI 1.023 - 1.099; P = 0.0014), and plasma pTau-181 (OR, 2.536; CI 1.358 – 4.736; P = 0.004) can predict brain amyloid positivity properly (AUC = 0.909, P = 0.703).

ROC, receiver operating curve; AUC, area under curve; CSF, cerebrospinal fluid.

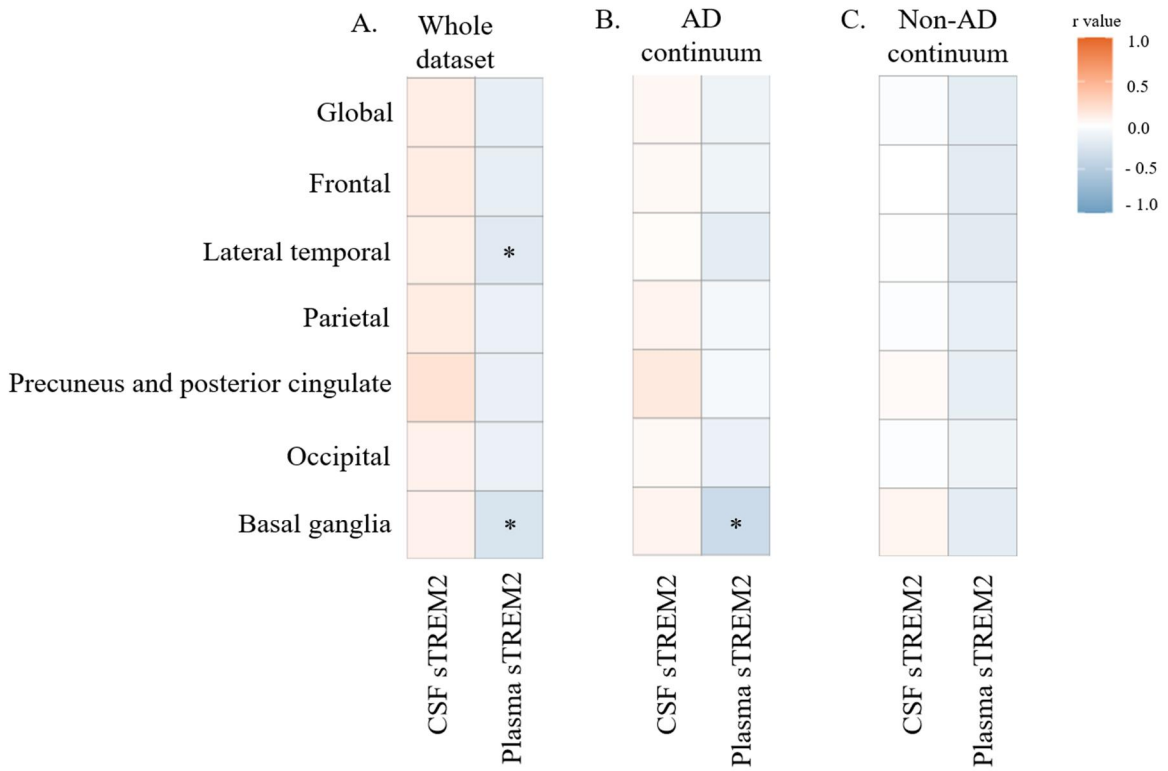
Figure 7B. ROC curves for plasma biomarkers and demographic factors.



Model II. Combination of demographic and plasma biomarkers, combination of plasma sTREM2 (OR, 1.000; CI 1.000; P = 0.100), plasma pTau-181(OR, 2.40; CI 2.431 – 2.631; P < 0.001), *AOPE* ε4 carrier (OR, 4.59; CI 3.98 – 5.60; P = 0.013), and MMSE (OR, 0.883; CI 0.761 – 1.025; P = 0.103) could predict brain amyloid positivity properly (AUC = 0.901, P = 0.703).

ROC, receiver operating curve; AUC, area under curve; CSF, cerebrospinal fluid; MMSE, mini-mental status examination

Figure 8. Correlation between regional amyloid burden and sTREM2.



Correlation between regional amyloid burden and sTREM2.

Spearman's correlation analysis was conducted.

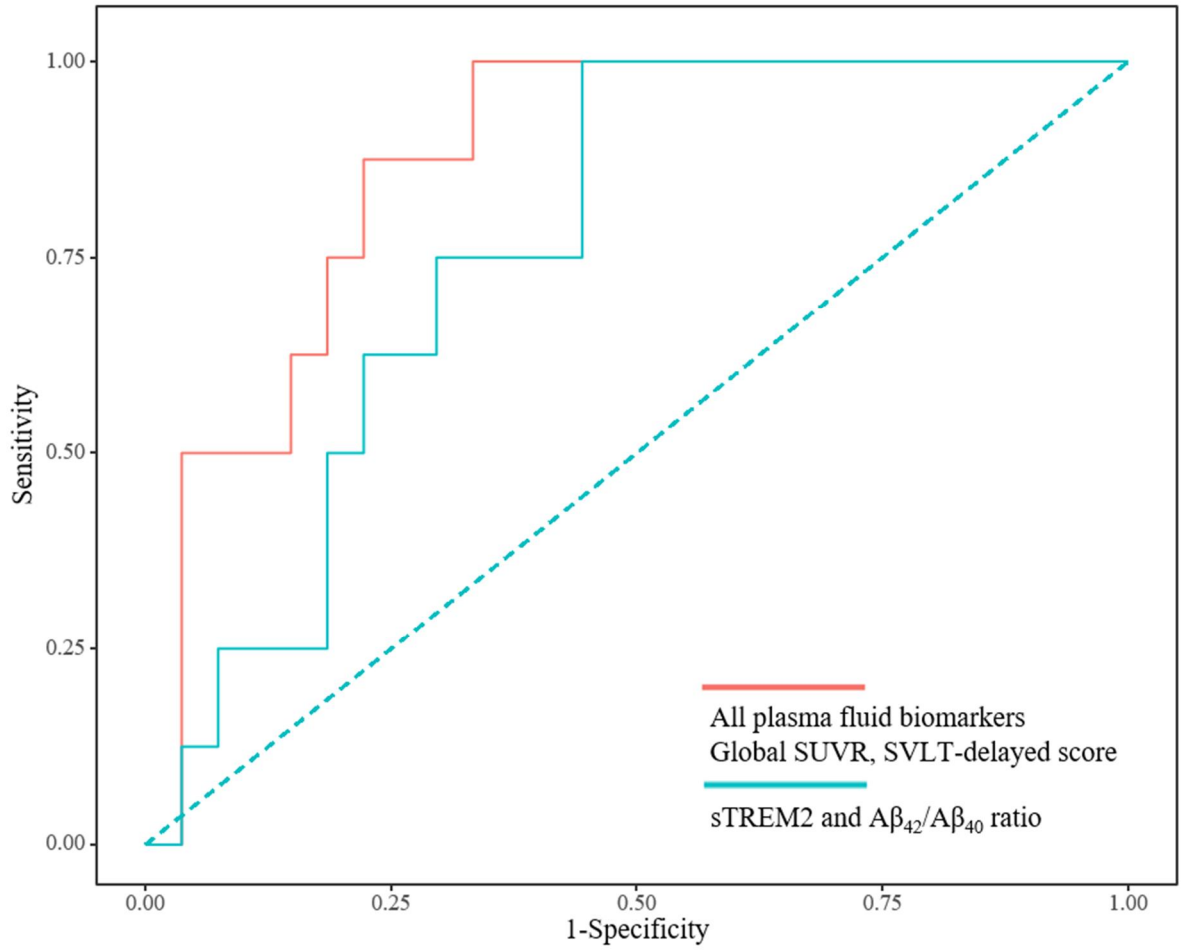
(A) Whole dataset

(B) AD continuum

(C) Suspected non-AD pathologic change

sTREM2, soluble triggering receptor expressed on myeloid cell 2; CSF, cerebrospinal fluid.

Figure 9. Models of cognitive decline in AD continuum.



Plasma sTREM2, $A\beta_{42}/A\beta_{40}$ ratio, pTau181, Neurogranin, NfL, telomere length, Global SUVR, SVLT-Delayed recall score were included in initial model. After back ward elimination, plasma sTREM2 and $A\beta_{42}/A\beta_{40}$ ratio were remained. The results from the ROC analysis revealed that the combination of plasma sTREM2 and $A\beta_{42}/A\beta_{40}$ ratio (AUC = 0.761, $P < 0.001$, red line) were not inferior to all plasma biomarkers with global SUVR and SVLT-delayed scores in predicting cognitive decline (AUC = 0.87, $P < 0.001$, DeLong's test $P = 0.153$, blue line).

AD, Alzheimer's disease; NfL, neurofilament light-chain, SUVR, standardized uptake value ratio; SVLT, Seoul verbal learning test; ROC, AUC, area under curve

Supplementary Appendix

This appendix was created by the authors to provide readers with additional information regarding their work.

Supplementary 1. The results of correlation analysis between sTREM2 and conventional AD biomarkers.

Supplementary 2. Prediction of amyloid positivity using conventional AD biomarkers.

Supplementary 3. The results of correlation analysis between sTREM2 and regional amyloid deposition

A. Whole dataset

Spearma's raw		CSF						Plasma							
		sTREM2	$A\beta_{42}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Total tau	NfL	sTREM2	$A\beta_{42}$	$A\beta_{40}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Neurogranin	NfL	Telomere
CSF	sTREM2	1													
	$A\beta_{42}$	0.02	1												
	$A\beta_{42}/A\beta_{40}$	-0.22*	0.37***	1											
	pTau-181	0.25*	-0.01	-0.47***	1										
	Total tau	0.44***	-0.02	-0.61***	0.83***	1									
	NfL	0.11	0.14	0.02	0.08	0.09	1								
Plasma	sTREM2	-0.19	0.12	0.1	-0.33***	-0.26*	0.11	1							
	$A\beta_{42}$	0.32***	-0.02	-0.09	-0.04	0.11	0.15	0.1	1						
	$A\beta_{40}$	0.28*	-0.04	-0.25*	-0.01	0.17	0.08	0.08	0.63***	1					
	$A\beta_{42}/A\beta_{40}$	-0.14	0.02	0.19	-0.04	-0.14	0.08	0.03	0.01	-0.68***	1				
	pTau-181	0.1	-0.34***	-0.35***	0.55***	0.45***	0.07	-0.02	0.05	0.04	0.01	1			
	Neurogranin	0.04	-0.17	-0.23*	0.06	0.2	-0.18	-0.12	0.12	0.30***	-0.32***	0.04	1		
	NfL	0.05	0.08	-0.05	-0.06	0.01	0.65***	0.29*	0.30***	0.28*	-0.06	0.18	-0.02	1	
	Telomere	-0.15	0.09	0.07	0.09	0.02	0.04	-0.17	-0.16	-0.01	-0.14	-0.01	-0.02	-0.05	1

p-value		CSF						Plasma							
		sTREM2	$A\beta_{42}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Total tau	NfL	sTREM2	$A\beta_{42}$	$A\beta_{40}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Neurogranin	NfL	Telomere
CSF	sTREM2	-													
	$A\beta_{42}$	0.886	-												
	$A\beta_{42}/A\beta_{40}$	0.044	0	-											
	pTau-181	0.019	0.95	0	-										
	Total tau	0	0.88	0	0	-									
	NfL	0.327	0.189	0.849	0.458	0.411	-								
Plasma	sTREM2	0.07	0.261	0.364	0.002	0.013	0.326	-							
	$A\beta_{42}$	0.003	0.827	0.42	0.683	0.311	0.171	0.337	-						
	$A\beta_{40}$	0.007	0.691	0.017	0.933	0.117	0.486	0.453	0	-					
	$A\beta_{42}/A\beta_{40}$	0.198	0.824	0.073	0.679	0.187	0.449	0.808	0.891	0	-				
	pTau-181	0.362	0.001	0.001	0	0	0.507	0.818	0.652	0.693	0.962	-			
	Neurogranin	0.708	0.119	0.034	0.571	0.068	0.095	0.249	0.261	0.004	0.003	0.716	-		
	NfL	0.647	0.444	0.648	0.609	0.919	0	0.007	0.004	0.007	0.609	0.093	0.843	-	
	Telomere	0.167	0.385	0.536	0.388	0.878	0.705	0.113	0.127	0.91	0.194	0.924	0.848	0.633	-

B. AD continuum

Spearma's raw		CSF						Plasma							
		sTREM2	$A\beta_{42}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Total tau	NfL	sTREM2	$A\beta_{42}$	$A\beta_{40}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Neurogranin	NfL	Telomere
CSF	sTREM2	1													
	$A\beta_{42}$	-0.09	1												
	$A\beta_{42}/A\beta_{40}$	-0.25	0.18	1											
	pTau-181	0.19	0.32*	-0.13	1										
	Total tau	0.51***	0.16	-0.36*	0.76***	1									
	NfL	0.24	0.02	-0.1	0.11	0.1	1								
Plasma	sTREM2	-0.16	0.09	-0.06	-0.24	-0.17	0.1	1							
	$A\beta_{42}$	0.35*	-0.15	-0.3	0.1	0.35*	0.11	0.04	1						
	$A\beta_{40}$	0.2	0.13	-0.34*	0.04	0.27	0.05	0.1	0.67***	1					
	$A\beta_{42}/A\beta_{40}$	0.07	-0.24	0.1	0.21	0.1	0.1	-0.15	0.09	-0.60***	1				
	pTau-181	0.04	-0.03	0.04	0.341	0.14	0	0.18	0.1	-0.06	0.25	1			
	Neurogranin	0.11	-0.17	-0.26	-0.15	0.14	-0.24	0.1	0.22	0.35*	-0.25	-0.03	1		
	NfL	0.2	-0.02	-0.2	-0.13	-0.05	0.56***	0.33*	0.29	0.24	-0.01	0.23	-0.02	1	
	Telomere	-0.1	0.12	0.03	0.1	0.03	0.05	-0.08	-0.16	0.12	-0.29	0.03	0.09	-0.1	1

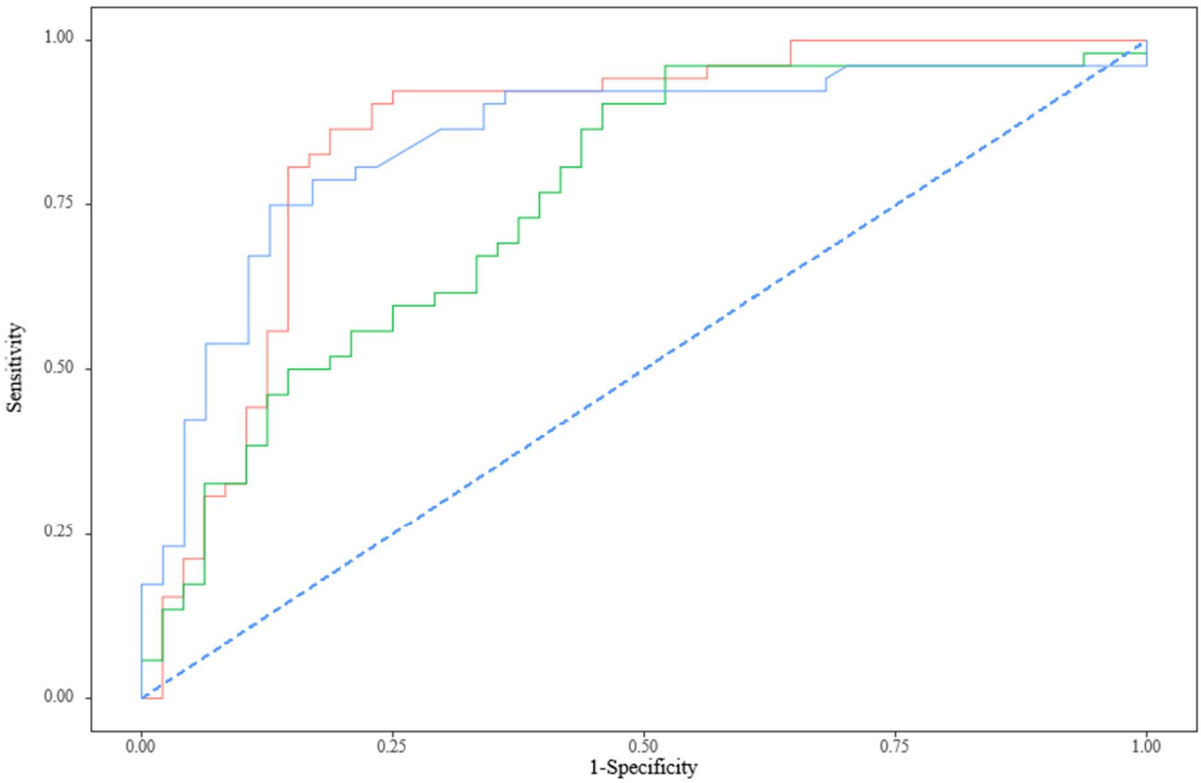
p-value		CSF						Plasma							
		sTREM2	$A\beta_{42}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Total tau	NfL	sTREM2	$A\beta_{42}$	$A\beta_{40}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Neurogranin	NfL	Telomere
CSF	sTREM2	-													
	$A\beta_{42}$	0.558	-												
	$A\beta_{42}/A\beta_{40}$	0.102	0.241	-											
	pTau-181	0.215	0.033	0.404	-										
	Total tau	0	0.285	0.017	0	-									
	NfL	0.116	0.899	0.514	0.495	0.507	-								
Plasma	sTREM2	0.302	0.543	0.709	0.118	0.262	0.526	-							
	$A\beta_{42}$	0.022	0.333	0.047	0.512	0.02	0.466	0.819	-						
	$A\beta_{40}$	0.183	0.408	0.025	0.819	0.075	0.762	0.5	0	-					
	$A\beta_{42}/A\beta_{40}$	0.669	0.12	0.499	0.169	0.532	0.535	0.315	0.546	0	-				
	pTau-181	0.776	0.838	0.799	0.067	0.368	0.984	0.238	0.533	0.719	0.095	-			
	Neurogranin	0.471	0.262	0.09	0.336	0.363	0.123	0.535	0.154	0.019	0.104	0.856	-		
	NfL	0.19	0.894	0.188	0.385	0.766	0	0.03	0.059	0.123	0.947	0.135	0.902	-	
	Telomere	0.511	0.44	0.863	0.503	0.855	0.764	0.604	0.289	0.442	0.059	0.871	0.58	0.52	-

C. Suspected non-AD pathological change

Spearma's raw		CSF						Plasma							
		sTREM2	$A\beta_{42}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Total tau	NfL	sTREM2	$A\beta_{42}$	$A\beta_{40}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Neurogranin	NfL	Telomere
CSF	sTREM2	1													
	$A\beta_{42}$	0.22	1												
	$A\beta_{42}/A\beta_{40}$	-0.06	0.19	1											
	pTau-181	0.21	0.59***	-0.35*	1										
	Total tau	0.41*	0.42***	-0.59***	0.79***	1									
	NfL	0.04	0.22	0.08	0.3	0.29	1								
Plasma	sTREM2	-0.12	-0.05	0	-0.11	-0.12	0.08	1							
	$A\beta_{42}$	0.33*	-0.02	0.02	-0.02	0.09	0.1	0.15	1						
	$A\beta_{40}$	0.38*	-0.31*	-0.26	-0.06	0.19	0.04	0.06	0.56***	1					
	$A\beta_{42}/A\beta_{40}$	-0.27	0.3	0.26	-0.03	-0.24	0.07	0.15	-0.02	-0.74***	1				
	pTau-181	-0.05	-0.05	-0.2	0.21	0.2	0.33*	0.16	0.13	0.23	-0.16	1			
	Neurogranin	-0.08	-0.19	-0.16	0	0.06	-0.09	-0.3	0.04	0.26	-0.35*	-0.09	1		
	NfL	0	0.1	-0.04	0.18	0.23	0.73***	0.22	0.3	0.29	-0.09	0.43***	0.02	1	
	Telomere	-0.15	0.09	0.13	0.02	-0.03	0.07	-0.31*	-0.18	-0.11	-0.01	-0.17	-0.16	0.01	1

p-value		CSF						Plasma							
		sTREM2	$A\beta_{42}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Total tau	NfL	sTREM2	$A\beta_{42}$	$A\beta_{40}$	$A\beta_{42}/A\beta_{40}$	pTau-181	Neurogranin	NfL	Telomere
CSF	sTREM2	-													
	$A\beta_{42}$	0.16	-												
	$A\beta_{42}/A\beta_{40}$	0.68	0.227	-											
	pTau-181	0.171	0	0.021	-										
	Total tau	0.006	0.004	0	0	-									
	NfL	0.792	0.159	0.606	0.049	0.054	-								
Plasma	sTREM2	0.448	0.75	0.985	0.481	0.42	0.591	-							
	$A\beta_{42}$	0.031	0.885	0.92	0.897	0.561	0.532	0.32	-						
	$A\beta_{40}$	0.012	0.041	0.084	0.702	0.208	0.812	0.705	0	-					
	$A\beta_{42}/A\beta_{40}$	0.08	0.051	0.093	0.862	0.113	0.674	0.318	0.876	0	-				
	pTau-181	0.757	0.734	0.2	0.18	0.186	0.03	0.301	0.417	0.132	0.286	-			
	Neurogranin	0.626	0.209	0.302	0.987	0.682	0.554	0.051	0.791	0.094	0.021	0.557	-		
	NfL	0.998	0.538	0.777	0.245	0.126	0	0.158	0.048	0.053	0.581	0.003	0.874	-	
	Telomere	0.338	0.569	0.393	0.884	0.841	0.631	0.041	0.255	0.486	0.968	0.279	0.299	0.956	-

Supplementary 2. Prediction of amyloid positivity using conventional AD biomarkers



Comparison between Aβ42/Aβ40 ratio and CSF p-tau 181, Plasma p-tau 181

1) Model CSF Aβ42/Aβ40 ratio

- optimal cutoff value : 0.0951
- AUC 0.76 (0.663 – 0.857)
- green, P = 0.902

2) Model CSF p-tau 181

- optimal cutoff value : 44.753 pg/ml
- AUC 0.846 (0.761 – 0.931)
- red, P < 0.001

3) Model Plasma p-tau 181

- optimal cutoff value : 3.14 pg/ml

- AUC 0.861 (0.784 – 0.939)

- blue, $P < 0.001$

Supplementary 3. Correlation between sTREM2 and regional amyloid burden

Spearman's raw	Whole dataset		AD		non-AD	
	CSF sTREM2	Plasma sTREM2	CSF sTREM2	Plasma sTREM2	CSF sTREM2	Plasma sTREM2
global	0.1	-0.17	0.04	-0.1	-0.03	-0.18
frontal	0.12	-0.17	0.03	-0.09	0	-0.19
temporal	0.09	-0.21*	0.01	-0.16	-0.01	-0.2
parietal	0.12	-0.14	0.06	-0.04	-0.02	-0.16
Precuneus & posterior cingulate	0.18	-0.15	0.14	-0.06	0.03	-0.17
occipital	0.09	-0.14	0.03	-0.11	-0.02	-0.12
basal ganglia	0.09	-0.27*	0.06	-0.35*	0.06	-0.18

p-value	Whole dataset		AD		non-AD	
	CSF sTREM2	Plasma sTREM2	CSF sTREM2	Plasma sTREM2	CSF sTREM2	Plasma sTREM2
global	0.314	0.097	0.811	0.513	0.833	0.224
frontal	0.256	0.09	0.841	0.567	0.982	0.191
temporal	0.366	0.038	0.943	0.294	0.94	0.181
parietal	0.257	0.191	0.695	0.797	0.877	0.285
Precuneus posterior cingulate	0.074	0.153	0.358	0.668	0.815	0.25
occipital	0.393	0.18	0.827	0.443	0.885	0.434
basal ganglia	0.368	0.008	0.682	0.017	0.833	0.224

논문 요약

알츠하이머병에 있어 sTREM2의 역할 규명

김형지

배경: 알츠하이머병은 치매를 유발하는 신경 퇴행성 질환 중에서 가장 흔한 병이다. 전통적인 아밀로이드 가설부터 시작하여 신경면역에 이르기까지 다양한 기전이 병을 유발하고, 악화시키는 것으로 알려져 있다. 최근의 연구들을 통해서 신경 면역이 점차 알츠하이머병의 병리에 있어 중요한 역할을 하는 것이 주목받기 시작하였다. 이러한 신경 면역에서는, 미아교세포의 역할이 중요한 요소로 작용을 하는 것으로 알려져 있다. 유전체 연구를 통해 triggering receptor expressed on myeloid cell 2 (TREM2) 유전자의 변이가 알츠하이머병을 포함한 신경퇴행성 질환의 위험도를 증가시키는 것이 알려짐에 따라, 미아교세포의 활성화와 상태의 변화가 알츠하이머병의 발생에서 핵심적인 역할을 담당하는 것으로 추정되고 있다. Soluble fragment of TREM2 (sTREM2)는 이러한 미아교세표의 활성화를 반영함과 동시에 알츠하이머 병의 진행 경과에서 환자의 상태에 따라 역동적인 변화를 보이는 것이 보고됨으로써 중요한 신경면역 생체표지자로 주목받고 있다. sTREM2는 막관통 단백질인 TREM2가 ADAM protease에 의해 단백질 분해를 거쳐 생성되며, 뇌척수액과 혈장 모두에서 검출될 수 있는 것으로 알려져 있다. 뇌척수액에서 발견되는 sTREM2는 특이적으로 미아교세포에서 유래된다. 반면, 혈장에서 검출되는 sTREM2는 기원이 다양하며, 주로 말초혈액의 단핵구 (peripheral blood mononuclear cells, PBMCs)가 기원이 되는 것으로 알려져 있다.

목적: 본 연구에서는 뇌척수액과 혈장의 sTREM2가 알츠하이머병의 병태생리적인 측면에서 가지는 역할을 규명해보고자 하였다. 이를 위해, 먼저 sTREM2가 기존에 아밀로이드 (A), 타우 (T), 및 신경퇴행 (N)으로 알려진 알츠하이머병의 생물표지자와 어떤 관련성을 가지는지를 분석하였다. 두 번째로, sTREM2가 뇌의 아밀로이드 침착과 함께 인지기능의 악화를 예측할 수 있는 잠재적인

생물표지자로써의 기능이 있을 것으로 가정하였다. 이를 통해 알츠하이머병에서 sTREM2가 기존의 AT(N) 생물표지자와 더불어 가질 수 있는 임상적인 의미를 규명하고자 하였다.

방법: 연구 대상환자는 서울아산병원 기억력장애 클리닉에 2018년에서 2020년에 방문한 환자중에서 모집하였다. 아밀로이드 양전자방출 단층촬영 (PET)검사를 통해 아밀로이드 양성 여부를 결정하였고, 신경심리검사를 시행하여 환자의 인지 상태를 진단하였다. 알려진 알츠하이머병의 생체표지자와 sTREM2를 뇌척수액 및 혈장에서 측정하였다.

결과: 뇌척수액의 sTREM2농도는 pTau-181과 양의 상관관계를 보였으나 ($\rho = 0.25$, $P = 0.019$), 혈장 sTREM2 농도는 음의 상관관계를 보여 ($\rho = -0.33$, $P = 0.002$), 뇌척수액과 혈장의 sTREM2가 다른 양상을 보였다. 그러나 뇌척수액과 혈장 sTREM2사이에서는 직접적인 연관성은 확인할 수 없었다. 아밀로이드 병리를 예측하는데 있어서는 뇌척수액 및 혈장의 sTREM2가 유의미한 통계적인 연관성을 확보하지 못하였고, 혈장 pTau-181이 가장 강력한 예측도를 보였다 (cutoff value, 3.14 pg/ml, AUC = 0.861, P <0.001). 아밀로이드 PET검사를 정량적으로 분석한 결과에서, 알츠하이머 환자 군에서 혈장 sTREM2가 기저핵 (basal ganglia)의 아밀로이드 침착과 음의 상관관계를 보였다 ($\rho = -0.35$, $P = 0.01$). 혈장 생물표지자를 이용하여 인지기능의 악화가 발생할 수 있는지를 예측한 결과에서는 혈장 sTREM2와 $A\beta_{42}/A\beta_{40}$ ratio의 조합이 통계적으로 의미있는 결과를 보였다(AUC = 0.761, P <0.001).

결론: 본 연구를 통해 뇌척수액의 sTREM2로 대표되는 신경염증이 알츠하이머병의 진행 과정에 있어 일정한 역할을 수행하고 있음을 확인할 수 있었다. 또한, 혈장의 sTREM2도 질환의 예후를 예측하는데 있어 의미가 있을 수 있음을 확인하였고, 기저핵과 같은 피질하조직의 아밀로이드 침착을 예측할 수 있는 생물표지자로써의 가능성도 확인할 수 있었다. 다만 본 연구는, sTREM2의 역할을 확증적으로 규명하기에는 제한이 있으므로, 추후 유전체 연구 및 다른 코호트와의 대조연구 등을 통해 의미를 확장해갈 필요가 있다.

중심단어: 알츠하이머병, 아밀로이드, 신경염증, 치매