TREM2 Levels Are Increased in Neurodegenerative Disease Patients and in an Alzheimer's Disease Mouse Model

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Research

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Abstract

Background: Triggering receptor expressed on myeloid cells 2 (TREM2) is expressed on microglia, and it plays an important role in neurodegenerative diseases, especially Alzheimer's disease (AD).This study aimed to elucidate the potential value of TREM2 as biomarker for the diagnosis of AD.

Methods: Serum TREM2 levels in human subjects and mice were detected by ELISA. TREM2 mRNA and protein expressions in leukocytes and mouse brain tissues were detected using real-time quantitative PCR(RT-qPCR) and western blot analysis. The cognitive ability of patients was measured by MMSE/MoCA score. The cognitive ability of mice was evaluated by the Morris water maze (MWM) test.

Results: Our results showed that the serum concentration and expression levels of TREM2 in leukocytes were increased both in neurodegenerative patients and in an AD model mice.TREM2 expression was correlated with the cognitive impairment in AD patients and APP/PS1 mice.

Conclusion: This study indicates that TREM2 is a novel potential biomarker for AD diagnosis.

Introduction

Alzheimer's disease (AD) is the most common type of neurodegenerative dementia worldwide[1]. Deposition of extracellular amyloid protein β(Aβ) producing amyloid plaques and intracellular tau aggregation producing neurofibrillary tangles are two typical pathological features of this disease[2,3].Patients progress from mild cognitive impairment (MCI) to dementia of the Alzheimer type (DAT), which typically takes approximately five years or longer[1]. There is a growing belief that AD is a heterogeneous disease affected by numerous factors, including age, hereditary factors, and environmental factors[4]. Although abundant work has been performed by researchers , there is no effective way to prevent and treat AD[5].

Microglia are the main neuroimmune cells, and they play a crucial role in the development and progression of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and prion diseases[6-10]. Researchers found that microglia proliferate and become active around the amyloid plaques in the brains of AD patients. The role of microglia in the progression of AD disease has not been fully elucidated. Accumulated evidence suggests that microglia may defend against the incidence of AD, as damaged microglial activities and abnormal microglial responses to β-amyloid are associated with an increased risk of AD[11].However, there is also abundant evidence that activated microglia can be harmful to neurons. Microglia secrete inflammatory factors directly or via activation of neurotoxic astrocytes, which can injure neurons [12].

Triggering receptor expressed on myeloid cells 2 (TREM2) is a transmembrane protein, that is primarily expressed on microglia. It plays an important role in regulating inflammatory signals and promoting microglial proliferation, phagocytosis and survival [13-15]. Rare variants in the *TREM2* gene have been shown to increasing the risk of microglia-mediated innate immune response in developing Alzheimer's

disease[16,17]. After proteolytic cleavage, soluble TREM2 (sTREM2) is released into the extracellular space and can be detected in the plasma and cerebrospinal fluid (CSF). Numerous experiments confirmed that AD patients presented higher sTREM2 levels in the CSF than cognitively normal individuals [18-20].These data suggest that TREM2 may be a potential biomarker for AD.

In this study, we detected TREM2 in the serum and leukocytes of patients at different stages of AD as well as other neurodegenerative disease patients. Moreover, we tested TREM2 in an AD mouse model and wild type (WT) mice and analyzed the correlation between cognition and the level of TREM2. Finally, we confirmed the diagnostic value of sTREM2 expression by relative operating characteristic curve (ROC) analysis.

Materials And Methods

Subjects and clinical assessment

A total of 330 participants were recruited in the present study. A total of 267 patients were diagnosed with Alzheimer's disease (AD), Parkinson's disease (PD) and vascular dementia (VaD). Moreover, according to the NINCDS-ADRDA diagnostic criteria, AD patients were categorized into the mild cognitive impairment (MCI) and dementia of Alzheimer type (DAT) groups.All of the AD patients were free from a family history of dementia. In addition, 63 healthy control(HC) subjects were included in this study. They were confirmed to be healthy and to be neurologically normal by assessment of medical history, general examination, and laboratory examinations. The cognitive status and severity of dementia of subjects was assessed by Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment Scale (MoCA) scores. The patient clinical data are shown in **Table 1**.This study was approved by the Ethics Committee of Xuanwu Hospital of Capital Medical University (Beijing, China), and all participants or their guardians signed informed consent.

Animals

AD model transgenic mice (APPswe/PS1dE9 mice) and wild type mice(C57BL/6J mice) were purchased from Beijing Huafukang Biotechnology Company. All animal experiments conformed to the National Institutes of Health guidelines and were approved by the ethics committee of Xuanwu Hospital of Capital Medical University.

Serum sample collection

Peripheral blood was taken from each subject by venipuncture.Mouse blood was collected from the retro-orbital plexus. Then the samples were allowed to clot for two hours at room temperature before centrifugation for 20 minutes at 1000×g. Then the samples were stored at -80°C for later use.

Leukocyte extraction

Leukocytes were isolated from peripheral blood by being placed in tubes with EDTA (an anticoagulant) containing Hank's Balanced Salt Solution (HBSS) (Solarbio,China), and then they were centrifuged for 10 minutes at 2000 rpm/min.Then, they were washed twice in the same way. The samples were stored at -80℃ for subsequent experiments.

ELISA

The serum concentrations of TREM2 in the human population and in mice were measured using human and mouse TREM2 ELISA kits respectively (Mlbio, China), according to the product instructions. Finally, the optical density (O.D.) was determined at 450 nm using a microtiter plate reader and the sample concentration was calculated according to the standard curve.

cDNA synthesis and real-time quantitative PCR (RT-qPCR)

RNA was extracted from leukocytes using TRI Reagent (Sigma-Aldrich,USA). Reverse transcription was performed to synthesize cDNA using 5X All-In-One RT MasterMix (Abm,Canada). Then real-time quantitative PCR (RT-qPCR) was conducted using TB Green® Premix Ex Taq™ II (TaKaRa,Japan) and a LightCycler 480 Real-time PCR system (Roche Applied Science, Germany).The RT-qPCR primers were as follows:

TREM2: forward primer:5’-GGTCAGCACGCACAACTTG-3’,

reverse primer:5’-CGCAGCGTAATGGTGAGAGT-3’.

GAPDH:forward primer:5’-GTCTCCTCTGACTTCAACAGCG-3’,

reverse primer: 5’-ACCACCCTGTTGCTGTAGCCAA-3’.

Western blotting

Brain tissues or leukocytes were lysed in 1% Triton X-100 lysis buffer (Solarbio, Beijing, China) supplemented with protease and phosphatase inhibitor cocktails (Thermo Scientific,USA). The protein concentration was determined using a BCA Protein Assay kit(Thermo Scientific, USA). Equal amounts of protein were loaded onto SDS-PAGE gels (with 10% SDS) and then were separated by electrophoresis. The seperated proteins were then transferred to PVDF membranes (Millipore, California, USA). The membranes were blocked with 5% w/v nonfat milk for 1 hour at room temperature. The membranes were then incubated overnight at 4 °C with a TREM2 antibody (1:500 dilution; Abcam) and a β-actin antibody (1:5000 dilution; ZSGB-BIO) in TBST containing 5% w/v nonfat milk. The cells were washed 3 × 5 minutes in TBST at room temperature. The membranes were incubated for 1 hour at room temperature with corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies (ZSGB-BIO, Beijing, China) in TBST containing 5% w/v nonfat milk. After extensive washing in TBST the protein bands were visualized using an Super ECL Detection Reagent (YEASEN,Shanghai,China) and then were imaged using a Tanon 4600SF system (China).

Morris water maze test

Mice were trained in a water maze for 5 consecutive days. The time spent reaching the platform (escape latency) was measured. The mice were gently guided to the platform if they were unable to reach the platform within 60 s and then were left on the platform for 20 s. After five days of training, the platform was removed from the pool, and the mice were subjected to an investigative trail session. Mice were allowed to swim for 60 s in search of a platform, and their motion trajectories were recorded by a video camera and analyzed by a computer video system.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software version 6.0 (CA, USA). Group differences in categorical data, such as age, gender, MMSE and MoCA grades were analyzed using one-way analysis of variance (ANOVA) test. Group differences between two groups were analyzed using Student's t-test. The correlations between the TREM2 level and MMSE/MoCA gardes were carried out using linear regression analysis.Sensitivity and specificity of measured variable for different groups were examined using a receiver operating characteristic curve (ROC) analysis under a nonparametric approach. All data are presented as the mean ±standard error of mean (SEM), and the level of significance was set to $P<0.05$.

Results

1.Soluble TREM2 was increased in different stage of AD and PD

The level of soluble TREM2 (sTREM2) in serum was measured by ELISA. Compared with the levels in the healthy control (HC) group, sTREM2 levels were increased in the MCI and DAT stages of AD patients (Fig.1A). Meanwhile, AD and PD but not VaD patients had higher sTREM2 concentrations than patients in the HC group. AD and PD patients had significantly higher sTREM2 concentrations than VaD patients (Fig.1B). Lower level of sTREM2 was observed in VaD group. To further explore the relationship between TREM2 and AD progression, we analyzed the correlation between sTREM2 and MMSE/MoCA scores(Fig.1C-F). There was a significantly negative correlation between sTREM2 and cognition (MMSE/MoCA) within the AD and PD group. Notably, this relation ship is stronger in AD than PD patients. These results elucidate that the level of sTREM2 is closely correlated with the cognitive ability of neurodegenerative disease.

2.The expression of TREM2 in leukocytes increased in neurodegenerative diseases

TREM2 expression levels in leukocytes were detected RT-qPCR and western blot. The results showed that compared to HC group, increased expression of TREM2 was observed in the leukocytes isolated from AD patients(both at the MCI and DAT stages) and PD patients, but not VaD patients in terms of mRNA(Fig.2A and B). In addition, we also found higher levels of TREM2 in DAT stage of AD patients, but not significant in MCI stage of AD patients in terms of protein level (Fig.2C).TREM2 level increased remarkably in PD

patients but not in VaD patients(Fig.2D). These results indicate that TREM2 expression is increased in neurodegenerative diseases.

3.TREM2 expression is elevated in AD model mice

We chose 2-,5- and 10-month-old AD model mice (APP/PS1 mice) and wild-type mice (WT mice) of the same age to evaluate their spatial learning and memory ability using a Morris water maze(MWM) test. According to the mean escape latencies (Fig.3A-C,G) and the time of across the platform(Fig.3D-F) during training days, 10-month-old APP/PS1 mice had distinct cognitive deficiencies compared with age matched WT mice. Then, we detected TREM2 expression in the hippocampus and the sTREM2 level in serum of different group mice. In particular, TREM2 expression in 5- and 10-month-old APP/PS1 mice was significantly elevated compared with that in WT mice(Fig.3H, I). Moreover, TREM2 expression showed a positive correlation with the escape latency of 10-month-old APP/PS1 mice (Fig.3L, $r^2 = 0.3811$, $p = 0.0325$). However, this correlation was not observed between the other groups of APP/PS1 and WT mice(Fig. 3J, K, $p > 0.05$).These results suggest that TREM2 expression increases with age in APP/PS1 and WT mice and that TREM2 expression is negatively correlated with cognition in APP/PS1 mice.

4.The diagnostic value of sTREM2 in neurodegenerative diseases

To better interpret these test results the diagnostic value of sTREM2 was further tested by ROC curve analysis. The sensitivity and specificity was 50.0 % and 63.60% respectively, and the area under the curve (AUC) was 0.5217 in discriminating AD from PD patients (Fig.4A).The sensitivity and specificity was 81.8 % and 83.3% respectively, and the area under the curve (AUC) was 0.8585 in discriminating AD from VaD patients (Fig.4B). The detailed data of diagnostic powers of the sTREM2 were showed in **Table2**.

Discussion

Age is one of the most important risk factors in developing AD. Depending on the age of onset, AD can be divided into early-onset Alzheimer's disease (EOAD)(patients younger than 65 years) and late-onset Alzheimer's disease (LOAD)(patients not older than 65 years)[21,22]. Genetic links between EOAD and three disease-causing genes, amyloid precursor protein (*APP*), presenilin1(*PS1*) and presenilin2 (*PS2*), have been identified in the past few decades[23,24]. However, the genetic causes of LOAD have not yet been completely explained yet. *TREM2*, a gene that encodes an immune receptor found in microglia, is closely related to LOAD[25,26].Our study provides evidence that there are increased levels of TREM2 in several neurodegenerative diseases, particularly AD.

First, the serum concentration and mRNA level of TREM2 were significantly increased in the MCI and DAT groups, and sTREM2 were associated with the cognitive ability of AD patients. Moreover, the serum concentration and leukocyte expression levels of TREM2 were also significantly increased in patients with PD, but they were decreased in VaD.These results suggest that higher sTREM2 may be associated with AD disease progression, and sTREM2 levels may be used as a potential predictive biomarker of neurodegenerative disease.

Second, we employed APP/PS1 mice to identify the relationship between TREM2 and cognitive competence. APP/PS1 mice are a transgenic AD model mice that are widely used in the study of the pathogenesis and treatment of AD. In this study, we found that 10-month-old APP/PS1 mice showed serious cognition impairment. Atrophy and neuronal inflammatory responses of the hippocampus and cortex are the typical features of AD. It is well known that the hippocampus of mice is a cognitive-related brain region. In this study we found that TREM2 expression was dramatically higher in the cortex of aging APP/PS1 mice than it was in controls. Furthermore, sTREM2 was negatively correlated with cognitive function, indicating that TREM2 may play an important role in the pathogenesis of AD.

Finally, we analyzed the diagnostic value of sTREM2 tested by ROC curve. AD, PD and VaD share many similarities in clinical manifestations and neuropathologic features. So the diagnosis of AD is a major challenge. According to the ROC curve analysis, the sensitivity and specificity of sTREM2 levels could differentiate AD patients from VaD patients to some extent, suggesting that TREM2 might act as a potential biomarker for AD. However, the elevated level TREM2 was observed in PD. Therefore, more data are needed to verify and determine the best diagnostic cut-off values to differentiate AD from other neurodegenerative diseases.

Although the relationship between TREM2 gene variation and AD has received extensive attention, more studies are required to validate this possible association [27,28]. In addition, sTREM2 levels in the cerebrospinal fluid of AD patients were elevated compared with those of healthy controls, and sTREM2 concentrations did not have the discriminative ability required for diagnostic procedures[29,30].

Conclusion

In conclusion, our study suggests that the elevated level of TREM2 may aggravate AD progression, and TREM2 is a potential diagnostic marker and therapeutic target for AD and other neurodegenerative diseases.

Abbreviations

TREM2: Triggering receptor expressed on myeloid cells 2

AD: Alzheimer’s disease

PD: Parkinson’s disease

VaD: vascular dementia

HC: healthy control

RT-qPCR: real-time quantitative PCR

MMSE: Mini-Mental State Examination

MoCA: Montreal Cognitive Assessment Scale

MWM: Morris water maze

Aβ: amyloid protein β

APP: amyloid precursor protein

PS1: presenilin1

PS2: presenilin2

MCI: mild cognitive impairment

DAT: dementia of the Alzheimer type

CSF: cerebrospinal fluid

WT: wild type

sTREM2: soluble TREM2

ROC: Receiver operating characteristic curve

AUC: area under the curve

CI: Confidence Interval

EDTA: ethylenediaminetetraacetic acid

HBSS: Hank's Balanced Salt Solution

ELISA: enzyme-linked immunosorbent assay

O.D. : optical density

PCR: polymerase chain reaction

SEM: standard error of mean

EOAD: early-onset Alzheimer’s disease

LOAD: late-onset Alzheimer’s disease

Declarations

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interests.

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Author contributions

Xiaomin Zhang and Jing Liu designed and performed experiments, analyzed data and drafted the manuscript. Min Cao performed some experiments and analyzed data. Tingting Yang analyzed data, Yaqi Wang and Yuli Hou provided statistical support, Qiao Song and Yuting Cui collected data and revised the manuscript. Peichang Wang provided financial support, instructed manuscript format and edited the final manuscript. All the co-authors contributed to revising the manuscript for intellectual content and approved the final version for publication.

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Ethics approval and consent to participate

All people were approved by the ethics committee of Xuanwu Hospital of Capital Medical University (Beijing, China), and written informed consent was obtained from all participants or their guardians.

The animal study protocol was approved by the ethics committee of Xuanwu Hospital of Capital Medical University.

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Tables

Table 1. Characterization of subjects in different groups.

Characteristics	HC(n=63)	MCI(n=74)	DAT(n=77)	PD(n=74)	VaD(n=42)
Age(years)					
Min	52	50	50	50	49
Max	80	83	85	85	89
Mean±SD	65.24±6.92	68.64±8.08	67.86±7.87	67.64±7.91	68.64±10.69
Gender					
Female,n(%)	29(46.0%)	39(52.7%)	44(57.1%)	44(59.5%)	25(59.5%)
Male,n(%)	34(54.0%)	35(47.3%)	33(42.9%)	30(40.5%)	17(40.5%)
MMSE					
Min	22	16	8	10	18
Max	29	28	26	28	26
Mean±SD	26.38±2.31	23.15±3.02	17.35±4.74	19.3±4.73	22.57±2.82
MoCA					
Min	21	10	7	12	17
Max	29	26	26	26	25
Mean±SD	25.70±2.39	20.89±3.47	19.45±4.81	19.81±3.66	21.10±2.07

Table 2. Diagnostic powers of the sTREM2 to discriminate between AD and the non-AD subjects.

Comparison group	Cut-off value	Sensitivity(%)	Specificity(%)	AUC	95% Confidence Inteval	P value
AD vs. PD	0.136	50.0	63.6	0.5217	0.4282- 0.6151	0.6458
AD vs. VaD	0.652	81.8	83.3	0.8585	0.7822 - 0.9348	<0.0001

Figures

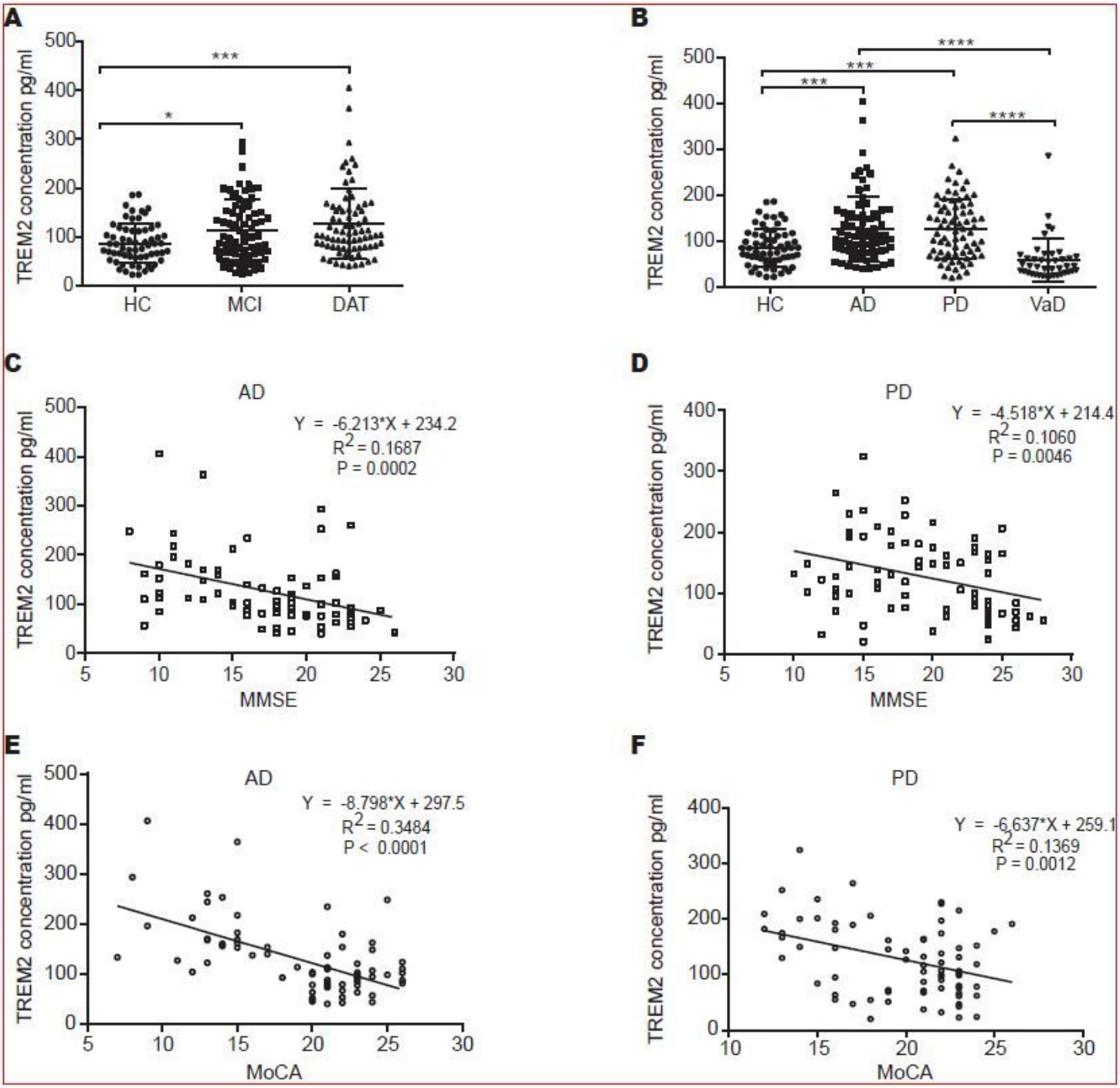

Figure 1

The level of soluble TREM2(sTREM2) in serum of different group subjects. A. sTREM2 increased in MCI (n = 74) and DAT (n = 77) patients compared with HC (n = 63) subjects (*p<0.05,***p < 0.005). B.Compared with HC (n = 63) subjects, sTREM2 higher in AD (n = 77) and PD (n = 74),but lower in VaD (n = 42) patients (***p < 0.005, ****p<0.0001). C.The correlation between sTREM2 and MMSE in AD patients was analyzed by linear regression analysis(R2 = 0.1687, P = 0.0002). D. The correlation between sTREM2 and MMSE in PD patients was analyzed by linear regression analysis(R2=0.1060, P=0.0046). E.The relationship between sTREM2 and MoCA in AD patients was analyzed by linear regression analysis(R2 =

0.3484 , P < 0.0001). F.The relationship between sTREM2 and MoCA in PD patients was analyzed by linear regression analysis(R2 = 0.1369,P = 0.0012).

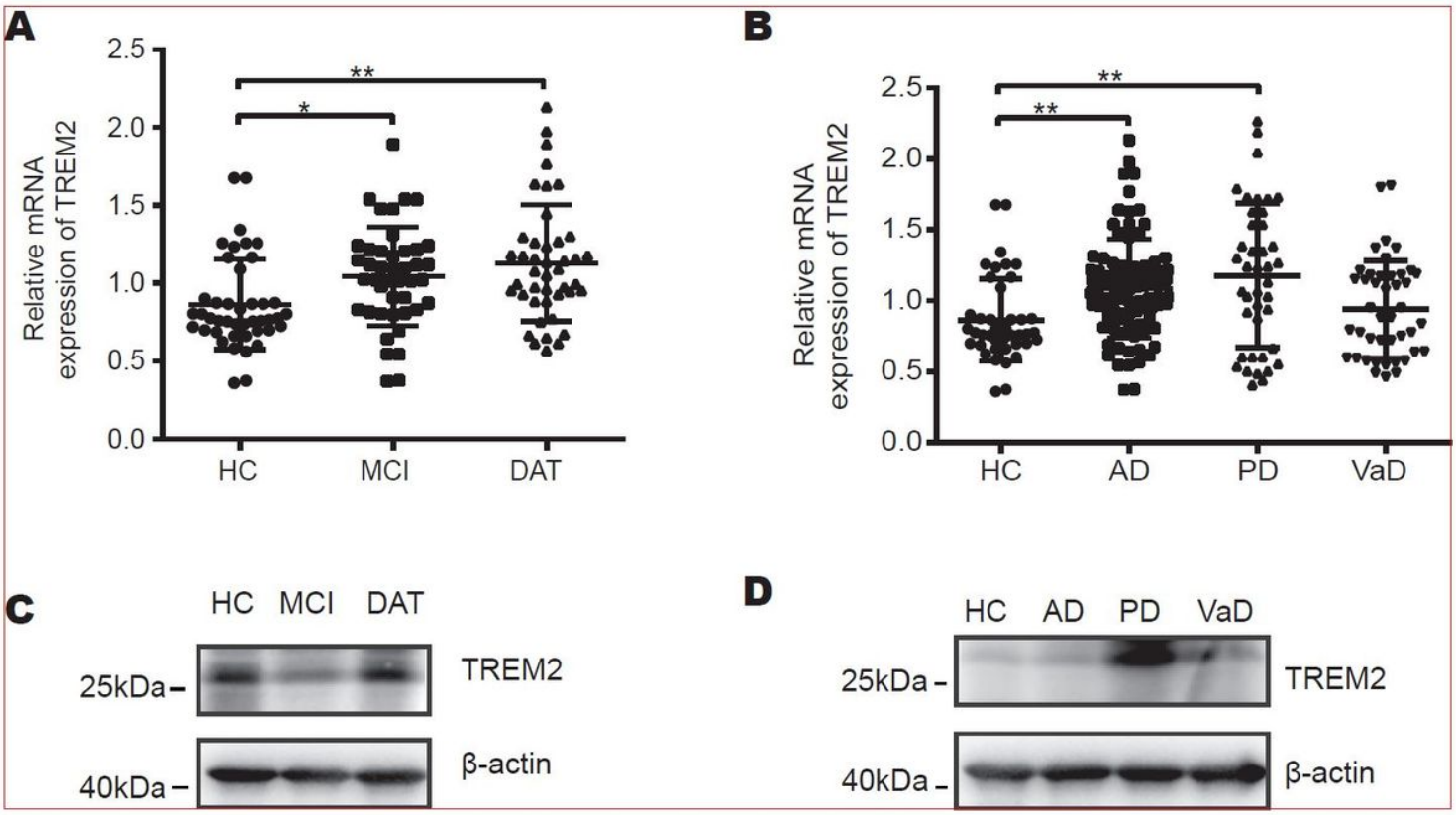

Figure 2

The mRNA and protein level of TREM2 in leukocytes of different group subjects. A. TREM2 was increased both in MCI (*p < 0.05) and DAT (**p < 0.01)patients compared with HC group in terms of mRNA. B. TREM2 was increased both in AD (*p < 0.05) and PD (*p < 0.05)patients compared with HC group in terms of mRNA, but there was no difference between VaD and HC groups (p > 0.05). C. TREM2 protein levels in the leukocytes of HC, MCI and DAT subjects. D. TREM2 protein levels in the leukocytes of HC, AD, PD and VaD subjects. Values represent mean ± SEM, *p < 0.05, **p < 0.01.

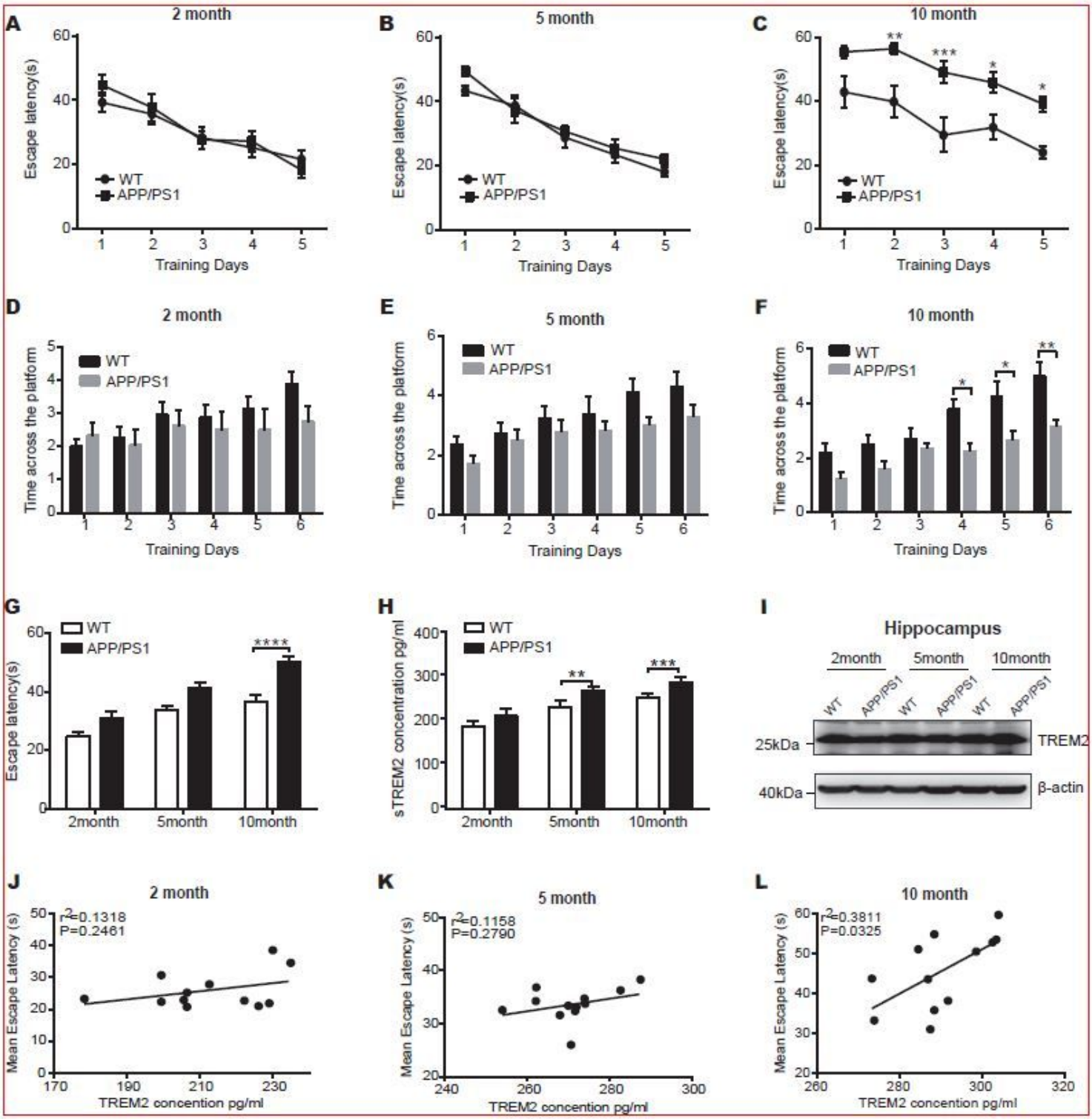

Figure 3

Assessment spatial cognitive ability and TREM2 expression in the hippocampus and serum of different groups of APP/PS1 and WT mice. A. The escape latency of APP/PS1 and WT mice at age of 2 month-old (n = 12). B.The escape latency of APP/PS1 and WT mice at age of 5 month-old (n = 12). C.The escape latency of APP/PS1 and WT mice at age of 10 month-old (n = 12). D. The time of across the platform of APP/PS1 and WT mice at age of 2 month-old (n = 12). E. The time of across the platform of APP/PS1 and WT mice at age of 5 month-old (n = 12). F. The time of across the platform of APP/PS1 and WT mice

at age of 10 month-old (n = 12). G. Average escape latency of APP/PS1 and WT mice at different ages during 1–5 days of training in MWM test. H. TREM2 levels of serum in different ages APP/PS1 and WT mice. I. TREM2 protein levels in the hippocampus of 2-, 5- and 10-month-old APP/PS1 and WT mice. J. Correlation between TREM2 expression in serum and cognitive ability in 2-month-old APP/PS1 mice (r2=0.1318, p=0.2461). K. Correlation between TREM2 expression in serum and cognitive ability in 5-month-old APP/PS1 mice (r2=0.1158, p=0.2790). L. Correlation between TREM2 expression in serum and cognitive ability in 10-month-old APP/PS1 mice (r2=0.3811, p=0.0325). Values represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001.

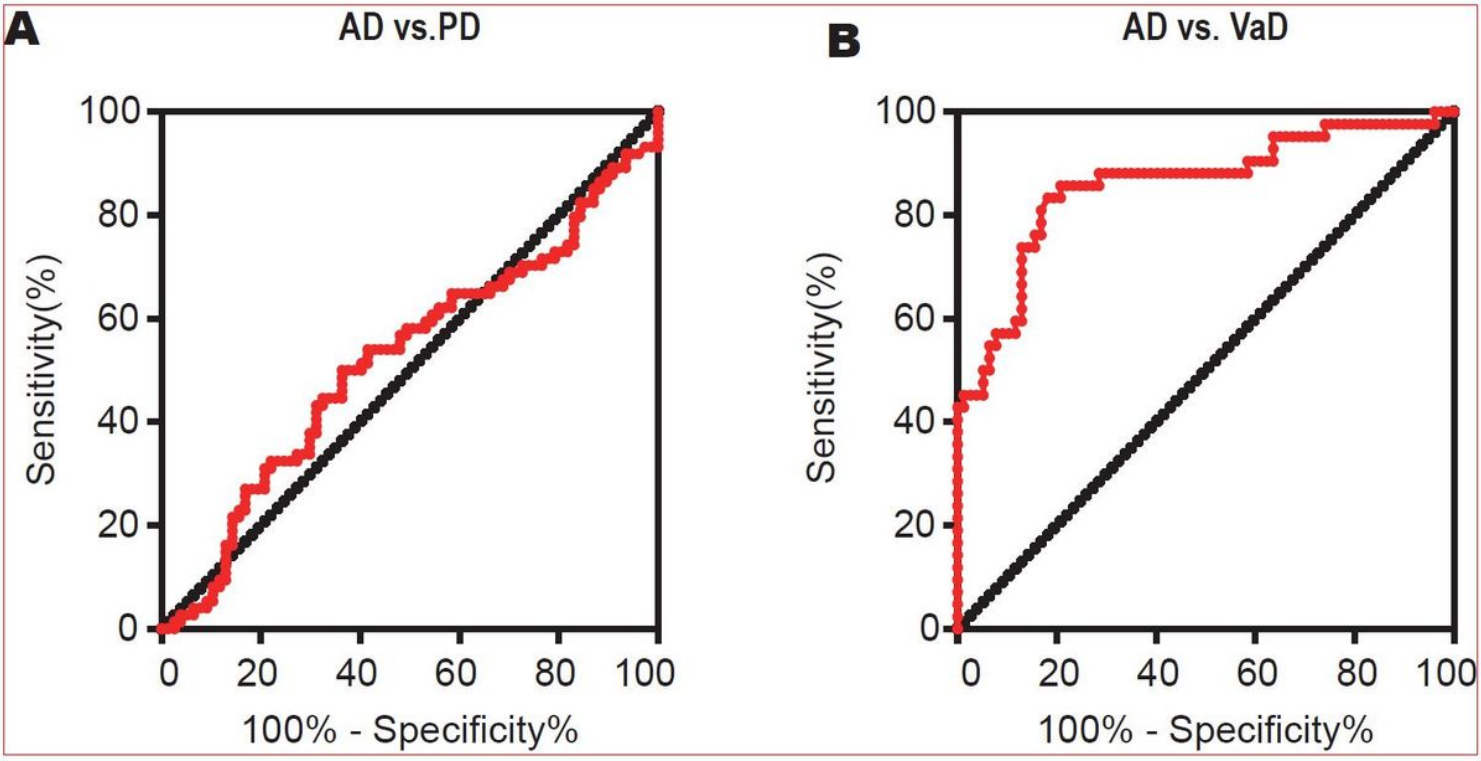

Figure 4

ROC curves of sTREM2 in the differential diagnosis of AD, PD and VaD. A. ROC curve of the sTREM2 were used to distinguish AD from PD. B. ROC curve of the sTREM2 were used to distinguish AD from VaD.